



**EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31**

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## SCIENTIFIC OPINION

### Scientific Opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2):

#### Aliphatic and aromatic hydrocarbons from chemical group 31<sup>1</sup>

#### EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)<sup>2,3</sup>

European Food Safety Authority (EFSA), Parma, Italy

#### ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 37 flavouring substances in the Flavouring Group Evaluation 25, Revision 2, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the ten substances [FL-no: 01.001, 01.027, 01.028, 01.033, 01.034, 01.038, 01.039, 01.046, 01.054 and 01.057] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For the remaining 27 candidate substances [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 10.078] no appropriate NOAEL was available and additional data are required. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. For five substances, the composition of the stereoisomeric mixture has to be specified further.

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1 On request from the Commission, Question No EFSA-Q-2010-01558, adopted on 19 May 2011.

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## SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to advise 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 evaluate 37 flavouring substances in the Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 37 flavouring substances belong to chemical group 31, Annex I of the Commission Regulation (EC) No 1565/2000.

The 37 candidate substances are aliphatic and aromatic hydrocarbons, which have been divided into eight subgroups: I) acyclic alkanes, II) acyclic alkenes, III) cyclohexene hydrocarbons, IVa) benzene hydrocarbons, IVb) naphthalene hydrocarbons, IVc) diphenylmethane, V) bi- and tricyclic, non-aromatic hydrocarbons and VI) macrocyclic, non-aromatic hydrocarbons.

Several of the 37 flavouring substances possess chiral centres and/or can exist as geometrical isomers. For five of the flavouring substances [FL-no: 01.021, 01.027, 01.032, 01.035 and 01.078] the stereoisomeric composition/composition of mixture has not been specified sufficiently.

Thirty-one of the 37 candidate substances belongs to structural class I, two belongs to structural class II and four to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Thirty-three out of the 37 candidate substances have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, 35 of the 37 flavouring substances in this group have intakes in Europe from 0.0012 to 28 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day), structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances. For limonene [FL-no: 01.001] and *l*-limonene [FL-no: 01.046] the intakes are 4000 and 2100 microgram/*capita*/day, which are above the threshold of concern value for structural class I (1800 microgram/person/day).

Combined intakes can be calculated for the substances in subgroup I (acyclic alkanes) and subgroup III (cyclohexene hydrocarbons) evaluated through the Procedure.

For subgroup I (acyclic alkanes), the combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Annex I), is 3.0

microgram/capita/day, which does not exceed the threshold of 1800 microgram/person/day. There are no supporting substances in subgroup I.

For subgroup III (cyclohexene hydrocarbons), the combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Annex I), is 6100 microgram/capita/day, which exceeds the threshold of 1800 microgram/person/day. The total combined intake of the candidate and six supporting substances (also from structural class I) is 42000 microgram/capita/day. This intake exceeds the threshold of 1800 microgram/person/day for a structural class I substance. However, limonene [FL-no: 01.001], *l*-limonene [FL-no: 01.046] and *d*-limonene (supporting substance [FL-no: 01.045]) accounts for approximately 40000 microgram/capita/day. The total combined intake of 42000 microgram/capita/day for the candidate and the supporting substances corresponds to 700 microgram/kg bw/day for a person with a body weight of 60 kg. Thus, based on the NOAEL for *d*-limonene of 215 mg/kg bw/day, a margin of safety of 307 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

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, there is evidence of an *in vivo* genotoxic and carcinogenic potential. However, the Panel concluded that the available data do not preclude an evaluation of the 37 candidate substances using the Procedure.

The available information on metabolism of the 37 candidate substances evaluated through the Procedure or the supporting substances for this FGE was very limited. Overall, only for 10 candidate substances it can be concluded that they will be metabolised into innocuous metabolites, [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 0.001, 01.027, 01.028, 01.039 and 01.046] from subgroup III. For six candidate substances [FL-no: 01.037, 01.050, 01.051, 01.053, 01.164 and 01.070] there are data, which show that they may be metabolised to toxic metabolites. For the remaining 21 candidate substances [FL-no: 0.021, 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], the information is too limited and it cannot be assumed that they are metabolised to innocuous metabolites.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is concluded that the 10 candidate substances which are expected to be metabolised to innocuous substances, [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 01.001, 01.027, 01.028, 01.039 and 01.046] from subgroup III, would not give rise to safety concerns at their estimated intakes arising from their use as flavouring substances based on the MSDI approach. For the remaining 27 candidate substances, [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 astructurally related supporting substances, no adequate NOAELs were available. Therefore, additional toxicological data are required.

The mTAMDI values for the 27 candidate substances from structural class I, for which use levels have been provided are in the range of 3100 to 3900 microgram/person/day. For each of the two candidate substances from structural class II and the four candidate substances from structural class III the mTAMDI is 3900 microgram/person/day. These values are above the threshold for structural class I, II and III of 1800, 540 and 90 microgram/person/day, respectively.

Accordingly, intake estimates according to the mTAMDI for the 33 candidate substances for which use levels have been provided exceed the thresholds of concern for the three structural classes, and more reliable exposure data are requested. On the basis of such additional data, the flavouring

substances should be considered using the Procedure. Subsequently, additional data might become necessary. For the four remaining candidate substances [FL-no: 01.001, 01.046, 01.070 and 01.078] use levels for food categories as outlined in Commission Regulation (EC) no 1565/2000, Annex III, are required.

In order to determine whether this conclusion could be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 32 flavouring substances. For five substances [FL-no: 01.021, 01.027, 01.032, 01.035 and 01.078] information on stereoisomeric composition/composition of mixture has not been specified sufficiently.

Thus, overall, for 27 candidate substances [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] additional toxicity data are required. For four of these, [FL-no: 01.021, 01.032, 01.035 and 01.078], additional information on composition requested. The final evaluation of the materials of commerce cannot be performed for one substance [FL-no: 01.027], pending further information on composition of mixture of stereoisomers. For nine candidate substances [FL-no: 01.001, 01.028, 01.033, 01.034, 01.038, 01.039, 01.046, 01.054 and 01.057] the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

## **KEYWORDS**

Hydrocarbons, aliphatic, alicyclic, aromatic, flavourings, safety, FGE.25, 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 2008/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

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

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

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

## HISTORY OF THE EVALUATION

FGE	Opinion Adopted by EFSA	Link	No. of Candidate Substances
FGE.25	1 April 2008	<a href="http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902222647.htm">http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902222647.htm</a>	32
FGE.25Rev1	23 September 2009	<a href="http://www.efsa.europa.eu/en/scdocs/scdoc/1334.htm">http://www.efsa.europa.eu/en/scdocs/scdoc/1334.htm</a>	34
FGE.25Rev2	18 May 2011		37

The present Revision of FGE.25, FGE.25Rev2, includes the assessment of three additional candidate substances [FL-no: 01.001, 01.021 and 01.046]. No toxicity or metabolism data were provided for these three substances. A survey in open literature did not result in further data.

Since the publication of FGE.25Rev1 new tonnage data for [FL-no: 01.035, 01.047 and 01.064] have become available (Flavour Industry, 2010a), which have been included in this revision.

Industry has also submitted additional information on stereoisomeric composition [FL-no: 01.027, 01.032, 01.034, 01.035, 01.050, 01.055, 01.056 and 01.060], composition of mixture [FL-no: 01.078] and missing ID-tests [FL-no: 01.078].

In the FGE.25 and FGE.25Rev1, the Panel considered that additional toxicity data were needed for 26 of the substances evaluated through the Procedure as no adequate toxicity study from which a NOAEL could be established was available, neither on the candidate substances nor on supporting substances. Additional toxicity and genotoxicity data have now become available for the supporting substance beta-myrcene [FL-no: 01.008].

Additional genotoxicity data have also now become available for one of the 26 substances [FL-no: 01.047] and on cedrene washed (unspecified cedrene).

The present revision of FGE.25, FGE.25Rev2 includes the evaluation of these data submitted by the Industry.

2-Methylbuta-1,3-diene [former FL-no: 01.049] was evaluated in FGE.25. For this substance the Panel concluded that it showed genotoxic potential *in vivo* and carcinogenic effects in experimental animals (IARC has also concluded that the substance is “possibly carcinogenic to humans” (group 2B) (IARC, 1999a). Therefore, this substance could not be evaluated through the Procedure and could not be considered safe when used as a chemically defined flavouring substance. Subsequently, the substance has been deleted from the Register (EC, 2008a).

## TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

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

“The European Commission requests the European Food Safety Authority to carry out a safety assessment on  $\beta$ -myrcene [FL-no: 01.008],  $\alpha$ -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.

## ASSESSMENT

### 1. Presentation of the Substances in Flavouring Group Evaluation 25, Revision 2

#### 1.1. Description

The present Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 37 aliphatic and aromatic hydrocarbons (candidate substances) from chemical group 31, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The candidate substances in the group have been divided into eight subgroups: I) acyclic alkanes, II) acyclic alkenes, III) cyclohexene hydrocarbons, IVa) benzene hydrocarbons, IVb) naphthalene hydrocarbons, IVc) diphenylmethane, V) bi- and tricyclic, non-aromatic hydrocarbons and VI) macrocyclic, non-aromatic hydrocarbons.

One flavouring substance, 2-methylbuta-1,3-diene [former FL-no: 01.049] evaluated in FGE.25 has been deleted from the Register of flavouring substances as it showed genotoxic potential *in vivo* and carcinogenic effects in experimental animals. Therefore this substance will not be further discussed as a candidate substance in the current Revision 2 of FGE.25 (FGE.25Rev2).

The 37 candidate substances under consideration, with their chemical Register names, FLAVIS - (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

A summary of the safety evaluation of the candidate substances under consideration in the present evaluation are listed in Table 2 and the supporting substances are listed in Table 3.

The 37 candidate substances are closely related structurally to 19 flavouring substances (supporting substances) evaluated at the 63<sup>rd</sup> JECFA meeting (JECFA, 2005c) in the groups of “Aliphatic and alicyclic hydrocarbons” and “Aromatic hydrocarbons”. The supporting substances are listed in Table 3.

## 1.2. Stereoisomers

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

Twenty-one of the 37 candidate substances possess chiral centres. For fifteen of these chiral substances the chemical names and CAS numbers specify the stereoisomers. For six of the substances [FL-no: 01.027, 01.034, 01.050, 01.055, 01.056 and 01.060] the stereoisomeric composition has been specified (EFFA, 2010a). However, for one substance [FL-no: 01.021] Industry has informed that it exist as a mixture of isomers (EFFA, 2011c). However, the Panel does not consider this information sufficient and requests data on the actual ratio (see Table 1).

Due to the presence and the position of double bonds several of the 37 candidate substances can exist as geometrical isomers. For four of these flavouring substances [FL-no: 01.027, 01.032, 01.035 and 01.078] Industry has informed that they exist as a mixture of isomers (EFFA, 2010a). However, the Panel does not consider this information sufficient and requests data on the actual ratios (see Table 1).

## 1.3. Natural Occurrence in Food

Thirty-three out of the 37 candidate substances have been reported to occur in various types of alcoholic beverages, chicken, egg (boiled), fish (raw), shrimps, guinea hen, lamb fat, cheese, milk, butter, various herbs, various fruits and vegetables, tea, mace and wort. Quantitative data on the natural occurrence in foods have been reported for 26 of these substances (TNO, 2000; TNO, 2010).

These reports include:

- Limonene [FL-no: 01.001]: up to 1.6 mg/kg in carrot, up to 0.3 in black currants, up to 1.4 mg/kg in tea and very high content in citrus oil.
- delta-Cadinene [FL-no: 01.021]: 1000 mg/kg in citrus fruits, 8000 mg/kg in ginger (EFFA, 2005a).
- alpha-Cedrene [FL-no: 01.022]: 0.0007 mg/kg in artichoke.
- 1(5),11-Guaiadiene [FL-no: 01.023]: 1.5 mg/kg in mango, trace amounts in basil and in pepper.

- beta-Bisabolene [FL-no: 01.028]: 33.3 mg/kg in parsley, up to 1.18 mg/kg in carrot, 0.003 mg/kg in artichoke, 0.0003 mg/kg in guava fruit, trace amounts in mace and in nutmeg.
- beta-Cubebene [FL-no: 01.030]: 0.08 mg/kg in grapefruit juice, up to 0.08 mg/kg in guava fruit, trace amounts in mace and in nutmeg.
- 1,2-Dihydro-1,1,6-trimethylnaphthalene [FL-no: 01.031]: 0.25 mg/kg in rum, up to 0.042 mg/kg in white wine.
- 2,2-Dimethylhexane [FL-no: 01.033]: up to 0.9 mg/kg in tea.
- 2,4-Dimethylhexane [FL-no: 01.034]: up to 2 mg/kg in tea.
- 2,6-Dimethylocta-2,4,6-triene [FL-no: 01.035]: 0.45 mg/kg in mango, 0.03 mg/kg in orange juice, up to 0.08 mg/kg in blackcurrants, trace amounts in sage.
- Dodec-1-ene [FL-no: 01.037]: up to 0.4 mg/kg in butter, up to 0.01 mg/kg in loquat, up to 0.01 mg/kg in passiflora.
- Dodecane [FL-no: 01.038]: up to 3.5 mg/kg in butter, up to 0.1 mg/kg in passiflora, 0.1 mg/kg in beans, 0.1 mg/kg in cocoa, 0.1 mg/kg in tea, up to 0.1 mg/kg in lamb, 0.08 mg/kg in dill, up to 0.05 mg/kg in strawberry, 0.02 mg/kg in chicken, up to 0.01 mg/kg in loquat, up to 0.01 mg/kg in papaya, 0.01 mg/kg in pea, up to 0.004 mg/kg in egg, 0.009 mg/kg in Guinea hen, 0.0006 mg/kg in raw fish, trace amounts in liquorice.
- delta-Elemene [FL-no: 01.039]: trace amounts in mandarin juice.
- Germacra-1(10),4(14),5-triene [FL-no: 01.042]: 0.001 mg/kg in mandarin juice.
- 3,7,10-Humulatriene [FL-no: 01.043]: up to 1.3 mg/kg in mango, up to 1.2 mg/kg in tea, up to 1.05 mg/kg in guava, 0.12 mg/kg in carrot, up to 0.12 in cheese, 0.2 mg/kg in grapefruit juice, up to 0.09 mg/kg in blackcurrants, 0.02 mg/kg in tamarind, up to 0.01 mg/kg in orange juice, up to 0.0002 mg/kg in artichoke, 0.0004 mg/kg in mandarin, trace amounts in nutmeg, in raspberry and in celery.
- Longifolene [FL-no: 01.047]: 0.0003 mg/kg in artichoke.
- 2-Methylnaphthalene [FL-no: 01.051]: 0.1 mg/kg in tea, 0.08 mg/kg in capsicum, up to 0.05 mg/kg in raspberry, up to 0.01 mg/kg in papaya, up to 0.01 mg/kg in passiflora, trace amounts in port wine.
- alpha-Muurolene [FL-no: 01.052]: 7.9 mg/kg in green tea, up to 0.009 mg/kg in mango, 0.005 mg/kg in bilberry, trace amounts in nutmeg.
- Naphthalene [FL-no: 01.053]: 0.1 mg/kg in tea, up to 0.06 mg/kg in capsicum annum (sweet pepper), 0.05 mg/kg in cocoa, up to 0.035 mg/kg in cheese, 0.02 mg/kg in raw fish, 0.02 mg/kg in beer, 0.01 mg/kg in grapefruit juice, up to 0.01 mg/kg in passiflora, up to 0.01 mg/kg in papaya, 0.008 mg/kg in soybean, up to 0.007 mg/kg in plumcot, 0.005 mg/kg in peanut, 0.005 mg/kg in peas, up to 0.005 mg/kg in plum, 0.005 mg/kg in bilberry, up to 0.005 mg/kg in raspberry, 0.004 mg/kg in water buffalo milk, 0.003 mg/kg in beans, 0.0029 mg/kg in skim milk powder, 0.001 mg/kg in kiwifruit, up to 0.001 mg/kg in mango, up to 0.0005 mg/kg in chicken, 0.0008 mg/kg in shrimps, trace amounts in peach, trace amounts in port wine.
- Pentadecane [FL-no: 01.054]: up to 2.3 mg/kg in butter, up to 0.4 mg/kg in mango, up to 0.346 mg/kg in cheese, 0.1 mg/kg in chicken, 0.1 mg/kg in tea, up to 0.05 mg/kg in

strawberry, up to 0.03 mg/kg in raw fish, 0.029 mg/kg in Guinea hen, up to 0.02 mg/kg in egg (boiled), 0.014 mg/kg in milk powder, up to 0.01 mg/kg in papaya, 0.02 mg/kg in tamarind, 0.00001 mg/kg in aubergine, trace amounts in liquorice.

- beta-Phellandrene [FL-no: 01.055]: up to 85 mg/kg in dill, up to 0.66 mg/kg in tea, up to 0.5 mg/kg in black currants, up to 0.4 mg/kg in mango, up to 0.1 mg/kg in papaya, 0.025 mg/kg in grapefruit juice, up to 0.01 mg/kg in passiflora, up to 0.005 mg/kg in plumcot, up to 0.002 mg/kg in redcurrants, trace amounts in cinnamon, trace amounts in nectarine.
- Tetradecane [FL-no: 01.057]: up to 1.9 mg/kg in butter, 0.3 mg/kg in liquorice, up to 0.3 mg/kg in mango, up to 0.3 mg/kg in dill, 0.021 mg/kg in guinea hen, up to 0.15 mg/kg in cheese, 0.1 mg/kg in cocoa, 0.1 mg/kg in tea, 0.088 mg/kg in passiflora, up to 0.05 mg/kg in strawberry, up to 0.01 mg/kg in papaya, up to 0.003 mg/kg in egg, 0.001 mg/kg in milk 0.0005 mg/kg in chicken, 0.0008 mg/kg in raw fish, trace amounts in thymus, aubergine, coconut and lamb.
- 4(10)-Thujene [FL-no: 01.059]: Up to 1000 mg/kg in caraway seed (oil), up to 1.9 mg/kg in blackcurrant, up to 5.2 mg/kg in carrot, up to 49000 mg/kg in cardamom (oil), up to 4000 mg/kg in coriander seed (oil), up to 4800 mg/kg in cumin seed (oil), 239000 mg/kg in pepper (oil) (different species), 334000 mg/kg in ginger (oil), up to 87600 mg/kg in laurel (oil), up to 510000 mg/kg in nutmeg (oil).
- 1,1,7-Trimethyltricyclo[2.2.1.0.(2,6)]heptane [FL-no: 01.060]: up to 50 mg/kg in pepper, trace amounts in cardamom.
- *cis*-3,7-Dimethyl-1,3,6-octatriene [FL-no: 01.064]: up to 13.6 mg/kg in guava fruit, up to 7.5 mg/kg in mango, 5.3 mg/kg in celery, 2.7 mg/kg in parsley, 2 mg/kg in dill, up to 0.6 mg/kg in tea, up to 0.5 mg/kg in papaya, up to 0.2 mg/kg in blackcurrant, 0.18 mg/kg in grapefruit, 0.05 mg/kg in cocoa, up to 0.01 mg/kg in passiflora, trace amounts in nectarine.
- 1-Octene [FL-no: 07.070]: up to 0.009 mg/kg in butter (1.7 mg/kg in heated butter), up to 0.001 mg/kg in boiled egg, 0.002 mg/kg in guinea hen.

According to TNO four of the substances, bisabola-1,8,12-triene [FL-no: 01.027], *l*-limonene [FL-no: 01.046], 2-cedrene [FL-no: 01.066], and 8(14)-cedrene [FL-no: 01.067] have not been reported to occur naturally in any food items (TNO, 2000; TNO, 2011).

## 2. Specifications

Purity criteria for the 37 substances have been provided by the Flavour Industry (EFFA, 2005a; EFFA, 2006o; EFFA, 2006p; Flavour Industry, 2006t; Flavour Industry, 2009t) (see Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for 32 candidate substances. Further information on stereoisomeric composition/composition of mixture is needed for five substances [FL-no: 01.021, 01.027, 01.032, 01.035 and 01.078] (see Section 1.2 and Table 1).

## 3. Intake Data

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

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

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

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

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

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

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

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population<sup>4</sup> (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present FGE.25Rev2 the total annual production volume of the 37 candidate substances from use as flavouring substances in Europe was reported to be approximately 51000 kg (EFFA, 2005a; EFFA, 2005b; EFFA, 2006o; EFFA, 2006p; EFFA, 2008b; Flavour Industry, 2006t). For the 19 supporting substances the total annual volume of production in Europe is approximately 330000 kg. *d*-limonene [FL-no: 01.045] accounts for 280000 kg and 47000 kg is accounted for by seven other supporting substances: [FL-no: 01.003, 01.004, 01.005, 01.006, 01.007, 01.008 and 01.020] (JECFA, 2005c).

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

On the basis of the annual volumes of production reported for the 37 candidate substances, the MSDI values for each of these flavourings have been estimated (Table 2).

Nearly 100 % of the total annual volume of production for the candidate substances is accounted for by two substances [FL-no: 01.001 and 01.046]. The estimated daily *per capita* intake from use as flavouring substance is 4000 and 2100 microgram, respectively. The daily *per capita* intakes for each of the remaining substances are less than 28 microgram (Table 2).

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

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

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

For 33 of the 37 candidate substances information on food categories and normal and maximum use levels<sup>5,6</sup> were submitted by the Flavour Industry (EFFA, 2005a; EFFA, 2006o; EFFA, 2007a). Thirty-three of the 37 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For three candidate substances no use levels have been submitted [FL-no: 01.001, 01.046 and 01.070] and for one substances [FL-no: 01.078] the use levels reported have not been in accordance with the food categories outlined in Annex III of the above mentioned Commission Regulation. For this reason these substances have been included in Table 3.1 as “no data for [FL-no: 01.001, 01.046, 01.078 and 01.070]”. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

**Table 3.1 Use of Candidate Substances**

Food category	Description	Flavourings used*
01.0	Dairy products, excluding products of category 2	33
02.0	Fats and oils, and fat emulsions (type water-in-oil)	33
03.0	Edible ices, including sherbet and sorbet	33
04.1	Processed fruits	33
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None of the 33
05.0	Confectionery	33
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	33
07.0	Bakery wares	33
08.0	Meat and meat products, including poultry and game	33
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	33
10.0	Eggs and egg products	None of the 33
11.0	Sweeteners, including honey	None of the 33
12.0	Salts, spices, soups, sauces, salads, protein products etc.	33
13.0	Foodstuffs intended for particular nutritional uses	33
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	33
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic	33

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

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

**Table 3.1 Use of Candidate Substances**

Food category	Description	Flavourings used*
	counterparts	
15.0	Ready-to-eat savouries	32 (except [FL-no: 01.044])
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	33

\*Information on use levels has not been provided for for of the 37 [FL-no: 01.001, 01.046, 01.070 and 01.078].

According to the Flavour Industry the normal use levels for the 33 candidate substances, for which use levels have been provided, are in the range of 2 - 20 mg/kg food, and the maximum use levels are in the range of 10 - 100 mg/kg (EFFA, 2002i; EFFA, 2005a; EFFA, 2006o; EFFA, 2007a) Table II.1.2, Annex II.

The mTAMDI values for the 27 candidate substances from structural class I (see Section 5) and for which use levels were provided, are for [FL-no: 01.059] 3100 microgram/person/day, for [FL-no: 01.044] 3500 microgram/person/day and 3900 microgram/person/day for each of the remaining 25 candidate substances. For each of the two candidate substances from structural class II and three candidate substances from structural class III (see Section 5) the mTAMDI is 3900 microgram/person/day.

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

#### 4. Absorption, Distribution, Metabolism and Elimination

Generally, the available data indicate that the aliphatic and aromatic hydrocarbons may participate in similar metabolic pathways. Being lipophilic and of relatively low molecular weight, these hydrocarbons may be assumed to be absorbed in the gastrointestinal tract. Subsequently, they can be oxidised to polar oxygenated metabolites, e.g. by CYP-450 enzymes. The phase I metabolites can then be conjugated and excreted mainly in the urine. The candidate and supporting substances are expected to be metabolised either by side chain oxidation or epoxidation of the exocyclic or endocyclic double bonds. Alkyl oxidation initially yields hydroxylated metabolites that may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites, which can also be excreted. If a double bond is present, intermediate epoxide metabolites may be formed, which are transformed either by hydrolysis to yield diols or by conjugation with glutathione to yield mercapturic acid derivatives. For the naphthalene hydrocarbons, in addition to epoxidation, formation of reactive quinones can be expected. The aromatic hydrocarbons that do not contain alkyl substituents can undergo ring hydroxylation to form phenolic metabolites that are subsequently conjugated with sulphate or glucuronic acid and excreted in the urine. The saturated alkanes in this group may be anticipated to be metabolised via omega and omega-1, -2, -3 or -4 oxidation. Whereas omega oxidation would ultimately lead to the formation of carboxylic acids, the other oxidations would give rise to secondary alcohols and ketones. The carboxylic acids may be expected to participate in the endogenous fatty acid metabolism.

However, for most of the eight subgroups (see Section 1.1 and Annex III) the information available was scarce and the similarity between candidate and supporting substances was limited. In addition, proper mass balance data were not available. The few mass balance data available indicated only slow elimination. For several subgroups no data were available at all. In Table 4.1 the final conclusion for each of the candidate substances have been presented together with a brief explanatory statement. For subgroup III there are only data for one supporting substance, *d*-limonene [FL-no: 01.045], which is oxidised in both side chains to yield alcohols and carboxylic acids, which may be conjugated and eliminated in the urine.

For more detailed information, see Annex III.

**Table 4.1 Can innocuous metabolites be expected to be formed based on available data?**

FL-no:	Substance name	Innocuous metabolites?
<b>Subgroup I: ACYCLIC ALKANES</b>		
01.033	2,2-Dimethylhexane	Yes
01.034	2,4-Dimethylhexane	Yes
01.038	Dodecane	Yes
01.050	3-Methylhexane	No (potential formation of neurotoxic gamma-diketone)
01.054	Pentadecane	Yes
01.057	Tetradecane	Yes
<b>Subgroup II: ACYCLIC ALKENES</b>		
01.032	2,3-Dihydrofarnesene	No (lack of supporting data)
01.035	2,6-Dimethylocta-2,4,6-triene	No (lack of supporting data)
01.037	Dodec-1-ene	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)
Deleted from the Register	2-Methylbuta-1,3-diene	No (known biotransformation to reactive metabolite responsible for toxicity and genotoxicity)
01.064	cis-3,7-Dimethyl-1,3,6-octatriene	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)
01.070	1-Octene	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)
01.078	2,4-Nonadiene	No (lack of supporting data)
<b>Subgroup III: CYCLOHEXENE HYDROCARBONS</b>		
01.055	beta-Phellandrene	No (lack of supporting data)
01.027	Bisabola-1,8,12-triene	Yes
01.028	beta-Bisabolene	Yes
01.039	delta-Elemene	Yes
01.001	Limonene	Yes
01.046	l-Limonene	Yes
<b>Subgroup IV: AROMATIC HYDROCARBONS</b>		
<b>Subgroup IVa: BENZENE HYDROCARBONS</b>		
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene	No (lack of supporting data)
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene	No (lack of supporting dat)
<b>Subgroup IVb: NAPHTHALENE HYDROCARBONS</b>		
01.051	2-Methylnaphthalene	No (known metabolism to toxic metabolites)
01.053	Naphthalene	No (known metabolism to toxic metabolites)
<b>Subgroup IVc: DIPHENYLMETHANE</b>		
01.036	Diphenylmethane	No (lack of supporting data)
<b>Subgroup V: BI- and TRICYCLIC, NON-AROMATIC HYDROCARBONS</b>		
01.021	Delta-Cadinene	No (lack of supporting data)
01.022	alpha-Cedrene	No (lack of supporting data)
01.023	1(5),11-Guaiadiene	No (lack of supporting data)

**Table 4.1 Can innocuous metabolites be expected to be formed based on available data?**

FL-no:	Substance name	Innocuous metabolites?
01.030	beta-Cubebene	No (lack of supporting data)
01.044	Isolongifolene	No (lack of supporting data)
01.047	Longifolene	No (lack of supporting data)
01.052	alpha-Muurolene	No (lack of supporting data)
01.056	alpha-Santalene	No (lack of supporting data)
01.059	4(10)-Thujene	No (lack of supporting data)
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane	No (lack of supporting data)
01.066	2-Cedrene	No (lack of supporting data)
01.067	8(14)-Cedrene	No (lack of supporting data)
<b>Subgroup VI: MACROCYCLIC, NON-AROMATIC HYDROCARBONS</b>		
01.042	Germacra-1(10),4(14),5-triene	No (lack of supporting data)
01.043	3,7,10-Humulatriene	No (lack of supporting data)

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

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

For the safety evaluation of the 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, all belonging to structural Cramer class I, 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 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 6.1). 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. Two candidate substances from subgroup III [FL-no: 01.001 and 01.046] have an estimated European daily *per capita* intakes of 4000 and 2100, respectively, which are above the threshold of concern of 1800 microgram/person/day for structural class I. These two candidate substances will therefore proceed to step A4 of the Procedure scheme.

### Step A4

The candidate substances [FL-no: 01.001 and 01.046] or their 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 Cramer structural class I have estimated European daily *per capita* intakes between 0.0085 and 28 microgram (Table 6.1). 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, respectively. 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) are available for any of the 27 candidate substances evaluated 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 27 substances.

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

The mTAMDI values for the 27 candidate substances, for which use levels have been provided, from structural class I and evaluated using the Procedure range from 3100 to 3900 microgram/person/day. No suitable information was submitted for the four remaining substances from structural class I. Hence for these four substances an mTAMDI cannot be calculated. For each of the two candidate substances from structural class II and the four candidate substances from structural class III the mTAMDI are 3900 microgram/person/day. The mTAMDI values for all 33 candidate flavouring substances for which suitable use levels are available, are above the thresholds for structural class I, II and III of 1800, 540 and 90 microgram/person/day, respectively.

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

For the 33 candidate substances, for which use levels have been provided, further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For four candidate substances [FL-no: 01.001, 01.046, 01.070 and 01.078] use levels for food categories as outlined in Commission Regulation (EC) no 1565/2000, Annex III, are required.

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

FL-no	EU Register name	MSDI ( $\mu\text{g}/\text{capita}/\text{day}$ )	mTAMDI ( $\mu\text{g}/\text{person}/\text{day}$ )	Structural class	Threshold of concern ( $\mu\text{g}/\text{person}/\text{day}$ )
01.001	Limonene	4000		Class I	1800
01.027	Bisabola-1,8,12-triene	0.024	3900	Class I	1800
01.028	beta-Bisabolene	2.7	3900	Class I	1800
01.033	2,2-Dimethylhexane	1.2	3900	Class I	1800
01.034	2,4-Dimethylhexane	1.2	3900	Class I	1800
01.038	Dodecane	0.012	3900	Class I	1800
01.039	delta-Elemene	0.012	3900	Class I	1800
01.046	l-Limonene	2100		Class I	1800
01.054	Pentadecane	0.61	3900	Class I	1800
01.057	Tetradecane	0.012	3900	Class I	1800
01.022	alpha-Cedrene	0.012	3900	Class I	1800
01.023	1(5),11-Guaiadiene	1.2	3900	Class I	1800
01.030	beta-Cubebene	0.012	3900	Class I	1800
01.032	2,3-Dihydrofarnesene	0.12	3900	Class I	1800
01.035	2,6-Dimethylocta-2,4,6-triene	9.1	3900	Class I	1800
01.037	Dodec-1-ene	0.024	3900	Class I	1800
01.042	Germacra-1(10),4(14),5-triene	0.012	3900	Class I	1800
01.043	3,7,10-Humulatriene	1.2	3900	Class I	1800
01.044	Isolongifolene	0.012	3500	Class I	1800
01.047	Longifolene	28	3900	Class I	1800
01.050	3-Methylhexane	0.061	3900	Class I	1800
01.052	alpha-Murolene	0.24	3900	Class I	1800
01.055	beta-Phellandrene	0.012	3900	Class I	1800
01.056	alpha-Santalene	0.012	3900	Class I	1800
01.059	4(10)-Thujene	14	3100	Class I	1800
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane	0.012	3900	Class I	1800
01.064	cis-3,7-Dimethyl-1,3,6-octatriene	14	3900	Class I	1800
01.066	2-Cedrene	0.97	3900	Class I	1800
01.067	8(14)-Cedrene	0.012	3900	Class I	1800
01.070	1-Octene	0.0085		Class I	1800
01.078	2,4-Nonadiene	6.1		Class I	1800
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene	0.0012	3900	Class II	540
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene	0.12	3900	Class II	540
01.021	delta-Cadinene	0.15	3900	Class III	90
01.036	Diphenylmethane	1.2	3900	Class III	90

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

FL-no	EU Register name	MSDI ( $\mu\text{g}/\text{capita}/\text{day}$ )	mTAMDI ( $\mu\text{g}/\text{person}/\text{day}$ )	Structural class	Threshold of concern ( $\mu\text{g}/\text{person}/\text{day}$ )
01.051	2-Methylnaphthalene	0.0012	3900	Class III	90
01.053	Naphthalene	0.013	3900	Class III	90

## 7. Considerations of Combined Intakes from Use as Flavouring Substances

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

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

The 27 candidate substances, for which additional data are requested, will not be included. The combined intakes have been calculated for the remaining candidate and supporting substances (i.e. five substances from subgroup I (acyclic alkanes) and five substances from subgroup III (cyclohexene hydrocarbons)).

The combined intakes have been calculated on the basis of the annual production volumes from use as flavouring substances in Europe (EFFA, 2005a; EFFA, 2005b; EFFA, 2006o; EFFA, 2006p; Flavour Industry, 2006t; JECFA, 2005c).

Subgroup I (acyclic alkanes): The combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Annex I), is 3.0 microgram/*capita*/day, which does not exceed the threshold of 1800 microgram/person/day. There are no supporting substances in subgroup I.

Subgroup III (cyclohexene hydrocarbons): The combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Annex I), is 6100 microgram/*capita*/day, which does exceed the threshold of 1800 microgram/person/day. The total combined intake of the five candidate and six supporting substances (also from structural class I) is approximately 42000 microgram/*capita*/day. This intake exceeds the threshold of 1800 microgram/person/day for a structural class I substance. However, together, limonene [FL-no: 01.001], *l*-limonene [FL-no: 01.046] and *d*-limonene (supporting substance [FL-no: 01.045]) account for approximately 40000 microgram/*capita*/day. The total combined intake of 42000 microgram/*capita*/day for the candidate and the supporting substances corresponds to 700 microgram/kg bw/day for a person with a body weight of 60 kg. Thus, based on the NOAEL for *d*-limonene of 215 mg/kg bw/day, a margin of safety of 307 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

## 8. Toxicity

### 8.1. Acute Toxicity

Data are available for seven candidate substances, 16 supporting substances and one structurally related substance (1-methyl-1,3-cyclohexadiene [FL-no: 01.077]). Oral LD<sub>50</sub> values in rat and mouse ranged from 500 - 13000 mg/kg body weight (bw).

The acute toxicity data are summarised in Annex IV, Table IV.1.

### 8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data are available for two candidate substances from subgroup IVb (naphthalene [FL-no: 01.053] and 2-methyl-naphthalene [FL-no: 01.051]), for two supporting substances from subgroup II (myrcene [FL no: 01.008] and undeca-1,3,5-triene [FL no: 01.061]), for one supporting substance from subgroup III (d-limonene [FL no: 01.045]), one supporting substance for subgroup IVb (1-naphthalene [FL no: 01.014]), for two supporting substances for subgroup V (pinene [FL no: 01.003] and camphene [FL no: 01.009]) (see also Table IV.2). In the text below the relevant studies are discussed.

#### *Subgroup II*

##### *Myrcene [01.008]*

##### *Mice, 90 day study (NTP, 2010b)*

Male and female B6C3F<sub>1</sub> mice (10/sex/group) were administered 0, 250, 500, 1000, 2000 or 4000 mg/kg bw of beta-myrcene by gavage 5 days a week for 14 weeks. Body weights and clinical observations were recorded weekly. Blood was collected from mice surviving to the end of the study for haematological analyses and micronuclei evaluation. Sperm morphology and vaginal cytology evaluations were conducted at the end of the study on animals in the control and three lowest dose groups. At necropsy, organ weights were measured and complete histopathological examination was performed on animals from the dose groups 0, 1000, 2000 and 4000 mg/kg bw, and all animals that died early.

All animals in the 4000 mg/kg bw group died within the first three days while 9 of 10 males and 8 of 10 females in the 2000 mg/kg bw group died prior to week 5. In animals that died prior to study termination, clinical signs included lethargy, abnormal breathing and/or thin appearance. The final mean body weights and body weight gain of the 1000 mg/kg bw males and 500 mg/kg bw females were significantly less than those of vehicle controls. Because of the low survival in the two top doses, further results are not reported for those doses.

A significant, approximately 15 - 20 % decrease in hematocrit, haemoglobin and erythrocyte count values was observed in the 1000 mg/kg bw dose group in both females and males at week 14. A dose-dependent significant increase in the relative liver weight (approximately 7 %, 6 % and 17 %) were observed for all doses in male mice, and a significant increase in absolute liver weight were observed for the low dose males only. In female mice a dose-dependent significant increase in relative kidney weight was observed at all doses (approximately 14 %, 12 % and 22 %), with a significant increase in absolute kidney weight in the 1000 mg/kg bw dose group only. Also for female mice, dose-dependent increases in absolute and relative liver weight (approximately 8 %, 17 % and 26 %) were observed, but the increase reached statistical significance only at 500 and 1000 mg/kg bw. There were no significant changes seen in the weights of the reproductive organs, in the sperm parameters, or in oestrous cyclicity at any dose level. No significant histopathological changes in other organs

examined, including kidney, were observed in mice receiving up to 1000 mg/kg bw beta-myrcene for 14 weeks.

As a significant dose-dependent increase in the relative kidney weight was observed for female mice at all treatment doses, no NOAEL for this study could be allocated.

*Rats, 90-day study (NTP, 2010b)*

Male and female F344N Fisher rats (10/sex/group) were administered 0, 250, 500, 1000, 2000 or 4000 mg/kg bw of beta-myrcene by gavage 5 days a week for 14 weeks. Body weights and clinical observations were recorded weekly. Blood was collected from rats surviving to the end of the study for clinical chemistry and haematological analyses. Sperm morphology and vaginal cytology evaluations were conducted at the end of the study on animals in the control and three lowest dose groups. At necropsy, organ weights were measured and complete histopathological examination was performed on animals from the dose groups 0, 2000 and 4000 mg/kg bw, and all animals that died early. Tissues were examined in the lower dose groups to a no-effect level, including renal pathology in all dose groups. Additionally sections of kidney from both sexes were stained using the Mallory-Heidenhain technique for investigation of hyaline droplet formation (indicative of development of alpha-2 $\mu$ -globulin).

All animals in the group receiving 4000 mg/kg bw beta-myrcene died within the first week of the study, except for one male that died at day 11. One male receiving 500 mg/kg bw, one male and one female receiving 1000 mg/kg bw and two males and four females receiving 2000 mg/kg bw died before the end of the study. Final mean body weight and mean body weight gains of males and females administered 500 mg/kg bw or more were significantly less than those of vehicle control. At termination at week 14, dose-related decreases in plasma creatinine concentration in both males and females were observed, statistically significant at 500 mg/kg bw and above in males and at 250 mg/kg bw and above in females. These decreases were suggested by the authors to be associated with the decreased body weight gains observed in treated rats. No other consistent changes in clinical chemistry parameters were found.

Absolute kidney and liver weights were significantly increased in both male and female rats receiving beta-myrcene. Also a dose-dependent increase in the relative liver and kidney weights were observed for males (25 - 150 % in kidney, 13 - 46 % in liver) and females (27 - 100 % in kidney, 13 - 67 % in liver). Microscopically, the incidence of renal tubular necrosis was significantly increased in all dosed groups of males and females, with increasing severity from minimal to moderate related to dose. Both control and treated rats showed development of chronic progressive nephropathy (CPN), although the incidence was higher in treated rats. Treatment-related increases in the incidences and severity of hyaline droplet accumulation were found in the 250, 500 and 1000 mg/kg bw males, accompanied by granular casts in the outer medulla of the kidney. Hyaline droplet formation was not observed in the 2000 mg/kg bw males, although the animals showed a high incidence of renal tubular necrosis, nephrosis and CPN. No evidence of hyaline droplet accumulation was found in female rats, however treated females showed both nephrosis and CPN. A significant increase in nephrosis was observed in the 1000 and 2000 mg/kg bw dose groups of both males and females, with a dose-related increase in severity from minimal to moderate. Nephrosis is an uncommon lesion defined as renal tubule epithelial degeneration and regeneration.

A dose-related effect of beta-myrcene in the nose was observed in both sexes as degeneration of the olfactory epithelium and necrosis of the respiratory epithelium (significant only at 2000 mg/kg bw) accompanied by chronic inflammatory change (significant at 1000 and 2000 mg/kg bw).

The incidence of splenic atrophy were significantly increased in both sexes receiving 2000 mg/kg bw, accompanied by thymic necrosis in one male and three females. In the mesenteric lymph node, the incidence of atrophy were increased in males receiving 2000 mg/kg bw and females receiving 1000 or

2000 mg/kg bw. The lymphoid changes in these organs were considered by the authors to be secondary to morbidity rather than a direct toxic effect of beta-myrcene.

It has been argued that increased hyaline droplet accumulation in male rats is characteristic of alpha-2 $\mu$ -globulin nephropathy (Hard et al., 1993), which is a male rat specific effect with little relevance for humans. The evidence provided for the mechanistic background of the hyaline droplet formation was considered too limited to completely disregard the nephrotoxic effects in male rats as irrelevant for humans. Involvement of alpha-2 $\mu$ -globulin accumulation (e.g. by immunohistochemical techniques) was not demonstrated. In addition, also renal toxicity was observed in the female animals. Therefore, based on the presence of renal tubular nephrosis and necrosis in all test groups, a NOAEL could not be assigned.

#### *Mice, 2-year carcinogenicity study (NTP, 2010b)*

Groups of B6C3F<sub>1</sub> mice (50/sex/group) were administered 0, 250, 500 or 1000 mg beta-myrcene/kg bw per day in corn oil by gavage once per day, five days a week for 104 (females) weeks or 105 weeks (males). The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Complete necropsies and microscopic examination were performed on all animals. Histological examinations were performed on all animals and tissues from all major organs were examined.

Mean body weights of males receiving 1000 mg/kg bw and females receiving 500 or 1000 mg/kg bw were less than those of the vehicle control. Survival of the high-dose group was significantly reduced for both males and females. Due to the high mortality in the 1000 mg/kg bw dose group, the results from this group are not described further.

In the liver there was an increase in hepatocellular carcinoma in both males and females, with a significant increase in incidence in males at the 500 mg/kg bw dose level (incidence 1 in controls, 4 in the 250 mg/kg bw group and 9 in the 500 mg/kg bw group) and females at 250 mg/kg bw, but not at 500 mg/kg bw. Males and to a lesser extent females showed an increase in hepatocellular adenoma in the liver at both 250 and 500 mg/kg bw. Liver hypertrophy was observed to increase with dose both in incidence and severity, reaching statistical significance only at 500 mg/kg bw in both males and females. Eosinophilic foci and cytoplasmic vacuolization were noted in both male and female treatment groups.

Treatment-related changes in other organs included increases in hyperplasia, inflammation, necrosis and ulcer of the forestomach, which were most likely attributable to gavage administration of an irritant substance. Bone marrow atrophy and mandibular and mesenteric lymph node atrophy was observed in both males and females at the 500 mg/kg bw dose. In addition, male mice showed atrophy of the spleen at the 500 mg/kg bw dose.

Taking into account the high sensitivity of this mice strain to tumour development in the liver, these effects were not regarded relevant to humans. A NOAEL of 250 mg/kg bw for beta-myrcene was allocated, based on the increase in bone marrow atrophy and lymph node atrophy observed in both males and females at 500 mg/kg bw dose.

#### *Rats, two-year carcinogenicity study (NTP, 2010b)*

A chronic two-year bioassay on beta-myrcene using the standard NTP protocol with F344/N rats was conducted. Doses were determined from the results of the prior 13-week subchronic study. Groups of F344/N rats (50/sex/group) were administered 0, 250, 500 or 1000 mg beta-myrcene/kg bw per day in corn oil by gavage once per day, five days a week for 104 weeks. The animals were observed twice per day and weighed once per week for 12 weeks and once per month thereafter. Complete necropsies and microscopic examination were performed on all animals. Histological examinations were performed on all animals and tissues from all major organs were examined.

Survival rates of females were comparable across all control and treatment groups. Survival of males in the low- and mid-dose groups was similar to that in the controls. However, no males in the high-dose group survived past 83 weeks of the study, due to renal toxicity. Body weight gain was significantly reduced in high-dose males and females, while the mean body weight of the 500 mg/kg females were less than those of the vehicle controls during much of the study but were similar by the end of the study. Due to the high mortality of males in the 1000 mg/kg bw group, the data from this dose group are not presented further.

The incidences of renal tubular adenoma and of renal tubule adenoma or carcinoma (combined) showed a significant and dose-dependent increase in the 250 and 500 mg/kg bw males. Two renal tubule adenomas occurred in the 1000 mg/kg bw females, the incidence being higher than the historical control mean for the laboratory. In males high increase in both the incidence and severity of renal tubular nephrosis were observed, already at the lowest dose, with 84 and 92 % incidence at 250 and 500 mg/kg bw, respectively. A significant but not dose related increase in papilla mineralisation, epithelial hyperplasia and inflammation was seen in the dose groups 250 and 500 mg/kg bw. In female rats a dose-dependent increase in nephropathy (CPN) were observed for the doses 250, 500 and 1000 mg/kg bw and a dose-dependent increase in the incidence and severity of renal tubule nephrosis at the doses 500 and 1000 mg/kg bw. In addition a non-dose related significant increase in renal tubular hyperplasia was reported at the doses (250, 500, 1000 mg/kg bw). Nephrosis, observed both in male and female rats, was reported by the authors to be an uncommon lesion defined as renal tubule epithelial degeneration and regeneration. This indicates that beta-myrcene might cause nephrotoxicity by a mechanism other than, or in addition to, alpha-2 $\mu$ -globulin nephropathy. The observation of renal neoplasms in female rats also suggests a mechanism of carcinogenesis that may be related to nephrosis and is distinct from the alpha-2 $\mu$ -globulin mechanism.

Other histopathological changes seen in the study included chronic active inflammation in the nose and forestomach, suggesting that the substance is an irritant. In females, the incidence of thyroid gland C-cell adenomas was significantly increased in the 250 mg/kg bw group, but the incidence did not increase with increasing dose. In the lung, the incidence of alveolar/bronchiolar adenoma in the 250 mg/kg bw group exceeded the control means, but was not observed at higher doses.

Due to the observation of renal tubular adenomas and carcinomas in all dose groups in male rats, accompanied by an increased incidence and severity of nephrosis in both sexes, a NOAEL for beta-myrcene from this study could not be allocated.

#### *Overall conclusion for the NTP study on beta-myrcene*

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 because of a lack of dose-response since nearly 100 % incidence of nephrosis was observed in rats already at the lowest dose of beta-myrcene.

#### *Subgroup III*

##### *d-Limonene [FL-no: 01.045]:*

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated the supporting substance *d*-limonene as an additive in its forty-first meeting in 1993, and withdrew the previous ADI for *d*-limonene and allocated an ADI “not specified” (JECFA, 1993a). This assessment was mainly based on an NTP study with *d*-limonene from 1990 (NTP 1990e). In the carcinogenicity study, F344 rats (n = 50/dose/sex) were treated with 0, 75 and 150 mg/kg *d*-limonene (males) and 0, 300 and 600 mg/kg (females) by gavage in corn oil for five days a week. B6C3F<sub>1</sub> mice (n = 50/dose/sex) were treated with 0, 250 and 500 mg/kg *d*-limonene (males) and 0, 500 and 1000 mg/kg (females) in corn oil by gavage for five days a week. In the high dose female rats, the survival was reduced. No effect

on survival or any other toxic effect was observed in the females of the low dose group. The major toxicological effect in the male rats was found in the kidneys where a dose dependent increase in both renal mineralization and epithelial hyperplasia, and a dose-dependent increase in renal tubular cell adenomas and adenocarcinomas was observed. These effects were accompanied with dose related increase in alpha-2 $\mu$ -globulin in the kidney of male rats. No increase in alpha-2 $\mu$ -globulin, kidney nephropathy or renal adenomas or carcinomas was found in female rats. Therefore, the toxicological effects seen in the male rat kidneys are due to alpha-2 $\mu$ -globulin accumulation related nephropathy specifically seen in male rats, which is not relevant for humans (Hard et al., 1993). A reduction in the mean body weights at the high dose group was observed for male and female rats (4 - 7 %) and high dose female mice (5 - 15 %). For the male rats this reduction in mean body weight may have been linked to the toxicity in the kidneys. Based on the decreased body weights in female mice, a NOAEL of 500 mg/kg bw/day (5 days/week) could be derived, but considering the decrease in survival in the female rats exposed at 600 mg/kg bw/day (5 days/week) an overall NOAEL of 300 mg/kg bw/day (5 days/week) should be derived from these NTP studies. This would correspond to 215 mg/kg bw/day for daily exposure.

#### *Subgroup IVb*

##### *Naphthalene [FL-no: 01.053]:*

For naphthalene [FL-no: 01.053] two unpublished 13 week oral studies on rats and mice were only available as summaries, referred to in US EPA (1998). Since the original data were not available it was not possible to evaluate the quality of the study. Groups of 20 rats, male and female, were given doses of 0, 25, 50, 100, 200 or 400 mg naphthalene/kg bw by gavage five days a week for 13 weeks. The NOAEL in this study was 100 mg/kg bw based on mean terminal bw decreases. Other findings were low incidence of lesions in exposed male kidneys and female thymuses. In the mouse study groups of 10 male and 10 female mice were given doses of 0, 12.5, 25, 50, 100 or 200 mg/kg bw following the same design as the rat study. Transient signs of toxicity occurred in the 200 mg/kg groups. No exposure-related lesions were observed in any organs examined. The authors considered the highest dose level to be a NOAEL, whereas the EPA judged the highest dose level to be a Lowest Observed Adverse Effect Level (LOAEL) for transient signs of toxicity.

Naphthalene [FL-no: 01.053] was given six times per week in food to 28 rats at a dose level varying between 10 and 20 mg/rat/day. Treatment was stopped after 700 days after reaching a total dose of 10 g/rat. Rats were observed until spontaneous death. The conclusion by the author was that naphthalene did not cause cancer in the study. It is however not possible to draw conclusions on carcinogenicity or chronic toxicity from the study since it is deficient in design and poorly reported (Schmähl, 1955).

Groups of male (76 - 112) and female (40 - 76) mice were given naphthalene [FL-no: 01.053] by gavage at dose levels of 0, 5.3, 53 or 133 mg/kg bw/day for 90 consecutive days. The study identified a LOAEL of 133 mg/kg bw/day and a NOAEL of 53 mg/kg bw/day based upon the significant decreases in absolute weight of brain, liver and spleen and relative weight of spleen in female mice. Histopathology was not conducted in this study (Shopp et al., 1984).

Naphthalene [FL-no: 01.053] was studied in a two-year NTP inhalation study in B6C3F<sub>1</sub> mice. The result showed no evidence of carcinogenic activity in male mice exposed to naphthalene, but some evidence of carcinogenic activity in female mice. Groups of male and female mice were exposed to 0 (75/group), 10 (75/group) or 30 ppm (150/group) naphthalene for six hours daily, five days/week for 105 weeks. No increased tumour incidence related to exposure was observed in male mice, but in females the incidence of pulmonary alveolar/bronchiolar adenomas was significantly greater in the high-dose group than in controls. In both male and female mice exposure to naphthalene caused increased incidences and severity of chronic inflammation, metaplasia of the olfactory epithelium and hyperplasia of the respiratory epithelium in the nose and chronic inflammation in the lungs (NTP, 1992g).

In a two-year NTP inhalation study in F344/N rats on the toxicology and carcinogenesis of naphthalene [FL-no: 01.053] it was concluded by NTP that there was clear evidence of carcinogenic activity based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose. Groups of 49 male and female rats were exposed to naphthalene by inhalation at concentrations of 0, 10, 30 or 60 ppm for six hours plus 12 minutes per day five days per week for 105 weeks. The highest exposure concentration is the maximum without naphthalene condensation. Additional groups of nine male and female rats were exposed to 10, 30 or 60 ppm for up to 18 months for evaluation of toxicokinetic parameters. At the exposure concentrations in the study approximately 20 - 30 % of the inhaled dose was metabolised by the rats. Naphthalene that was not absorbed during exposure was presumed to be exhaled. The daily doses were estimated to be 0, 3.6 - 3.9, 10.7 - 11.4 and 20.1 - 20.7 mg/kg bw. Survival of all exposed groups was similar to that of controls. Mean body weights of exposed animals were less than those of controls. Incidences of adenoma of the respiratory epithelium were associated with exposure and increased with dose, as did incidences of neuroblastoma of the olfactory epithelium. Increased incidences of non-neoplastic lesions of the nose associated with exposure to naphthalene included atypical hyperplasia, atrophy, chronic inflammation, hyaline degradation of epithelium, hyperplasia, squamous metaplasia, goblet hyperplasia of respiratory epithelium and glandular hyperplasia and squamous metaplasia. There were no ocular abnormalities in the rats, although naphthalene is a known ocular irritant. Unlike mice, naphthalene was not carcinogenic in the rat lung. Higher rates of metabolism in the mouse lung compared to the rat lung may have been a contributing factor to this species difference in response (NTP, 2000d).

#### *Conclusion on carcinogenicity for naphthalene*

The Panel agreed with the European Union Risk Assessment Report (EU-RAR, 2003) in its evaluation of the long-term carcinogenicity studies on naphthalene. It was concluded that overall the balance of evidence indicates that naphthalene is not genotoxic. In the two-year rat study, inhalation of naphthalene produced an increase in the incidence of respiratory epithelial adenomas and olfactory epithelial neuroblastomas in both males and females. All these tumours occurred at sites where dose-related non-neoplastic inflammatory changes in the nose and respiratory epithelium also occurred. In view of the negative results obtained in the *in vivo* genotoxicity studies (see Section 8.4), these tumours are considered to arise via a non-genotoxic, thresholded mechanism. In mice an increase in the incidence of benign alveolar/bronchiolar adenomas in females was observed after inhalation of naphthalene. These tumours are similarly considered to arise via a non-genotoxic procedure, and are unlikely to be of relevance to human health due to the well-known species differences in pulmonary metabolism, mouse lung preparations being able to metabolise naphthalene at a substantially greater rate (up to 100-fold) than those from hamster, rat or monkey. Although the significance of these inhalation studies for the evaluation of naphthalene used as a flavouring substance in food is questionable, the Panel noted that they did not provide a NOAEL for the non-neoplastic toxic effects on the olfactory and respiratory tissues preceding the tumour formation.

#### *2-Methylnaphthalene [FL-no: 01.051]:*

In a valid study by Murata *et al.* (1997) groups of 50 male and 50 female mice were fed diets containing 0, 0.075 % or 0.15 % 2-methylnaphthalene [FL-no: 01.051] for 81 weeks. The doses were calculated to correspond to 50 - 54 and 108 - 114 mg/kg bw/day, respectively. Pulmonary alveolar proteinosis developed in all treated animals differing significantly from controls. No tumours were seen. No NOAEL could be derived from this study (Murata *et al.*, 1997).

Cutaneous application of methylnaphthalene, a mixture of 1-methylnaphthalene and 2-methylnaphthalene in acetone, at doses of 29.7 or 118.8 mg/kg bw painted on the shaved backs of B6C3F<sub>1</sub> mice twice a week for life was followed by lesions in the lungs. Control mice received acetone in the same manner. The lesions were diagnosed as endogenous lipid pneumonia. Frequency of pneumonia was 0/4 control, 3/11 low dose and 31/32 high dose animals (Emi and Konishi, 1985).

The results suggest that lipid pneumonia could result from systemic administration, and that the lung may be a site for naphthalene toxicity in mice as is discussed in the NTP studies reported above.

#### *Conclusion on carcinogenicity for 2-methylnaphthalene*

The data available, principally from a valid 81-week feeding study in mice, do not indicate that 2-methylnaphthalene [FL-no: 01.051] is carcinogenic.

The toxicity data are summarised in Annex IV, Table IV.2.

### **8.3. Developmental / Reproductive Toxicity Studies**

Developmental and reproductive studies are available for one candidate substance, naphthalene [FL-no: 01.053] (*subgroup IVb*). Studies have been performed on mice, rats and rabbits. Pregnant mice were given 0 or 300 mg naphthalene/kg/day on gestation days 7 - 14. The conclusion was drawn that naphthalene toxicity is manifest before a high level of developmental toxicity occurs. The study does not assess skeletal or visceral foetal development and it is not possible to derive a NOAEL from the study (Booth et al., 1983; Plasterer et al., 1985).

Groups of 25 - 26 pregnant rats were administered naphthalene [FL-no: 01.053] by gavage at dose levels of 0, 50, 150 or 450 mg/kg bw on gestational days 6 - 15. Clinical signs of toxicity were observed in all dams, but they subsided in the two lowest dose groups. Decreased body weight and body weight gain were observed in the two highest dose groups. Foetal growth, viability and morphological development were not significantly affected by treatment. There was no significant treatment related effect on implantation, foetal survival or resorption. The authors concluded that the lowest dose level was a LOAEL for maternal toxicity and that 450 mg/kg bw was a NOAEL for foetal developmental toxicity (Navarro et al., 1991).

Groups of 25 - 27 pregnant rabbits were administered naphthalene [FL-no: 01.053] by gavage at dose levels of 0, 20, 80 or 120 mg/kg bw on gestational days 6 - 19. No treatment-related signs of maternal toxicity were observed. No statistically significant differences were found between control and treated groups in average live litter size, foetal body weight or incidence of malformations. The developmental NOAEL was considered to be 120 mg/kg bw/day in this study.

Groups of 18 pregnant rabbits were given naphthalene [FL-no: 01.053] by gavage at doses of 0, 40, 200 or 400 mg/kg bw on gestational days 6 - 18. Treatment-related clinical signs were observed in a dose-related manner. Maternal NOAEL was not determined. No statistically significant changes were apparent in the mean number of corpora lutea, foetal body weights, viable foetuses, total implantations, post-implantational loss or foetal sex distribution when comparing control or treatment groups. No treatment-related effects were found on examining foetuses for visceral and skeletal abnormalities. Foetal developmental NOAEL was determined as 400 mg/kg bw (Naismith & Matthews, 1986).

#### *Conclusion*

In the tested animal species, mice, rats and rabbits, clinical symptoms of maternal toxicity appeared before signs of reproductive or developmental toxicity were apparent. Developmental NOAELs varied between 120 mg/kg bw/day (rabbit) and 450 mg/kg bw/day (rat, rabbit).

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

### **8.4. Genotoxicity Studies**

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<sup>7</sup>: [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 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 mammals 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.

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<sup>7</sup> 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.

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 micronucleous 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-Rodrigues 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 this substance 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 IV.4). 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.

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

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. However, the Panel concluded that the available data do not preclude evaluating the 37 candidate substances using the Procedure.

## 9. Conclusions

The present revision of FGE.25, Revision 2 includes the assessment of three additional candidate substances [FL-no: 01.001, 01.021 and 01.046] compared to FGE.25Rev1. The FGE.25Rev2 deals with 37 flavouring substances in total.

In the previous version of the present FGE, the Panel considered that additional toxicity data were needed for 26 of the substances evaluated through the Procedure as no adequate toxicity study from which a NOAEL could be established was available, neither on the candidate substances nor on supporting substances. Additional toxicity data have now become available for the structurally related substance beta-myrcene [FL-no: 01.008].

However, no overall NOAEL from the NTP study on beta-myrcene could be allocated due to the observation renal toxicity in male and female rats at all dose groups. Therefore, additional toxicological data are still required.

The 37 candidate substances are aliphatic and aromatic hydrocarbons from chemical group 31, which have been divided into eight subgroups: I) acyclic alkanes, II) acyclic alkenes, III) cyclohexene hydrocarbons, IVa) benzene hydrocarbons, IVb) naphthalene hydrocarbons, IVc) diphenylmethane, V) bi- and tricyclic, non-aromatic hydrocarbons and VI) macrocyclic, non-aromatic hydrocarbons.

Several of the 37 flavouring substances possess chiral centres and/or can exist as geometrical isomers. For five of the flavouring substances [FL-no: 01.021, 01.027, 01.032, 01.035 and 01.078] the stereoisomeric composition/composition of mixture has not been specified sufficiently.

Of the 37 candidate substances 31 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.* (1978).

Thirty-three out of the 37 candidate substances have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, 35 of the 37 flavouring substances in this group have intakes in Europe from 0.0012 to 28 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day), structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances. For limonene [FL-no: 01.001] and *l*-limonene [FL-no: 01.046] the intakes are 4000 and 2100 microgram/*capita*/day, which are above the threshold of concern value for structural class I (1800 microgram/person/day).

Combined intakes can be calculated for the substances in subgroup I (acyclic alkanes) and subgroup III (cyclohexene hydrocarbons) evaluated through the Procedure.

For subgroup I (acyclic alkanes): The combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Annex I), is 3.0 microgram/capita/day, which does not exceed the threshold of 1800 microgram/person/day. There are no supporting substances in subgroup I.

For subgroup III (cyclohexene hydrocarbons): The combined intake of the five candidate substances, all from structural class I and evaluated via the A-side of the Procedure (Annex I), is 6100 microgram/capita/day, which exceeds the threshold of 1800 microgram/person/day. The total combined intake of the candidate and six supporting substances (also from structural class I) is 42000 microgram/capita/day. This intake exceeds the threshold of 1800 microgram/person/day for a structural class I substance. However, limonene [FL-no: 01.001], *l*-limonene [FL-no: 01.046] and *d*-limonene (supporting substance [FL-no: 01.045]) accounts for approximately 40000 microgram/capita/day. The total combined intake of 42000 microgram/capita/day for the candidate and the supporting substances corresponds to 700 microgram/kg bw/day for a person with a body weight of 60 kg. Thus, based on the NOAEL for *d*-limonene of 215 mg/kg bw/day, a margin of safety of 307 can be calculated, and accordingly these substances are not expected to be of safety concern at the estimated level of intake.

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, there is evidence of an *in vivo* genotoxic and carcinogenic potential. However, the Panel concluded that the available data do not preclude an evaluation of the 37 candidate substances using the Procedure.

The available information on metabolism of the 37 candidate substances evaluated through the Procedure or the supporting substances for this FGE was very limited. Overall, only for the following 10 candidate substances it can be concluded that they will be metabolised into innocuous metabolites, [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 0.001, 01.027, 01.028, 01.039 and 01.046] from subgroup III. For six candidate substances there are data, which show that they may be metabolised to toxic metabolites [FL-no: 01.037, 01.050, 01.051, 01.053, 01.164 and 01.070]. For the remaining 21 candidate substances [FL-no: 0.021, 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], the information is too limited and it cannot be assumed that they are metabolised to innocuous metabolites.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is concluded that the 10 candidate substances which are expected to be metabolised to innocuous substances, [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] from subgroup I and [FL-no: 01.001, 01.027, 01.028, 01.039 and 01.046] from subgroup III, would not give rise to safety concerns at their estimated intakes arising from their use as flavouring substances based on the MSDI approach. For the remaining 27 candidate substances, [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 structurally related supporting substances, no adequate NOAELs were available. Therefore, additional toxicological data are required.

The mTAMDI values for the 27 candidate substances from structural class I, for which use levels have been provided are in the range of 3100 to 3900 microgram/person/day. For each of the two candidate substances from structural class II and the four candidate substances from structural class III the mTAMDI is 3900 microgram/person/day. These values are above the threshold for structural class I, II and III of 1800, 540 and 90 microgram/person/day, respectively.

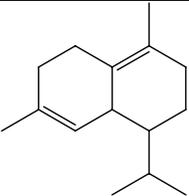
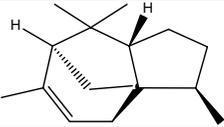
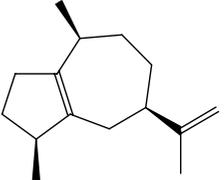
Accordingly, intake estimates according to the mTAMDI for the 33 candidate substances for which use levels have been provided exceed the thresholds of concern for the three structural classes, and more reliable exposure data are requested. On the basis of such additional data, the flavouring substances should be considered using the Procedure. Subsequently, additional data might become necessary. For the four remaining candidate substances [FL-no: 01.001, 01.046, 01.070 and 01.078] use levels for food categories as outlined in Commission Regulation (EC) no 1565/2000, Annex III, are required.

In order to determine whether this conclusion could be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 32 flavouring substances. For five substances [FL-no: 01.021, 01.027, 01.032, 01.035 and 01.078] information on stereoisomeric composition/composition of mixture has not been specified sufficiently.

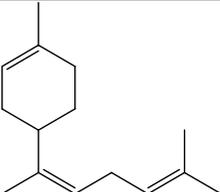
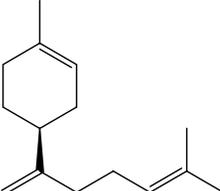
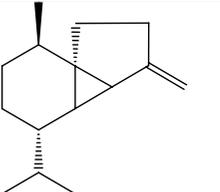
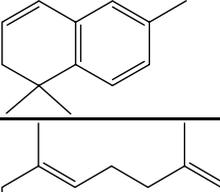
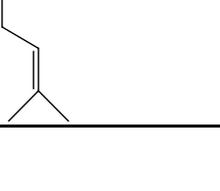
Thus, overall, for 27 candidate substances [FL-no: 01.021, 01.022, 01.023, 01.030, 01.031, 01.032, 01.035, 01.036, 01.037, 01.042, 01.043, 01.044, 01.047, 01.050, 01.051, 01.052, 01.053, 01.055, 01.056, 01.058, 01.059, 01.060, 01.064, 01.066, 01.067, 01.070 and 01.078] additional toxicity data are required. For four of these, [FL-no: 01.021, 01.032, 01.035 and 01.078], additional information on composition requested. The final evaluation of the materials of commerce cannot be performed for one substance [FL-no: 01.027], pending further information on composition of mixture of stereoisomers. For nine candidate substances [FL-no: 01.001, 01.028, 01.033, 01.034, 01.038, 01.039, 01.046, 01.054 and 01.057] the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

**TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 25, REVISION 2**

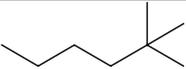
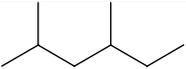
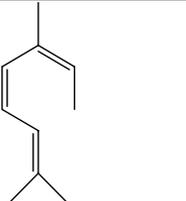
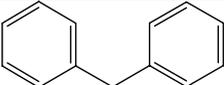
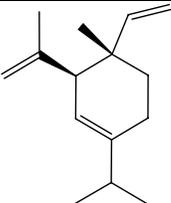
**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 2**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
01.001	Limonene		2633 491 138-86-3	Liquid C <sub>10</sub> H <sub>16</sub> 136.23	Insoluble Soluble	178 MS 95 %	1.4760- 1.4820 0.843-0.851	With respect to specific gravity it is noted that limonene and l-limonene are submitted by different applicants.
01.021	delta-Cadinene		10982 29350-73-0	Liquid C <sub>15</sub> H <sub>24</sub> 204.36	Freely soluble	286 MS 95 %	1.497-1.503 0.917-0.923	Mixture of beta-Cadinene (CAS-no 523-47-7) and mainly the racemic mixture (+)-delta-Cadinene (CAS-no 483-76-1) and (-)-delta-Cadinene (CAS-no 60305-17-1), and some alpha-, gamma- and epsilon isomers (EFFA, 2011c). Composition of the stereoisomeric mixture to be specified.
01.022	alpha-Cedrene		10985 469-61-4	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	119 (13 hPa) MS 95 %	1.500-1.506 0.932-0.938	
01.023	1(5),11-Guaiadiene		11003 3691-12-1	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	78 (3 hPa) MS 95 %	1.493-1.499 0.897-0.903	

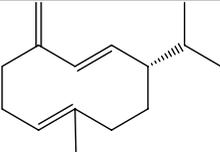
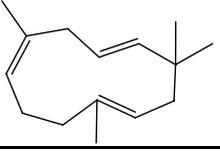
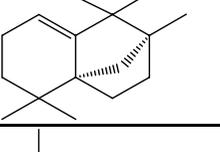
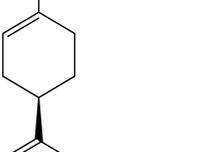
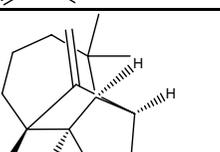
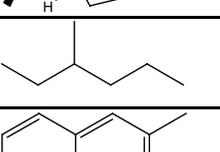
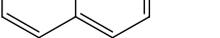
**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 2**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
01.027	Bisabola-1,8,12-triene		17627-44-0	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	99 (5 hPa) MS 95 %	1.483-1.489 0.912-0.918	Racemate of (R)- and (S)-isomers, mixture of (E)- and (Z)-isomers (EFFA, 2010a). CASm in Register refers to the racemate. Composition of stereoisomeric mixture to be specified.
01.028	beta-Bisabolene		495-61-4	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	130 (13 hPa) MS 95 %	1.489-1.495 0.879-0.885	
01.030	beta-Cubebene		10989 13744-15-5	Solid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	284 60 MS 95 %	n.a. n.a.	
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene		30364-38-6	Liquid C <sub>13</sub> H <sub>16</sub> 172.27	Practically insoluble or insoluble Freely soluble	115 (24 hPa) MS 95 %	1.542-1.548 0.942-0.948	
01.032	2,3-Dihydrofarnesene		7681-88-1	Liquid C <sub>15</sub> H <sub>26</sub> 206.37	Practically insoluble or insoluble Freely soluble	130 (15 hPa) NMR 95 %	1.468-1.474 0.817-0.823	Mixtures of (E)- and (Z)-isomers (EFFA, 2010a). CASm in Register does not specify stereoisomeric composition. Composition of the stereoisomeric mixture to be specified.

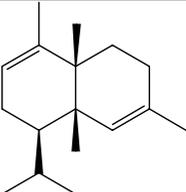
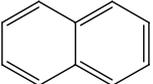
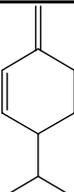
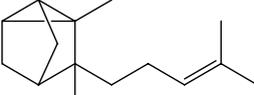
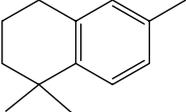
**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 2**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
01.033	2,2-Dimethylhexane		590-73-8	Liquid C <sub>8</sub> H <sub>18</sub> 114.23	Practically insoluble or insoluble Freely soluble	107 MS 95 %	1.390-1.396 0.693-0.699	
01.034	2,4-Dimethylhexane		589-43-5	Liquid C <sub>8</sub> H <sub>18</sub> 114.23	Practically insoluble or insoluble Freely soluble	109 MS 95 %	1.390-1.396 0.697-0.703	Racemate (EFFA, 2010a).
01.035	2,6-Dimethylocta-2,4,6-triene		673-84-7	Liquid C <sub>10</sub> H <sub>16</sub> 136.24	Practically insoluble or insoluble Freely soluble	75 (13 hPa) MS 95 %	1.539-1.545 0.809-0.815	Mixtures of (E)- and (Z)- isomers (EFFA, 2010a). CASm in Register does not specify stereoisomeric composition. Composition of the stereoisomeric mixture to be specified.
01.036	Diphenylmethane		11847 101-81-5	Solid C <sub>13</sub> H <sub>12</sub> 168.24	Practically insoluble or insoluble Freely soluble	262 27 MS 95 %	n.a. n.a.	
01.037	Dodec-1-ene		10992 112-41-4	Liquid C <sub>12</sub> H <sub>24</sub> 168.23	Practically insoluble or insoluble Freely soluble	213 MS 95 %	1.425-1.431 0.755-0.761	
01.038	Dodecane		112-40-3	Liquid C <sub>12</sub> H <sub>26</sub> 170.34	Practically insoluble or insoluble Freely soluble	216 MS 95 %	1.417-1.423 0.746-0.752	
01.039	delta-Elemene		10996 20307-84-0	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	107 (13 hPa) MS 95 %	1.480-1.486 0.856-0.862	

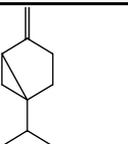
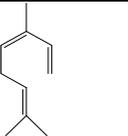
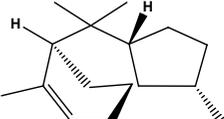
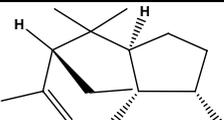
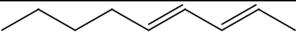
**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 2**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
01.042	Germacra-1(10),4(14),5-triene		23986-74-5	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	308 MS 95 %	1.507-1.513 0.896-0.892	
01.043	3,7,10-Humulatriene		11004 6753-98-6	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	123 (13 hPa) MS 95 %	1.499-1.505 0.889-0.895	
01.044	Isolongifolene		1135-66-6	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	120 (16 hPa) MS 95 %	1.495-1.501 0.926-0.932	
01.046	l-Limonene		2633 491 5989-54-8	Liquid C <sub>10</sub> H <sub>16</sub> 136.23	Insoluble Soluble	177 MS 95 %	1.469 - 1.473 0.837 - 0.841	With respect to specific gravity it is noted that limonene and l-limonene are submitted by different applicants.
01.047	Longifolene		475-20-7	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	115 (13 hPa) MS 95 %	1.498-1.504 0.929-0.935	
01.050	3-Methylhexane		589-34-4	Liquid C <sub>7</sub> H <sub>16</sub> 100.20	Practically insoluble or insoluble Freely soluble	92 MS 95 %	1.385-1.391 0.684-0.690	Racemate (EFFA, 2010a).
01.051	2-Methylnaphthalene		11010 91-57-6	Solid C <sub>11</sub> H <sub>10</sub> 142.20	Practically insoluble or insoluble Freely soluble	241 35 MS 95 %	n.a. n.a.	

**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 2**

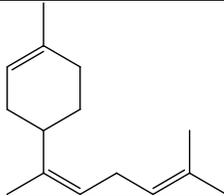
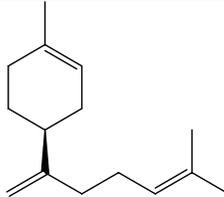
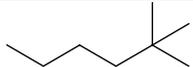
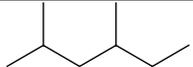
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
01.052	alpha-Muurolene		11011 10208-80-7	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	118 (17 hPa) MS 95 %	1.502-1.508 0.911-0.917	
01.053	Naphthalene		11014 91-20-3	Solid C <sub>10</sub> H <sub>8</sub> 128.17	Practically insoluble or insoluble Freely soluble	218 80 MS 95 %	n.a. n.a.	
01.054	Pentadecane		629-62-9	Liquid C <sub>15</sub> H <sub>32</sub> 212.42	Practically insoluble or insoluble Freely soluble	270 10 MS 95 %	1.428-1.434 0.765-0.771	
01.055	beta-Phellandrene		11017 555-10-2	Liquid C <sub>10</sub> H <sub>16</sub> 136.24	Practically insoluble or insoluble Freely soluble	174 MS 95 %	1.476-1.482 0.839-0.845	Racemate (EFFA, 2010a).
01.056	alpha-Santalene		512-61-8	Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	112 (9 hPa) MS 95 %	1.480-1.486 0.895-0.901	Register name to be changed to (-)-alpha-Santalene (EFFA, 2010a).
01.057	Tetradecane		629-59-4	Liquid C <sub>14</sub> H <sub>30</sub> 198.39	Practically insoluble or insoluble Freely soluble	252 5 MS 95 %	1.422-1.428 0.759-0.765	
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene		475-03-6	Liquid C <sub>13</sub> H <sub>18</sub> 174.29	Practically insoluble or insoluble Freely soluble	245 MS 95 %	1.519-1.525 0.932-0.938	

**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 25, Revision 2**

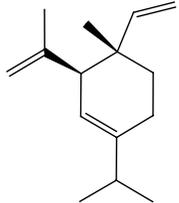
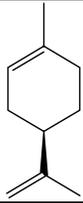
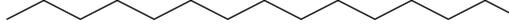
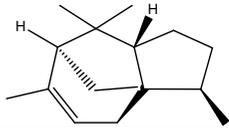
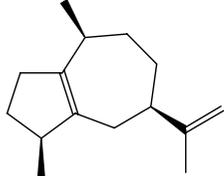
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
01.059	4(10)-Thujene		11018 3387-41-5	Liquid C <sub>10</sub> H <sub>16</sub> 136.24	Practically insoluble or insoluble Freely soluble	165 MS 95 %	1.463-1.469 0.840-0.846	Assay value: 70 % 4(10)-thujene, 6 % alpha-pinene, 19 % beta-pinene, 1 % myrcene, < 4 % not identified (EFFA).
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane		508-32-7	Solid C <sub>10</sub> H <sub>16</sub> 136.24	Practically insoluble or insoluble Freely soluble	152 66 MS 95 %	n.a. n.a.	Racemate (EFFA, 2010a).
01.064	cis-3,7-Dimethyl-1,3,6-octatriene		3338-55-4	Liquid C <sub>10</sub> H <sub>16</sub> 136.24	Practically insoluble or insoluble Freely soluble	177 MS 95 %	1.483-1.489 0.796-0.802	Synonym: cis-beta-ocimene.
01.066	2-Cedrene			Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	82 (11 hPa) MS 95 %	1.495-1.501 0.928-0.934	CASrn 35944-22-0 to be introduced in the Register.
01.067	8(14)-Cedrene			Liquid C <sub>15</sub> H <sub>24</sub> 204.35	Practically insoluble or insoluble Freely soluble	118 (13 hPa) MS 95 %	1.498-1.504 0.930-0.936	CASrn 22567-43-7 or 35964-52-4 to be clarified.
01.070	1-Octene		4293 111-66-0	Liquid C <sub>8</sub> H <sub>16</sub> 112.22	Insoluble Soluble	121 IR NMR MS 97 %	1.410-1.416 0.718-0.722	
01.078	2,4-Nonadiene		4292 56700-78-8	Liquid C <sub>9</sub> H <sub>16</sub> 124.23	Insoluble Slightly soluble	155 MS 79 %	1.446 0.755	Mixture of isomers: (2E,4E)-nonadiene: 79 % (1E,3E)-nonadiene: 9.7 % (1E,3Z)-nonadiene: 1.1% Other isomers including (2E,4Z)-nonadiene: 9.3 %.  Minimum assay 79 % (EFFA, 2010a). Composition of the mixture to be specified further.

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

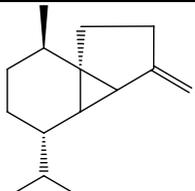
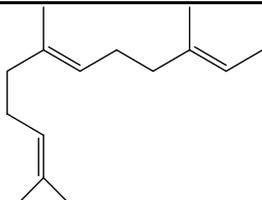
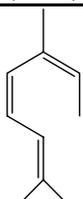
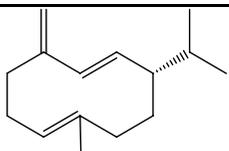
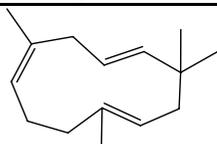
**TABLE 2: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)**
**Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)**

FL-no	EU Register name	Structural formula	MSDI 1) ( $\mu\text{g}/\text{capita}/\text{day}$ )	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	Bisabolone-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)	

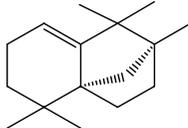
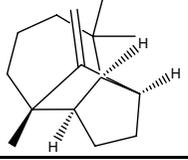
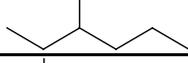
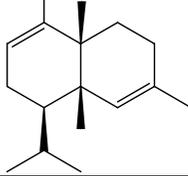
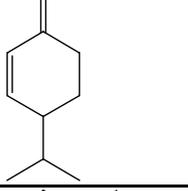
**Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)**

FL-no	EU Register name	Structural formula	MSDI 1) ( $\mu\text{g}/\text{capita}/\text{day}$ )	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		

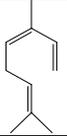
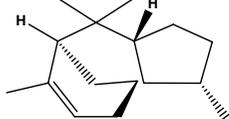
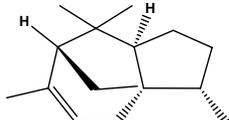
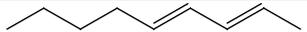
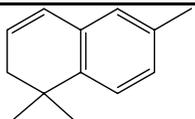
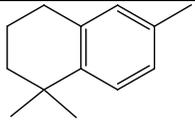
**Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)**

FL-no	EU Register name	Structural formula	MSDI 1 ( $\mu\text{g}/\text{capita}/\text{day}$ )	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
01.030	beta-Cubebene		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
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		

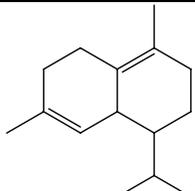
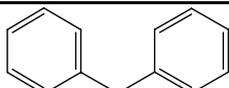
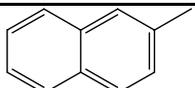
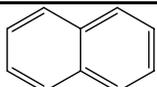
**Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)**

FL-no	EU Register name	Structural formula	MSDI 1 ( $\mu\text{g}/\text{capita}/\text{day}$ )	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
01.044	Isolongifolene		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
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		

**Table 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 ( $\mu\text{g}/\text{capita}/\text{day}$ )	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
01.059	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		
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		

**Table 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 ( $\mu\text{g}/\text{capita}/\text{day}$ )	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
01.021	delta-Cadinene		0.15	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
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) =  $\mu\text{g}/\text{capita}/\text{day}$ .

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

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

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

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

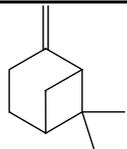
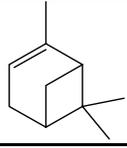
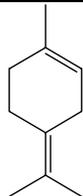
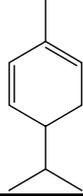
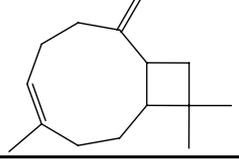
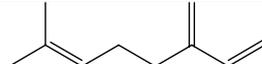
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

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

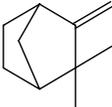
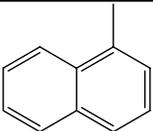
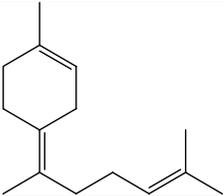
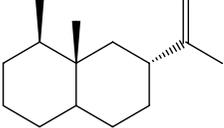
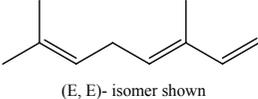
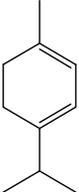
8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

**TABLE 3: SUPPORTING SUBSTANCES SUMMARY (JECFA, 2005C)**

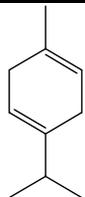
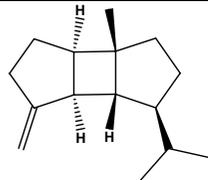
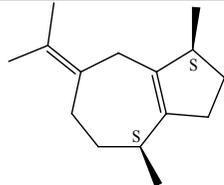
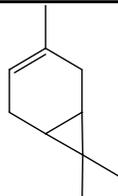
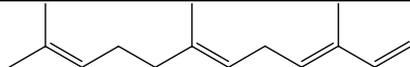
**Table 3: Supporting Substances Summary (JECFA, 2005c)**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
01.003	Pin-2(10)-ene		2903 2114 127-91-3	1330 JECFA specification (JECFA, 2005b)	1300	No safety concern a) Category B b)	JECFA name: beta-Pinene. EFSA conclusion: Additional data required (EFSA, 2011j).
01.004	Pin-2(3)-ene		2902 2113 80-56-8	1329 JECFA specification (JECFA, 2005b)	1800	No safety concern a) Category B b)	JECFA name: alpha-Pinene. EFSA conclusion: Additional data required (EFSA, 2011j).
01.005	Terpinolene		3046 2115 586-62-9	1331 JECFA specification (JECFA, 2005b)	660	No safety concern a) Category B b)	
01.006	alpha-Phellandrene		2856 2117 99-83-2	1328 JECFA specification (JECFA, 2005b)	79	No safety concern a) Category B b)	
01.007	beta-Caryophyllene		2252 2118 87-44-5	1324 JECFA specification (JECFA, 2005b)	330	No safety concern a) Category B b)	EFSA conclusion: Additional data required (EFSA, 2011j).
01.008	Myrcene		2762 2197 123-35-3	1327 JECFA specification (JECFA, 2005b)	290	No safety concern a) Category B b)	EFSA conclusion: Additional data required (EFSA, 2011j).

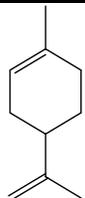
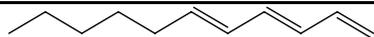
**Table 3: Supporting Substances Summary (JECFA, 2005c)**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
01.009	Camphene		2229 2227 79-92-5	1323 JECFA specification (JECFA, 2005b)	13	No safety concern a) Category B b)	EFSA conclusion: Additional data required (EFSA, 2011j).
01.014	1-Methylnaphthalene		3193 11009 90-12-0	1335 JECFA specification (JECFA, 2005b)	0.73	No safety concern a)	EFSA conclusion: Additional data required (EFSA, 2011j).
01.016	1,4(8),12-Bisabolatriene		3331 10979 495-62-5	1336 JECFA specification (JECFA, 2005b)	13	No safety concern a)	JECFA name: Bisabolene.
01.017	Valencene		3443 11030 4630-07-3	1337 JECFA specification (JECFA, 2005b)	53	No safety concern a)	EFSA conclusion: Additional data required (EFSA, 2011j).
01.018	beta-Ocimene	 (E, E)- isomer shown	3539 11015 13877-91-3	1338 JECFA specification (JECFA, 2005b).	55	No safety concern a)	JECFA name: 3,7-Dimethyl-1,3,6-octatriene. EFSA conclusion: Additional data required (EFSA, 2011j).
01.019	alpha-Terpinene		3558 11023 99-86-5	1339 JECFA specification (JECFA, 2005b)	28	No safety concern a)	JECFA name: p-Mentha-1,3-diene.

**Table 3: Supporting Substances Summary (JECFA, 2005c)**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
01.020	gamma-Terpinene		3559 11025 99-85-4	1340 JECFA specification (JECFA, 2005b).	1200	No safety concern a)	JECFA name: p-Mentha-1,4-diene.
01.024	beta-Bourbonene		11931 5208-59-3	1345 JECFA specification (JECFA, 2005b)	ND	No safety concern a)	EFSA conclusion: Additional data required (EFSA, 2011j). MSDI based on USA production figure.
01.026	1(5),7(11)-Guaiadiene		88-84-6	1347 JECFA specification (JECFA, 2005b)	0.012	No safety concern a)	JECFA name: Guaiene. EFSA conclusion: Additional data required (EFSA, 2011j).
01.029	delta-3-Carene		3821 10983 13466-78-9	1342 JECFA specification (JECFA, 2005b)	290	No safety concern a)	EFSA conclusion: Additional data required (EFSA, 2011j).
01.040	alpha-Farnesene		3839 10998 502-61-4	1343 JECFA specification (JECFA, 2005b)	0.61	No safety concern a)	EFSA conclusion: Additional data required (EFSA, 2011j).

**Table 3: Supporting Substances Summary (JECFA, 2005c)**

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
01.045	d-Limonene		2633 491 5989-27-5	1326 JECFA specification (JECFA, 2005b)	34000	No safety concern a)	ADI not specified (JECFA, 2006a).
01.061	Undeca-1,3,5-triene		3795 16356-11-9	1341 JECFA specification (JECFA, 2005b)	0.24	No safety concern a)	JECFA name: 1,3,5- Undecatriene. EFSA conclusion: Additional data required (EFSA, 2011j).

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

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

3) No safety concern at estimated levels of intake.

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

a) (JECFA, 2005c).

b) (CoE, 1992).

ND) No intake data reported.

## ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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

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

- can the flavourings be predicted to be metabolised to innocuous products<sup>8</sup> (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous<sup>9</sup> (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

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

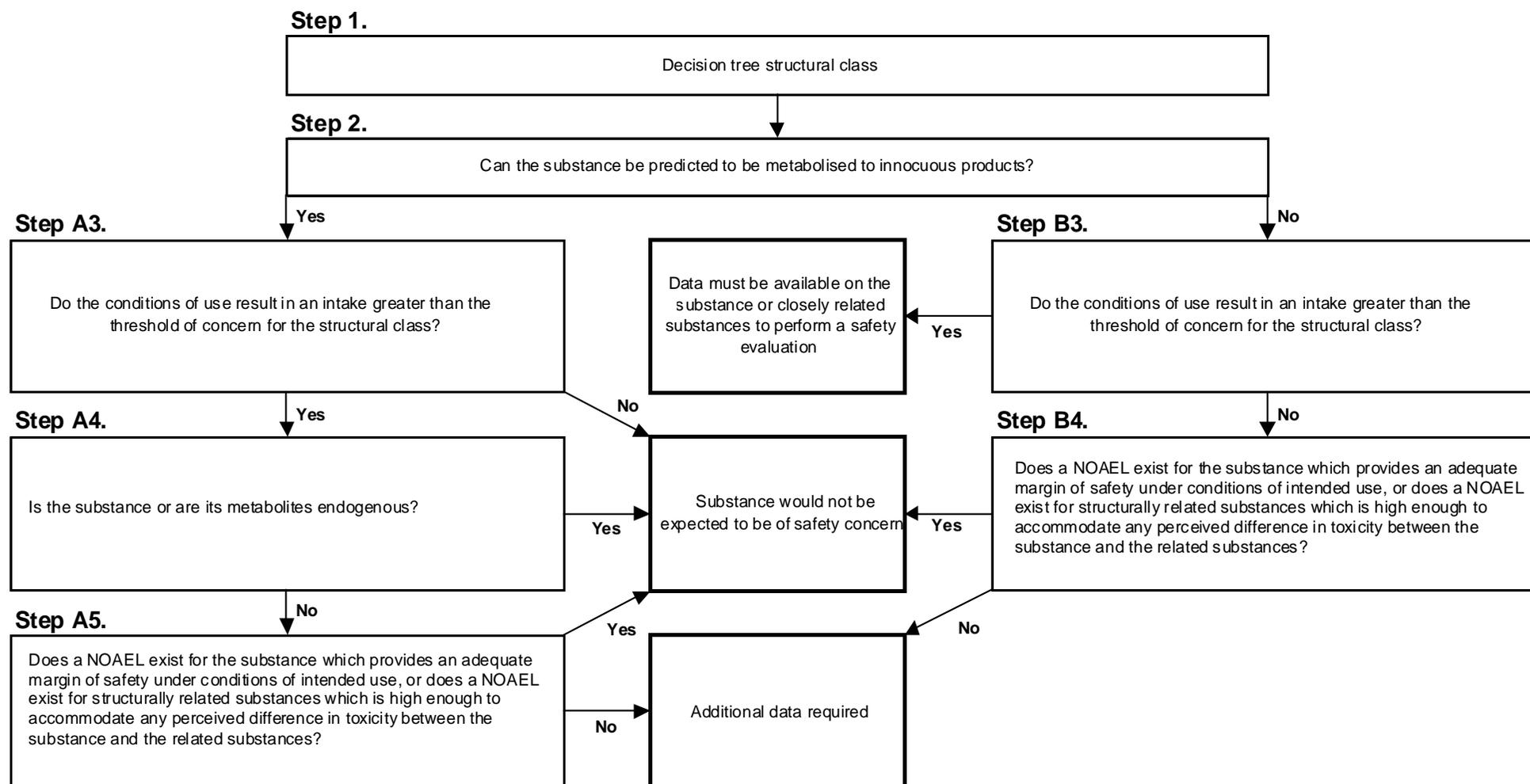
The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

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

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

### Procedure for Safety Evaluation of Chemically Defined Flavouring Substances



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

## ANNEX II: USE LEVELS / MTAMDI

### II.1 Normal and Maximum Use Levels

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

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

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

The “normal and maximum use levels” are provided by Industry for the 33 of the 37 candidate substances in the present flavouring group (Table II.1.2).

**Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.25Rev2 (EFFA, 2005a; EFFA, 2006o; EFFA, 2007a).**

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
01.021	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
01.022	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
01.023	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
01.027	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
01.028	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
01.030	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
01.031	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
01.032	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
01.033	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 II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.25Rev2 (EFSA, 2005a; EFSA, 2006o; EFSA, 2007a).**

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	
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25	
01.034	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	
01.035	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	
01.036	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	
01.037	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	
01.038	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	
01.039	7	5	10	7	-	10	4	10	2	2	-	-	5	10	5	10	20	5	
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25	
01.042	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	
01.043	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	
01.044	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	-	5	
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	-	25	
01.047	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	
01.050	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	
01.051	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	
01.052	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	
01.053	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	
01.054	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	
01.055	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	
01.056	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	
01.057	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	
01.058	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	
01.059	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	5	15	5	
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	8	75	25	
01.060	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	
01.064	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	
01.066	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	
01.067	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	

## II.2 mTAMDI Calculations

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

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

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

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

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

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

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be	Food		

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

Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories
placed in categories 01.0 - 15.0	

The mTAMDI values (see Table II.2.3) are presented for each of the 33 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2005a; EFFA, 2006o; EFFA, 2007a). The mTAMDI values are only given for the highest reported normal use levels.

**Table II.2.3 Estimated intakes based on the mTAMDI approach**

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
01.001	Limonene		Class I	1800
01.027	Bisabolol-1,8,12-triene	3900	Class I	1800
01.028	beta-Bisabolene	3900	Class I	1800
01.033	2,2-Dimethylhexane	3900	Class I	1800
01.034	2,4-Dimethylhexane	3900	Class I	1800
01.038	Dodecane	3900	Class I	1800
01.039	delta-Elementene	3900	Class I	1800
01.046	l-Limonene		Class I	1800
01.054	Pentadecane	3900	Class I	1800
01.057	Tetradecane	3900	Class I	1800
01.022	alpha-Cedrene	3900	Class I	1800
01.023	1(5),11-Guaiadiene	3900	Class I	1800
01.030	beta-Cubebene	3900	Class I	1800
01.032	2,3-Dihydrofarnesene	3900	Class I	1800
01.035	2,6-Dimethylocta-2,4,6-triene	3900	Class I	1800
01.037	Dodec-1-ene	3900	Class I	1800
01.042	Germacrene-1(10),4(14),5-triene	3900	Class I	1800
01.043	3,7,10-Humulatriene	3900	Class I	1800
01.044	Isolongifolene	3500	Class I	1800
01.047	Longifolene	3900	Class I	1800
01.050	3-Methylhexane	3900	Class I	1800
01.052	alpha-Muurolene	3900	Class I	1800
01.055	beta-Phellandrene	3900	Class I	1800
01.056	alpha-Santalene	3900	Class I	1800
01.059	4(10)-Thujene	3100	Class I	1800
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane	3900	Class I	1800
01.064	cis-3,7-Dimethyl-1,3,6-octatriene	3900	Class I	1800
01.066	2-Cedrene	3900	Class I	1800
01.067	8(14)-Cedrene	3900	Class I	1800
01.070	1-Octene		Class I	1800
01.078	2,4-Nonadiene		Class I	1800
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene	3900	Class II	540
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene	3900	Class II	540
01.021	delta-Cadinene	3900	Class III	90
01.036	Diphenylmethane	3900	Class III	90
01.051	2-Methylnaphthalene	3900	Class III	90
01.053	Naphthalene	3900	Class III	90

## ANNEX III: METABOLISM

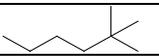
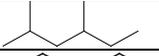
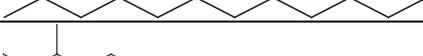
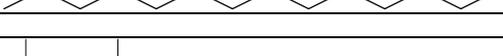
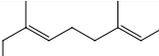
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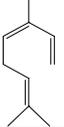
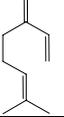
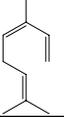
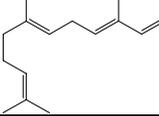
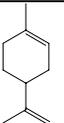
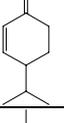
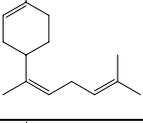
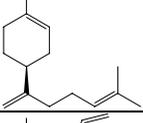
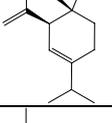
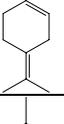
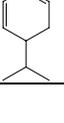
### III.1. Introduction

This group of flavouring substances is very diverse with respect to the chemical structures. In order to facilitate the evaluation of the metabolism aspects of the individual substances, the candidate substances in the group have been divided into eight subgroups: I) acyclic saturated hydrocarbons [FL-no: 01.033, 01.034, 01.038, 01.050, 01.054 and 01.057], II) acyclic unsaturated hydrocarbons [FL-no: 01.032, 01.035, 01.037, 01.064, 01.070 and 01.078], III) cyclohexene hydrocarbons [FL-no: 01.001, 01.027, 01.028, 01.039, 01.046, and 01.055], IVa) benzene hydrocarbons [FL-no: 01.031 and 01.058], IVb) naphthalene hydrocarbons [FL-no: 01.051 and 01.053], IVc) diphenylmethane [FL-no: 01.036], V) bi- and tricyclic, non-aromatic hydrocarbons [FL-no: 01.021, 01.022, 01.023, 01.030, 01.044, 01.047, 01.052, 01.056, 01.059, 01.060, 01.066 and 01.067] and VI) macrocyclic, non-aromatic hydrocarbons [FL-no: 01.042 and 01.043]. From the evaluation of flavouring substances as carried out by the JECFA in 2004, a group of supporting substances has been identified. These supporting substances have also been allocated to subgroups in the same way as has been indicated for the candidate substances. The allocation of the candidate and supporting substances is shown in Table III.1.

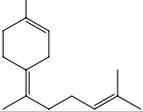
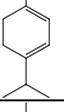
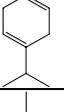
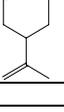
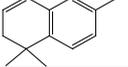
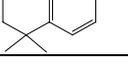
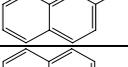
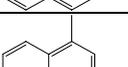
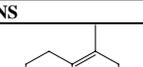
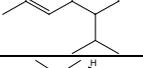
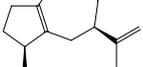
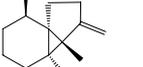
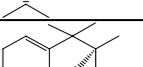
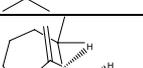
**Table III.1 Subgroups. The supporting substances are listed in brackets.**

FL-no	EU Register name	Structural formula	Structural class
<b>I: ACYCLIC ALKANES</b>			
01.033	2,2-Dimethylhexane		I
01.034	2,4-Dimethylhexane		I
01.038	Dodecane		I
01.050	3-Methylhexane		I
01.054	Pentadecane		I
01.057	Tetradecane		I
<b>II: ACYCLIC ALKENES</b>			
01.032	2,3-Dihydrofarnesene		I
01.035	2,6-Dimethylocta-2,4,6-triene		I
01.037	Dodec-1-ene		I

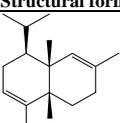
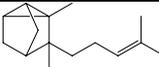
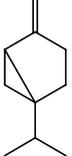
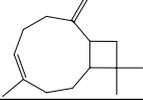
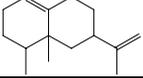
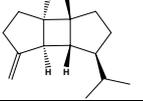
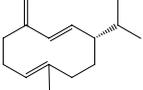
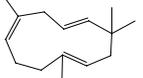
**Table III.1 Subgroups. The supporting substances are listed in brackets.**

FL-no	EU Register name	Structural formula	Structural class
Deleted from the Register	2-Methylbuta-1,3-diene		I
01.064	cis-3,7-Dimethyl-1,3,6-octatriene		I
01.070	1-Octene		I
01.078	2,4-Nonadiene		I
(01.008)	(Myrcene)		I
(01.018)	(beta-Ocimene)		I
(01.040)	(alpha-Farnesene)		I
(01.061)	(Undeca-1,3,5-triene)		I
<b>III: CYCLOHEXENE HYDROCARBONS</b>			
01.001	Limonene		I
01.046	l-Limonene		I
01.055	beta-Phellandrene		I
01.027	Bisabola-1,8,12-triene		I
01.028	beta-Bisabolene		I
01.039	delta-Elemene		I
(01.005)	(Terpinolene)		I
(01.006)	(alpha-Phellandrene)		I

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

FL-no	EU Register name	Structural formula	Structural class
(01.016)	(1,4(8),12-Bisabolatriene)		I
(01.019)	(alpha-Terpinene)		I
(01.020)	(gamma-Terpinene)		I
(01.045)	(d-Limonene)		I
<b>IV: AROMATIC HYDROCARBONS</b>			
<b>IVa: BENZENE HYDROCARBONS</b>			
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene		II
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene		II
<b>IVb: NAPHTHALENE HYDROCARBONS</b>			
01.051	2-Methylnaphthalene		III
01.053	Naphthalene		III
(01.014)	(1-Methylnaphthalene)		III
<b>IVc: DIPHENYLMETHANE</b>			
01.036	Diphenylmethane		III
<b>V: BI- and TRICYCLIC, NON-AROMATIC HYDROCARBONS</b>			
01.021	Delta-Cadinene		III
01.022	alpha-Cedrene		I
01.023	1(5),11-Guaiadiene		I
01.030	beta-Cubebene		I
01.044	Isolongifolene		I
01.047	Longifolene		I

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

FL-no	EU Register name	Structural formula	Structural class
01.052	alpha-Muurolene		I
01.056	alpha-Santalene		I
01.059	4(10)-Thujene		I
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane		I
01.066	2-Cedrene		I
01.067	8(14)-Cedrene		I
(01.003)	(Pin-2(10)-ene)		I
(01.004)	(Pin-2(3)-ene)		I
(01.007)	(beta-Caryophyllene)		I
(01.009)	(Camphene)		I
(01.017)	(Valencene)		I
(01.024)	(beta-Bourbonene)		I
(01.026)	(1(5),7(11)-Guaiaiene)		I
(01.029)	(delta-3-Carene)		I
<b>VI: MACROCYCLIC, NON-AROMATIC HYDROCARBONS</b>			
01.042	Germacre-1(10),4(14),5-triene		I
01.043	3,7,10-Humulatriene		I

For the majority of the substances no information of biotransformation had been submitted. Also data on structural analogues were scarce. Therefore, an additional search was carried out. The additional information retrieved has been included in the following text.

### III.2. Absorption, Distribution and Elimination

#### Acyclic Alkanes (Candidate and Structurally Related Supporting Substances from Subgroup I)

No data on absorption, distribution and elimination were submitted for any of the candidate substances in this subgroup. Some studies on saturated alkanes were retrieved from the additional data search, but apart from two oral studies (Olson et al., 1986; Serve et al., 1995) and one subcutaneous study (Manini et al., 1999), in all other studies retrieved, inhalation was the route of exposure. From these studies (Bahima et al., 1984; Perbellini et al., 1986; Perbellini et al., 1982; Fedtke and Bolt, 1987; Holmberg et al., 1977), absorption through the membranes of the inhalatory tract was observed. Absorption rates and distribution ratios were dependent on blood/air and blood/tissue partition coefficients (Imbriani et al., 1985; Nilsen et al., 1988). In addition, one inhalation study by (Dahl, 1989) showed that more branched isomers were less well absorbed than less branched or unbranched isomers. More saturated hydrocarbons were less well absorbed than unsaturated and more volatile substances were less well absorbed than less volatile (reversely correlated to chain length), but this may not be a relevant difference for oral exposure situations. The main purpose of these studies was however to study metabolism, but these inhalation exposure studies do in general not provide appropriate mass balance data. For that reason, apart from the two oral and one inhalation study (Dahl, 1989) which give insight in mass balance, the studies by (Bahima et al., 1984; Perbellini et al., 1986; Perbellini et al., 1982; Fedtke and Bolt, 1987) and (Manini et al., 1999) will be discussed in the section on biotransformation.

Male and female rats were given a dose of 2 ml/kg or 1.4 g/kg *n*-octane by gavage and urine was collected for up to 48 hours. Urine samples were treated with glucuronidase/sulphatase and the liberated metabolites were analysed by gas chromatography. Several oxidised metabolites in the urine could be found, but quantitative data were not given (Olson et al., 1986). A similar study was carried out with *n*-nonane by Serve *et al.* (Serve et al., 1995), but again, no quantitative data were provided.

Groups of three or four male F 344 rats were exposed to [4-<sup>14</sup>C] or [5-<sup>14</sup>C]-labelled iso-octane (= 2,2,4-trimethyl pentane (trivial name)) and [1-<sup>14</sup>C]-octane vapours at approximately 1 and 350 ppm (~ 4.76 or 1700 mg/m<sup>3</sup> for both substances) by the nose for two hours. During the experiment exhalant was drawn through a bubbler train for sampling. Urine and faeces were collected at the same times, except that none were collected at one and two hours. For iso-octane, all rats exposed to 350 ppm were exposed to C4-labeled substance, but three of four low-exposure rats were exposed to C5-labeled iso-octane. Values for exhaled <sup>14</sup>CO<sub>2</sub> were 0.36; 0.31; and 0.52 % of inhaled <sup>14</sup>C. For the single rat exposed to C4-labeled iso-octane the corresponding value was 2 %<sup>10</sup>. Values for all rats were averaged regardless of position of label. The validity of conclusions regarding low production of <sup>14</sup>CO<sub>2</sub> from iso-octane would have been enhanced by exclusive use of C5-labeled material.

For both *n*-octane and iso-octane the metabolised fraction was higher at low compared to high inhaled concentrations. For octane the major route of elimination was as carbon dioxide (15 % of the radioactive dose within 70 hours). For iso-octane the major route of excretion was urine. Half of the octane-introduced <sup>14</sup>C that was retained at the end of the two-hour exposure period was eliminated 5 - 10 hours post exposure and the exhalation of radiolabel became undetectable after 30 hours after which 75 - 85 % of the label was eliminated. For iso-octane the time to eliminate half of the label was 15 hours and was not completed at the end of the observation period. Based on a discussion of papers from other research groups, the study authors suggest that for straight-chain hydrocarbons, breakdown of the carbon skeleton with the release of CO<sub>2</sub> is an important metabolic pathway. The route of excretion for *n*-octane in this study was markedly affected by the concentrations of the inhaled vapour. The ratio of total exhaled <sup>14</sup>CO<sub>2</sub>: total <sup>14</sup>C in urine was 5:1 after inhalation at 1 ppm but about 1:1 after inhalation of 350 ppm (Dahl, 1989).

<sup>10</sup> In an additional study (not reported in detail), three rats were exposed to C4- or C5-labeled iso-octane at about 350 ppm. More <sup>14</sup>CO<sub>2</sub> was exhaled during exposure to the C4-labeled material (0.07% of the inhaled amount) than after exposure to the C5-labeled material (0.03 %). This observation also confirms that C5 labelled iso-octane is less well metabolised to CO<sub>2</sub> than C4 labelled iso-octane.

### Acyclic Alkenes (Candidate and Structurally Related Supporting Substances from Subgroup II)

When given to male Japanese White rabbits by gavage at a dose of 670 mg/kg bw per day for two days, approximately 25 % of the total administered amount of myrcene [FL no: 01.008] (19 g to six rabbits) could be recovered from the urine over a period of three days following administration (Ishida et al., 1981). Only metabolites of myrcene were identified. The fate of the remaining part of the dose is unclear.

Following intraperitoneal injection of 64 mg [4-<sup>14</sup>C]-2-methylbuta-1,3-diene/kg to F344 rats and B6C3F<sub>1</sub> mice, the majority of radioactivity (≈ 54 and 47 %, respectively) was excreted unchanged in the expired air or as urinary metabolites (≈ 32 and 33 %, respectively) over the 24 hours collection period. Less than 2 % of the radioactivity was recovered as CO<sub>2</sub> for both species, and 0.2 and 7.2 % in rats and mice, respectively, of the radioactivity was recovered in the faeces over the same time period. Radioactivity remaining in the carcass and tissues amounted to only 3.1 and 1.7 % in rats and mice, respectively. From the tissues examined, the highest concentration was found in the kidneys but in both species the renal concentration of radioactivity was only twice as high as the concentration in blood. Total percentage of the dose recovered was ≈ 91 % for both rats and mice (Buckley et al., 1999).

### Cyclohexene Derivatives (Candidate and Structurally Related Supporting Substances from Subgroup III)

Data were only available for one supporting substance in subgroup III, namely *d*-limonene [FL-no: 01.045].

Following the oral administration of [9-<sup>14</sup>C]-*d*-limonene to male Wistar rats by stomach tube at a dose of 800 mg/kg bw, radioactivity was determined in blood, tissues (fat not included), excreta, bile and expired air. The animals were sacrificed at 48 hours post dosing. Radioactivity reached a peak plasma level at two hours post dosing, and after maintaining high levels for 10 hours, declined to negligible levels at 48 hours. In most tissues, peak levels of radioactivity were reached within two hours post dosing indicating rapid distribution. The liver, kidney and adrenals contained the highest levels of radioactivity (higher than blood or serum); other tissues (including brain) contained less than 0.2 % of the administered radioactivity. Hardly any radioactivity could be detected at 48 hours post dosing. Whole body autoradiography confirmed these findings. At 48 hours post-dosing, about 60 % of the administered radioactivity was recovered from the urine, 5 % from faeces and 2 % from exhaled air as CO<sub>2</sub>. Approximately 25 % of the administered radioactivity was excreted in the bile during 24 hours after administration. Total recovery of radioactivity was less than 100 % and as there was hardly any radioactivity present in the tissues at 48 hours, this could point to loss of volatile <sup>14</sup>C from the excreta or to the elimination of volatile <sup>14</sup>C-compounds other than CO<sub>2</sub> (Igimi et al., 1974). When a similar radioactive dose of [9-<sup>14</sup>C]-*d*-limonene was given to male rabbits, 72 % and 7 % of the radioactivity was excreted in the urine and faeces during 72 hours, respectively (Kodama et al., 1974).

In an additional study with several species (rats, hamsters, guinea pigs, rabbits, dogs and humans) dosed orally with [9-<sup>14</sup>C]-*d*-limonene, urinary excretion of radioactivity in rodents and rabbits comprised 82 - 96 % of the dose within 72 hours and faecal excretion 2 - 9 %. The total excretion rate in dogs was somewhat lower (77 % *via* urine and 9 % *via* faeces within 72 hours), while two human volunteers excreted 55 - 83 % of the administered dose in the urine. Faecal excretion in humans was not measured, but may have been considerable in the person with lower urinary excretion as this person developed a diarrhoea shortly after administration. In all species, most excretion occurred within the first 24 hours (Kodama et al., 1976).

*In vitro*, the solubility of *d*-limonene in blood and olive oil was high, but low in water, which suggests a high respiratory uptake and accumulation in adipose tissues (Falk et al., 1990a). Indeed, uptake was rapid and high (68 %) in an experiment in which human volunteers were exposed to *d*-limonene in air at 225 and 450 mg/m<sup>3</sup> for 2 hours while doing light physical exercise. The absorbed *d*-limonene was metabolised rapidly. Elimination followed a triphasic pattern, with a short half-life in blood immediately after exposure (2.6 minutes) but a long half-life during the late elimination phase (12.5 hours), which indicates slow elimination from adipose tissues. Approximately 1 % of the total uptake was eliminated unchanged in expired air, while approximately 0.003 % was eliminated unchanged in urine (Falk-Filipson et al., 1993).

## Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup IV)

This subgroup is further divided into three groups, one with benzene derivatives (di- and tetrahydronaphthalenes (IVa), one with naphthalene derivatives (IVb) and one with one candidate substance, diphenylmethane (IVc). For the candidates of subgroup IVb only one supporting substance has been submitted (1-methylnaphthalene), but for both naphthalene and 2-methylnaphthalene many toxicity data are available, and extensive reviews on these substances have been published recently (EU-RAR, 2003; Shelby and Witt, 1995).

### *Subgroup IVa (benzene derivatives):*

No data were available on any of the candidate substances in this group. No supporting substances were suggested.

### *Subgroup IVb (naphthalene derivatives):*

#### Naphthalene [FL-no: 01.053]:

Bock *et al.* (1979) reported that naphthalene was rapidly absorbed from isolated rat intestinal loops *in situ*, predominantly unchanged, into the portal blood. Approximately 84 % of the naphthalene related substances in the blood was naphthalene, the remainder were metabolites; mainly naphthalene-1,2-dihydrodiol and 1-naphthol, 40 % of which was recovered as conjugates; predominantly with glucuronic acid (Bock *et al.*, 1979).

Three groups of Sprague-Dawley rats were treated orally with a single administration of 2 mg <sup>14</sup>C-naphthalene. The dose per unit body weight was not stated (Bakke *et al.*, 1985). One group of 16 animals was bile duct cannulated, the second group of four germ-free animals was uncannulated and the third group of 13 animals was standard uncannulated rats. Urine, faeces and bile were collected for 72 hours after administration of naphthalene. In the standard uncannulated rats 75.6 % of the radioactivity was recovered in the urine in 24 hours. At 72 hours approximately 83 % of the radioactivity had been recovered in the urine, 6 % in the faeces and 4 % remained in the carcass with no account made for the remaining radioactivity. In the cannulated rats the 24 hours urine and bile contained 30 % and 66.8 % of the <sup>14</sup>C dose, respectively (faecal data not presented for 24 hours time period). At 72 hours, approximately 30 % of the radioactivity had been recovered in the urine, 68 % was contained in the bile, less than 1 % was excreted in the faeces and 0.2 % remained in the carcass.

When a single dose of 20 mg/kg bw of naphthalene-[ring-U-<sup>3</sup>H] was administered intraperitoneally in olive oil to outbred male IMP:Wist rats (n = 54), urine and faeces were the main routes of elimination of the radioactivity with more than 88 % of the radioactivity excreted during the first 72 hours. The maximum level of radioactivity in plasma was observed at two hours post-dosing followed by biphasic clearance (t<sub>1/2</sub> = 0.8 and 99 hours for phase I and phase II, respectively). The highest concentrations during the first hour following dose administration were detected in fat, liver and kidneys, with a gradual decline of radioactivity in all tissues examined (Kilanowicz *et al.*, 1999).

Male Sprague-Dawley rats were given [1-<sup>14</sup>C] naphthalene (100 mg/kg) via i.p. injection. Of the administered dose, 20 - 30 % was excreted in the 0 - 24 hours urine, while 3-11 % was found in the 24 - 48-hours urine. Unconjugated metabolites accounted for 5 - 20 % of the metabolites excreted, while 80 - 95 % of the metabolites were excreted as acidic conjugates (Horning *et al.*, 1980).

Urinary excretion of premercapturic acids and mercapturic acids represents a major excretory pathway for naphthalene in mice and rats (Chen and Dorough, 1979; Stillwell *et al.*, 1978; Pakenham *et al.*, 2002). Urine was collected for 24 hours after intraperitoneal administration of 0, 1.56, 3.12, 6.25, 12.5, 25, 50, 100 and 200 mg/kg radiolabelled [<sup>14</sup>C]-naphthalene dissolved in corn oil to male Sprague Dawley rats and Swiss Webster mice, four animals in each group. The percentage of the administered dose excreted as mercapturic

acids in the urine for mice (25 – 34 %) and rats (24 – 35 %) did not differ with increasing dose (Pakenham et al., 2002). Studies with chimpanzees (Summer et al., 1979b) and with rhesus monkeys (Rozman et al., 1982) indicated that urinary excretion of mercapturic acids is probably less important in primates. Boyland and Sims reported that only traces of mercapturic acids were detected in the urine of three human subjects after oral administration of 500 mg naphthalene, while for rats, mice, hamsters and rabbits, 1-naphthyl-(pre)mercapturic acid was abundantly present in the urine (Boyland and Sims, 1958).

#### 2-Methylnaphthalene [FL-no: 01.051]:

Following intraperitoneal injection of C57BL/6J mice with a single 400 mg/kg dose of [8-<sup>14</sup>C]-2-methylnaphthalene, the majority of radioactivity was distributed in fat, liver, kidney and lung, in order of decreasing concentration. Maximal plasma and tissue levels were achieved one (liver), two (plasma; fat) or four (kidneys) hours after dose administration and the  $t_{1/2}$  of elimination of radioactivity from blood was approximately three hours (Griffin et al., 1982).

When 10 mg/kg bw 2-[1-<sup>3</sup>H]-methylnaphthalene was orally administered to guinea pigs, the highest concentrations of radioactivity were found in the gall bladder, kidneys and liver three hours after dosing. Lower concentrations were observed in the blood, brain, heart, lung and spleen. At that time the total gastrointestinal tract contained 27 % of the dose while the urine contained 23 %. At 24 hours only some retention was observed in the liver, gall bladder and kidney, with 11 % in faeces and 79 % in the urine. After 48 hours 12 % was recovered from faeces and 72 % from the urine, and 1 % of the dose was recovered from the tissues investigated (Teshima et al., 1983).

#### *Subgroup IVc (diphenylmethane):*

After intraperitoneal administration of the candidate flavouring substance diphenylmethane [FL-no: 01.036], metabolites have been shown in urine and faeces. Only a small fraction of the dose (up to 6.8 %) was recovered after 24 hours (DeLoach et al., 1978; Stocklinski et al., 1979). The studies were too incomplete to estimate if total elimination is slow, or that this apparently slow elimination is indicated because quantitatively important metabolites were not studied.

### **Bi- and Tricyclic, Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup V)**

No data on absorption on candidate substances in this group were submitted. Some data were retrieved after database search on the candidate substance longifolene [FL-no: 01.047] and on several supporting substances including alpha- and beta-pinene [FL-no 01.004 and 01.003], caryophyllene [FL-no: 01.007] and delta-3-carene [FL-no: 01.029].

Asakawa *et al.* (1986) studied the metabolism of (+)-longifolene and caryophyllene in rabbits after gavage dosing. At two days after administration, metabolites of all of these substances could be detected in the urine, from which it can be concluded that these substances are absorbed. As no mass balance data were given, the extent of absorption cannot be assessed (Asakawa et al., 1986).

Male albino rabbits (6/group) were administered single gavage doses of 400 - 700 mg/kg bw of (+)-alpha-pinene, (-)-alpha-pinene, (±)-alpha-pinene, (-)-beta-pinene or delta-3-carene. Urine of individual animals was collected for three days. The animals excreted bicyclic terpene hydrocarbon metabolites as glucuronic acid conjugates or as further oxidised metabolites, notably carboxylic acids (Ishida, 1981).

*In vitro* data on the solubility of alpha-pinene [FL-no: 01.004], beta-pinene [FL-no: 01.003] and delta-3-carene [FL-no: 01.029] in blood, olive oil and water suggest a high respiratory uptake and accumulation in adipose tissues. For alpha-pinene this is supported by a high estimated brain/blood partition coefficient of 18 (Falk et al., 1990a). Experiments in which human volunteers were exposed to (+)- and (-)-alpha-pinene or delta-3-carene in air at 225 and 450 mg/m<sup>3</sup> for two hours while doing light physical exercise confirmed that uptake was rapid and high for these agents (58 – 60 % for (+)- and (-)-alpha-pinene and 70 % for delta-3-

carene), and that they were metabolized rapidly. Elimination followed a triphasic pattern, with (+)- and (-)-alpha-pinene exhibiting a rapid initial (distribution) phase (4.8 and 5.6 minutes, respectively), a rapid second distribution phase (38 and 40 minutes, respectively), and a slow elimination phase (695 and 555 minutes, respectively). Triphasic elimination was also observed for delta-3-carene with half-lives of 4.5, 35 and 1800 minutes for the initial, rapid and slow phases, respectively. It was estimated that it would require over two or six days to eliminate alpha-pinene or delta-3-carene, respectively, from the body. The long half-lives indicate slow elimination from adipose tissues. Less than 0.001 % of the total uptake of alpha-pinene or delta-3-carene was eliminated unchanged in the urine, while 7.5 - 7.8 % and 3 % of the inhaled amount of the alpha-pinenes and delta-3-carene were exhaled (Falk et al., 1990b; Falk et al., 1991).

In another study, humans were exposed for four or six hours to atmospheres containing a mixture of volatile organic substances, which included alpha-pinene, at total concentrations of 12 or 24 mg/m<sup>3</sup>. At a concentration of 24 mg/m<sup>3</sup> for the total volatiles, the air concentration of alpha-pinene was 0.775 mg/m<sup>3</sup>. The mean pre-exposure blood concentration of alpha-pinene of 0.035 microg/l increased to an average concentration of 1.9 microg/l during the 4-hours exposure (50 - 240 minutes). Thereafter (330 - 450 minutes), the mean blood concentration decreased to 0.15 microg/l. Changes proportional to those observed at 24 mg/m<sup>3</sup> were recorded at 12 mg/m<sup>3</sup> exposure. Similar results were recorded for the 6-hours exposure. Plasma elimination for alpha-pinene was best described with a three-exponential curve, with half-lives ranging from 0.22 - 7.8 minutes, 19 - 58 minutes and > 150 minutes for the initial, mid and terminal phases, respectively (Ashley and Prah, 1997).

In the urine of sawmill workers exposed to an atmosphere containing 31 - 210 mg/m<sup>3</sup> alpha-pinene [FL-no: 01.004], 2 - 17 mg/m<sup>3</sup> beta-pinene [FL-no: 01.003] and 6 - 90 mg/m<sup>3</sup> delta-3-carene [FL-no: 01.029] for three days, *cis* and *trans*-verbenol were identified as metabolites. They were excreted as conjugates, probably with glucuronic acid. The authors suggested that these metabolites were formed by hydroxylation of alpha-pinene (Eriksson and Levin, 1990). Analysis of urinary metabolites eliminated by human volunteers within four hours following a 2-hours exposure to 10 - 450 mg alpha-pinene/m<sup>3</sup> revealed that alpha-pinene is indeed eliminated as *cis*- and *trans*-verbenol, in a ratio of 1:10, within 20 hours following exposure (Levin et al., 1992). In a more extensive metabolic study, urine was collected from sawmill workers at the end of an eight - nine hours work shift or from chamber-exposed individuals. Following hydrolysis of glucuronic acid conjugates, several pinene biotransformation products were found (Eriksson and Levin, 1996).

### **Macrocyclic, Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup VI)**

No data were submitted on any of the two candidate substances in this subgroup [FL-no: 01.042 and 01.043]. No supporting substances were identified.

### **III.3. Conclusions on Absorption, Distribution and Elimination**

Based on the available studies it may be concluded that the candidate alkane substances (subgroup I) will be absorbed after oral exposure. The extent of absorption is not known, but may be high, given their lipophilic character and their low molecular weight. The ease with which these substances cross the membranes of the respiratory tract further supports the assumption that these substances will also be absorbed after oral intake. Data indicate that straight-chain alkanes will predominantly be broken down to carbon dioxide at low dose levels. At high dose levels, biotransformation becomes saturated and other metabolites will be generated, which will be excreted via the urine. As illustrated by for iso-octane, metabolism of (highly) branched alkanes to carbon dioxide is less favourable, and their metabolites will be eliminated more slowly via the urine.

For the candidate or supporting substances in subgroup II (acyclic alkenes), data were only available for the supporting substance beta-myrcene [FL-no: 01.008] and for the structurally related substance 2-methylbuta-1,3-diene (former candidate substance (See "History of the evaluation")). Given the narrow range of

molecular weights of the candidate substances (all between ~ 68 and 206 D) and their lipophilic nature (Log  $K_{ow}$  values e.g. 2.4 (2-methylbuta-1,3-diene), 6.1 (dodec-1-ene), 4.17 (beta-myrcene) or 4.8 (*cis*-3,7-dimethyl-1,3,6-octatriene)), it must be assumed that these candidate flavouring substances will be absorbed from the gastrointestinal tract, at least to some extent, if not completely. Mass balance data are also incomplete. At least for beta-myrcene it has been shown its metabolites will be excreted via urine in an amount of 25 % of the dose within three days after dosing. For 2-methylbuta-1,3-diene a fairly complete mass balance of elimination has been presented, which shows also elimination via the exhaled air (~ 50 % of the dose), but as this substance was administered via the intraperitoneal dose route, the elimination pattern may be different after oral dosing with a lower fraction of the dose exhaled, e.g. because of more efficient first-pass biotransformation after oral dosing.

For the candidate or supporting substances in subgroup group III (cyclohexene derivatives), data were only available for *d*-limonene [FL-no: 01.045], which is actually a supporting substance. For this substance, data show a considerable absorption from the gastrointestinal tract. In humans, elimination of the substance followed a triphasic pattern, but still 55 - 83 % of an oral dose could be found in the urine within 72 hours post-dosing, with the major part excreted within 24 hours. Also in other species urinary elimination was most important accounting for up to 82 - 96 % of the dose within 72 hours. Hence, it may be argued that monocyclic cyclohexene derivatives, such as *d*-limonene, administered orally are absorbed and distributed throughout the body. Following oral administration to humans, *d*-limonene was distributed preferentially to fatty tissues, as indicated by a high oil-blood partition coefficient and a long half-life during the slow elimination phase. Because of the limited molecular weight of the candidate substances in this group (range ~ 136 - 204 D) and their lipophilic character (e.g. log  $K_{ow}$  values for beta-phellandrene, beta-bisabolene and *d*-limonene are *ca.* 4.7, 7.1 or 4.6, respectively) it may be assumed that all of the substances in this group will be absorbed to some degree, although the extent of absorption for individual substances cannot be accurately estimated from these physico-chemical properties. For the substances in subgroup III with conjugated double bonds no data are available, but based on the structural similarity with the other substances in subgroup III, it may be anticipated that these substances will also be absorbed.

No data are available on the absorption, distribution and elimination of any candidate or supporting substance in subgroup IVa (benzene hydrocarbons). There are several data on the candidate substances in subgroup IVb (naphthalene hydrocarbons). The data show that naphthalene [FL-no: 01.053] will be absorbed after oral administration and will be eliminated predominantly via the urine, in the form of various metabolites, conjugated with e.g. glucuronic acid or with glutathione. In bile duct cannulated animals, considerable elimination into the bile was also observed, but as in intact animals urinary elimination exceeds by far faecal excretion, the material eliminated via the bile is reabsorbed. The glucuronide conjugate of 1-naphthol represents a significant urinary metabolite of naphthalene. Glutathione conjugates of naphthalene are catabolised to (pre)mercapturic and mercapturic acids, particularly in rodents, before excretion in bile or urine. In primates and humans, conjugation with glutathione seems to be less important. Less data are available for 2-methylnaphthalene [FL-no: 01.051], but the available data show that 2-methylnaphthalene is also absorbed from the gastrointestinal tract and eliminated predominantly via the urine. For both substances the data indicate that 85 - 89 % of the dose is excreted within three days after dosing. With respect to the one candidate substance (diphenylmethane [FL-no: 01.036]) in subgroup IVc, it may be concluded that the metabolites of this substance will be eliminated from the body after intraperitoneal injection. No conclusions can be made on absorption from the gastrointestinal tract and the rate of elimination as any oral ADME studies and mass-balance data are available.

For the substances in subgroup V (bi- and tricyclic, non-aromatic hydrocarbons), the available data from oral studies are few. These studies have only addressed the excretion of some supporting and one candidate substance (longifolene) in the form of metabolites via the urine. No mass-balance data were provided. So these studies only show that these substances will be absorbed to some extent. The same may be anticipated for the (other) candidate substances in this subgroup. For the supporting substances alpha- and beta-pinene and delta-carene information on kinetics is available from humans exposed via inhalation, in occupational settings. These studies show that these substances can be absorbed after inhalation exposure and that metabolites will be excreted into the urine e.g. as glucuronide conjugates. The elimination follows a triphasic

pattern with rather long terminal half-lives. The absorbed amount will be eliminated within several days. Based on the lipophilic character of these substances it may be anticipated that they will preferentially distribute in the adipose tissues, which is supported by the slow terminal elimination rates.

No data were submitted on candidate or supporting substances in subgroup VI (macrocyclic, non-aromatic hydrocarbons). The two substances in this group have a molecular weight of ~ 204. They are also rather lipophilic (estimated log  $K_{ow}$  are 4.6 and 7.0 for [FL-no: 01.042] and [FL-no: 01.043], respectively). Based on these data it may be assumed that these two candidate substances will be absorbed from the gastrointestinal tract. For humulatriene, given the rather high  $K_{ow}$ , the extent of absorption may be relatively low.

### III.4. Metabolism

#### Acyclic Alkanes (Candidate and Structurally Related Supporting Substances from Subgroup I)

##### Oral

Male and female rats were given a dose of 1400 mg *n*-octane per kg bw by gavage and urine was collected for up to 48 hours. Urine samples were treated with glucuronidase/sulphatase and the liberated metabolites were analysed by gas chromatography. Compounds found in urine were 2-octanol, 3-octanol, 5-oxohexanoic acid and 6-oxoheptanoic acid. In female rats 2-octanol was found to be the major urinary metabolite and 5-oxohexanoic acid was the major metabolite in males. The authors state that in contrast to *n*-hexane and *n*-heptane, *n*-octane was not metabolised to a ketone, diketone or a diol derivative. The structures of the keto acids that were produced indicate that metabolic oxidation is occurring on both ends of the *n*-octane molecule. A former metabolism study of *n*-heptane did yield *gamma*-valerolactone, which was presumed to have been formed on the GC column from 4-hydroxy-1-pentanoic acid, which would involve a 2 carbon loss. The authors speculate that with *n*-heptane, *n*-octane and perhaps higher straight chain hydrocarbon homologues, a major metabolic pathway involves the formation of acids with loss of carbon (Olson et al., 1986).

The metabolism of *n*-nonane has been studied in rats after oral administration of 800 mg/kg bw over a 48 hours excretion period. In the urine the following metabolites were detected: 2-, 3- and 4-nonanol, 4-nonanone, 2,5-hexanedione, *gamma*-valerolactone, delta-hexanolactone and delta-heptanolactone and 5-methyl-2-(3-oxobutyl)furan. The authors also reported the formation of 1-heptanol, but no explanation for this metabolite is available. Keto-acids, as observed with *n*-octane could not be detected after administration on *n*-nonane (Serve et al., 1995).

##### Other Routes of Exposure

Rats were exposed to *n*-heptane in a concentration of 8100 mg/m<sup>3</sup> in the air for six hours/day five days/week for 12 weeks. After exposure the animals were kept in a metabolism cage to collect urine samples during the next 18 hours for the first week of the experiment and subsequently at the end of every five-day exposure period. Primary metabolites of *n*-heptane in the urine were 1-, 2-, 3-, and 4-heptanol, of which the 2- and to a lesser extent the 3-isomer were quantitatively the most important. Subsequent oxidations of these two secondary alcohols provided 2- and 3-heptanone and the diols 2,6- and 2,5-heptanediol. In follow-up oxidations, final products were 5-hydroxy-2-heptanone, 6-hydroxy-2-heptanone, 2,6- and 2,5-heptanedione and 6-hydroxy-3-heptanone, which was de-acetylated to yield 4-hydroxy-pentanoic acid. This metabolite was detected in the urine as *gamma*-valerolactone. The alcoholic metabolites were rapidly excreted as sulphate and glucuronide conjugates. Although 2,5-heptanedione is a *gamma*-diketone, no signs of peripheral neuropathy were observed. The authors speculated that the plasma levels of this ketone did not become high enough to trigger this effect because of the rapid conjugation of the precursor alcohols which was supported by the minute amounts in which they were present in the urine, although their (conjugated) precursors reached much higher concentrations (Bahima et al., 1984).

In Sprague-Dawley rats exposed to vapours (7680 mg/m<sup>3</sup>) of n-heptane for six hours, main urinary metabolites were 2- and 3-heptanol. Other metabolites detected were 2- and 3-heptanone, 4-heptanol, 2,5-heptanedione, gamma-valerolactone, 2,6-dimethyl-2,3-dihydropyran and 2-ethyl-5-methyl-2,3-dihydrofuran. In humans, occupationally exposed to n-heptane (5 - 196 mg/m<sup>3</sup>) 2- and 3-heptanol were found in the urine. Other metabolites detected were 2- and 4-heptanone and 2,5-heptanedione. In human urinary samples no ring-closure products could be detected, but it is noted that the human exposure was less than that of the rats. In rat tissues (blood, muscle, kidney, nervous tissue, liver), levels of 2-heptanol were between 0.2 and 2 mg/l. Levels of n-heptane in the same tissues were around 20 mg/l (Perbellini et al., 1986).

After oral administration of n-octane (1400 mg/kg bw) to rats in the urine the following metabolites could be detected within 48 hours after dosing: 2- and 3-octanol and 5-keto-hexanoic and 6-keto-heptanoic acid. In contrast to heptane, n-octane was not converted into mono- or diketones or into diols (Olson et al., 1986). Based on this study and the data for n-heptane, the authors argued that for n-heptane and higher straight-chain alkanes, formation of carboxylic acids and subsequent loss of carbon may be a major metabolic pathway. However, the quantitative contribution of de-carboxylation was not thoroughly studied (e.g. no studies with radioactive material) and no mass balances were provided. It has been proposed that n-octane is also converted into 1-octanol and further into octanoic acid, but no detailed information on these studies was submitted (Low et al., 1987).

Groups of three or six rats (Sprague-Dawley, 300 - 600 g), groups of two male rabbits (New Zealand, 1600 - 2000 g) and one male monkey (*Maccaca mulatta*, 1600 - 2000 g) were subjected to single inhalatory treatments of 5000 ppm (~ 18 000 mg/m<sup>3</sup>) n-hexane for 6, 12 and 24 hours. After treatment animals were kept in metabolic cages for the following 72 hours. Urine from treated and control animals were collected during treatment and at various intervals thereafter. For rats blood was collected during and after treatment. Urine samples were subjected to enzymatic and then acid hydrolysis and analysed by gas chromatography. In urine of rats, 2-hexanol, 3-hexanol, 2,5-dimethylfuran, gamma-valerolactone and 2,5-hexanedione along with methyl n-butylketone were found. The metabolites detected in urine from rabbit and monkey were 2-hexanol, 3-hexanol, methyl n-butylketone and 2,5-hexanedione. 1-hexanol and 2,5-hexanediol were not detectable in any of the species. In rats peaks of 2,5-hexanedione and 2,5-dimethylfuran in urine were preceded by analogous peaks in blood, while blood concentrations of n-hexane, methyl n-butylketone and 2-hexanol "peaked" immediately after termination of exposure (Perbellini et al., 1982).

Male rats were exposed to n-hexane by inhalation at concentrations ranging from approximately 180 to approximately 11000 mg/m<sup>3</sup> for 8 hours in an all-glass exposure system. Urinary kinetics for the metabolites 1-hexanol, 2-hexanol, 3-hexanol, 2 hexanone, 2,5-hexanedione and 4,5-dihydroxy-2-hexanone were assessed. Urine samples were collected up to 48 hours following the start of exposure. Amounts of metabolites were linearly dependent on the exposure concentrations, up to an exposure of about 1070 mg/m<sup>3</sup>. Above this the metabolite excretion indicated saturation kinetics in the metabolism. 2-Hexanol amounted to about twice the excretion of all other metabolites excreted. In quantity 4,5-dihydroxy-2-hexanone was the second metabolite, the amount in urine being about 10 times higher than that of excreted 2,5-hexanedione. 2-Hexanol and 4,5-dihydroxy-2-hexanone accounted for 90 % of the total excretion (Fedtke and Bolt, 1987).

A male volunteer was exposed to a mean n-hexane atmosphere of 217 ppm (~ 800 mg/m<sup>3</sup>) for 4 hours. The occurrence of 4,5-dihydroxy-2-hexanone as a metabolite in urine of the man was confirmed. Twenty-six hours after exposure the excretion of 4,5-dihydroxy-2-hexanone reached a level that was four times higher than the excretion of 2,5-hexanedione. The authors conclude that formation of 4,5-dihydroxy-2-hexanone may be viewed as a route of detoxification, and also state that it is clear that 4,5-dihydroxy-2-hexanone is responsible for an artificial appearance of 2,5-hexanedione after drastic acid treatment of human urine, presumably via 2,5-dimethylfuran as intermediate (Fedtke and Bolt, 1987).

Groups of three or four male F 344 rats were exposed to [4-<sup>14</sup>C]-labelled iso-octane (2,2,4-trimethyl pentane) and [1-<sup>14</sup>C]-octane vapours at approximately 1 and 350 ppm (~ 4.76 or 1700 mg/m<sup>3</sup> for both substances) for two hours. During the experiment, exhalant was drawn through a bubbler train for sampling. Urine and

faeces were collected at the same times, except that none were collected at one or two hours. Exhalation of  $^{14}\text{CO}_2$  appeared to be much more important for n-octane than for iso-octane, from which a larger part of the radioactivity is eliminated via urine. Based on this observation and on a discussion of papers from other research groups, the study authors suggest that for straight-chain hydrocarbons, breakdown of the carbon skeleton with the release of  $\text{CO}_2$  is an important metabolic pathway. The route of excretion for n-octane in this study was markedly affected by the concentrations of the inhaled vapour. The ratio of  $^{14}\text{CO}_2$ :  $^{14}\text{C}$  in urine was 5:1 after inhalation at 1 ppm but 1:1 after inhalation of 350 ppm (Dahl, 1989). The radioactivity eliminated via the urine was not identified.

Hexane was given to rats via subcutaneous injections during three consecutive days. Urinary metabolites were various hexanols, 5-hydroxy-2-hexanone, 4,5-dihydroxy-2-hexanone, 2,5-hexanedione, 2-hexanone and the ring closure products *gamma*-valerolactone and 2,5-dimethylfuran. It was noted that most of the alcoholic metabolites were excreted as conjugates (in particular glucuronide) (Manini et al., 1999). Such conjugation has also been reported in the other alkane biotransformation studies discussed in this Annex. The study authors (Manini et al., 1999) further demonstrated that the cyclization products (i.e. the lactones and furans) may be formed under the acidic conditions during deconjugation procedures for sample treatment.

### *Conclusions on the metabolism of subgroup I substances*

The substances in subgroup I can be expected to be metabolised through omega-oxidation, which will lead to the formation of alcohols, and after subsequent oxidation to carboxylic acids, which may be further oxidised via beta-oxidation to yield carbon dioxide. Extensive mass balance data are not available, but in some of the studies described above some excretion of carboxylic acids and carbon dioxide has been reported. Formation of keto-acids has also been reported, and these may be formed from omega-oxidation at one end of the molecule and omega-1 or -2 oxidation at the other end. More interest has been put in the identification of metabolites which did not undergo substantial chain shortening. These metabolites will arise from oxidation of the non-terminal carbon atoms, e.g. via omega-1, -2, -3 or even -4 oxidations. The resulting secondary alcohols can be conjugated with e.g. glucuronic acid and excreted via the urine, or can be further oxidised to yield ketones. A subsequent introduction of another secondary hydroxyl group may result in the formation of diketones. With n-hexane, the resulting 2,5-hexanedione (a *gamma*-diketone) has been demonstrated to be responsible for hexane-induced neurotoxicity. Neurotoxicity is a common feature of *gamma*-diketones, and for that reason, the candidate substance 3-methylhexane [FL-no: 01.050] cannot be anticipated to be metabolised into innocuous compounds. In addition, it is known that methyl-branching of the carbon chain potentiates the neurotoxicity of the *gamma*-diketone (Topping et al., 1994; EFSA, 2004e). The two other hexane derivatives in this group [FL-no: 01.033 and 01.034] cannot be oxidised to *gamma*-diketone, due to the presence of methyl groups on the C2 or C5 carbon atoms. Therefore, these substances may be metabolised to innocuous metabolites. For the longer chain alkanes in this group, it would require oxidation of the more central carbon atoms in order to be converted into *gamma*-diketones. Such oxidations are less favourable than omega, omega-1 or -2 oxidations, and therefore it is concluded that oxidation of these higher alkanes will not result in toxicologically relevant levels of *gamma*-diketones, also because of rapid conjugation of the precursor alcohols. For heptane it was shown that this substance does not result in neurotoxicity (Bahima et al., 1984), although the *gamma*-diketone itself (2,5-heptanedione) is known to be neurotoxic (Topping et al., 1994). The remaining three candidate flavouring substances in this group i.e. [FL-no: 01.038, 01.054 and 01.057] can be expected to be metabolised to innocuous products.

### **Acyclic Alkenes (Candidate and Structurally Related Supporting Substances from Subgroup II)**

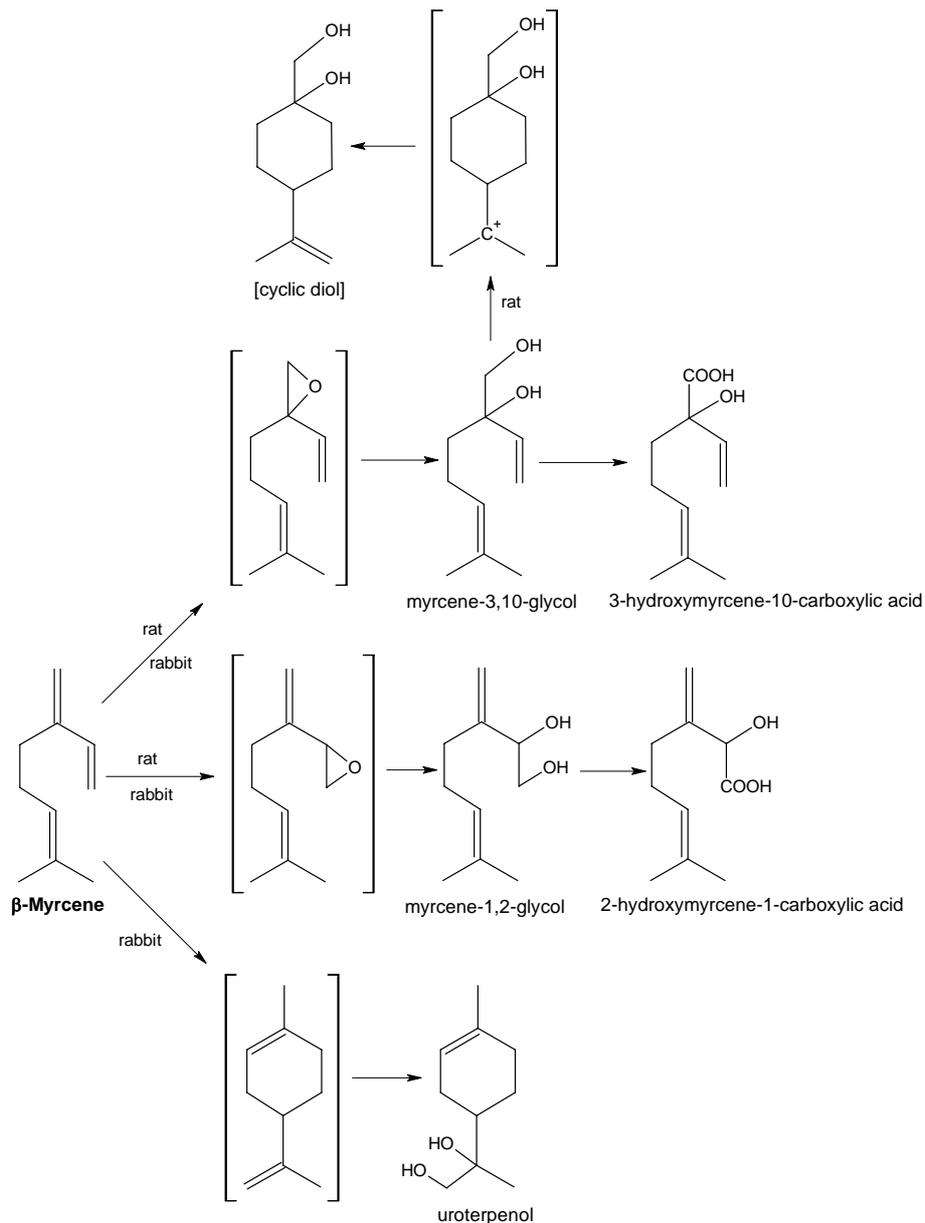
For the substances in this group, information is only available on the biotransformation of beta-myrcene ([FL-no: 01.008]; a supporting substance) and 2-methylbuta-1,3-diene (a structurally related substance). Some additional information on the related industrial chemical 1,3-butadiene has also been added. Two of the substances in this group (dodec-1-ene; [FL-no: 01.037] and 1-octene [FL-no: 01.070]) bear a terminal double bond without any other structural features (e.g. hydroxyl groups, methyl substituents). No further data for these or similar substances are available. The metabolic aspects of terminal double bond substances have been discussed in FGE.07 and FGE.18 (EFSA, 2004e; EFSA, 2006b). The relevant parts of these FGEs will also be included in the current discussion.

*Beta-Myrcene [FL-no: 01.008]*

In the urine of rabbits, orally administered beta-myrcene via gavage (670 mg/kg bw/day for two days), 25 % of the total amount administered could be recovered from the urine within three days post-dosing, and > 80 % of the myrcene-derived substances were neutral metabolites; the rest were acidic substances. The main metabolites identified were myrcene-3,10-glycol, myrcene-1,2-glycol and uroterpenol (as acetate) (40.7, 20.8 and 11.8 %, respectively, of the neutral metabolites). Additionally, the glycols underwent further oxidation to yield 2-hydroxymyrcene-1-carboxylic acid and 3-hydroxymyrcene-10-carboxylic acid (no quantitative data were given for these acidic metabolites). The authors suggested that uroterpenol (or limonene-8,9-diol) may have been formed from limonene, which is derived from cyclization of myrcene in the acidic conditions of the rabbit stomach (Ishida et al., 1981). A graphic representation of beta-myrcene metabolites has been presented in Figure III.1.

When rats were administered 800 mg/kg bw per day of beta-myrcene orally via gavage for 20 days, the principal metabolites isolated from the urine were 10-hydroxylinalool (or myrcene-3,10-glycol) and, to a lesser extent, 7-methyl-3-methylene-oct-6-ene-1,2-diol (or myrcene-1,2-glycol). Other minor metabolites included the hydroxy-acids of both the 3,10- and 1,2-glycols (10-carboxylinalool (or 3-hydroxymyrcene-10-carboxylic acid) and 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid (or 2-hydroxymyrcene-1-carboxylic acid), respectively) and a cyclic diol, 1-hydroxymethyl-4-isopropenylcyclohexanol (or *p*-menth-8-ene-1,7-diol), formed by intramolecular cyclization of an open chain metabolite (Madyastha & Srivatsan, 1987). It was demonstrated that the biotransformation of beta-myrcene was cytochrome P450 (CYP)-mediated and that it could be enhanced by pre-treatment of animals with phenobarbital (Madyastha & Srivatsan, 1987).

Aside from being a substrate for CYP enzymes, myrcene has also been shown to induce these enzymes at high dose levels (1000 mg/kg bw/day orally for three days), especially those from the CYP2B (phenobarbital-inducible) subfamily (De-Oliveira et al., 1997a). At lower dose levels (40 mg/kg bw/day intraperitoneally for three days) such induction was not observed (Austin et al., 1988).



**Figure III.1.** Metabolism of beta-myrcene in rats and rabbits.

### 2-Methylbuta-1,3-diene

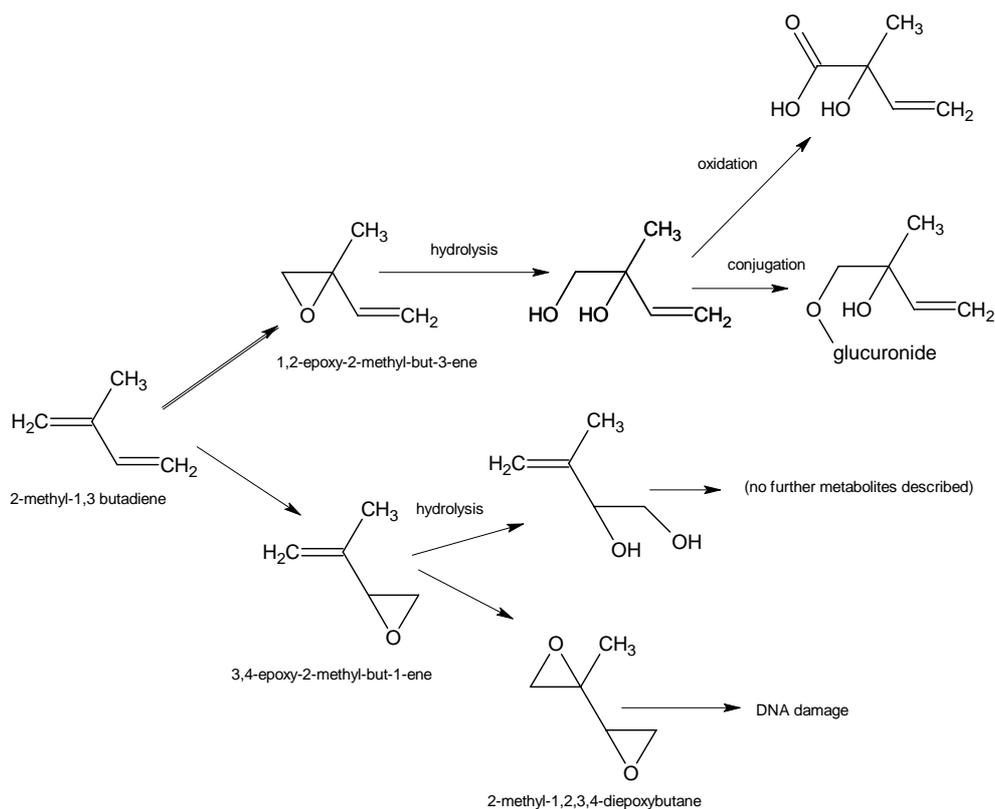
In a sequence of inhalation exposure studies in which rats and mice were exposed to 2-methylbuta-1,3-diene in the air in concentration of 13 - 11 000 mg/m<sup>3</sup>, the metabolism of isoprene became saturated at a level of ca. 700 mg/m<sup>3</sup> in the rat and ca. 840 mg/m<sup>3</sup> in the mouse. Maximal rates of metabolism were 130 micromole/kg bw/hour in the rat and 400 micromole/kg bw/hour in the mouse. It was demonstrated that 2-methylbuta-1,3-diene is also produced endogenously at rates of 1.9 micromole/(hour × kg) in rats and 0.4 micromole/(hour × kg) in mice. Part of the endogenous 2-methylbuta-1,3-diene is exhaled by animals, but it is metabolised extensively. The rate of metabolism of endogenously produced and systemically available 2-methylbuta-1,3-diene is 1.6 micromole/(hour × kg) in rats and 0.3 micromole/(hour × kg) in mice, respectively (Peter et al., 1987). The authors quoted literature demonstrating that 2-methylbuta-1,3-diene is also endogenously produced in humans (Gelmont *et al.*, 1981 as cited in (Peter et al., 1987)).

Liver microsomes from mice, rats, rabbits and hamsters metabolise 2-methylbuta-1,3-diene to the corresponding monoepoxides, 1,2-epoxy-2-methyl-3-butene (major) and 3,4-epoxy-2-methyl-1-butene (minor). Both monoepoxides are hydrolysed to their respective diols (for chemical structures, see Figure III.2). The main metabolite of 2-methylbuta-1,3-diene (i.e. 1,2-epoxy-2-methyl-3-butene) exhibited a half-life of 75 minutes in aqueous environments; whereas the minor metabolite, 3,4-epoxy-2-methyl-1-butene (14 - 25 % with respect to the main metabolite) is more stable (half-life not specified). The kinetic constants for the formation of the major epoxide metabolite (trans-2-methyl-3-butene-1,2-diol) of 2-methylbuta-1,3-diene were determined in the four test species as apparent  $K_m = 0.06 - 0.2$  mM and  $V_{max} = 0.24 - 1.79$  nmol trans-2-methyl-3-butene-1,2-diol/mg protein  $\times$  min. The minor metabolite, 3,4-epoxy-2-methyl-1-butene, was further epoxidised to the diepoxide, 2-methyl-1,2:3,4-diepoxibutane, by microsomes of all rodents studied. The authors argued that the latter diepoxide metabolite could be responsible for the genotoxic and carcinogenic activity of 2-methylbuta-1,3-diene, as the two mono-epoxies were not reported to be genotoxic (Del Monte et al., 1985; Longo et al., 1985).

The *in vitro* metabolism of 2-methylbuta-1,3-diene was investigated in rat, mouse and human liver microsomes and in microsomes derived from cell lines expressing eight different human cytochrome P-450 enzymes. Human CYP2E1 showed the highest rates for formation of the monoepoxides, 1,2-epoxy-2-methyl-3-butene and 3,4-epoxy-2-methyl-1-butene, and CYP2B6 showed the second highest rate. Only CYP2E1 catalysed formation of the diepoxide, 2-methyl-1,2:3,4-diepoxibutane. With human liver microsomes in the presence of an epoxide hydrolase inhibitor, the formation of 1,2-epoxy-2-methyl-3-butene was four times faster than the formation of the 3,4-epoxy-2-methyl-1-butene, which is comparable to the results in rats and mice obtained by others (e.g. Del Monte *et al.*, 1985; Longo *et al.*, 1985). The rates of monoepoxide formation from isoprene and diepoxide formation from either monoepoxide intermediate were strongly correlated with the microsomal activity of CYP2E1, rather than with the activities of the other CYP enzymes, and both monoepoxides were equally good substrates for the formation of the diepoxide.

Also, species differences with regard to the role of epoxide hydrolase were investigated by comparing the epoxidation of 2-methylbuta-1,3-diene by rat, mouse and human liver microsomes. When an epoxide hydrolase inhibitor was used, similar rates of monoepoxide formation in mouse, rat and human liver microsome systems were measured. However, without epoxide hydrolase inhibition, the total amount of 1,2-epoxy-2-methyl-3-butene measured at the end of the incubation period was twice as high for mouse as for rat liver microsomes and 30 times as high for mouse as for human liver microsomes in which formation of this epoxide was reduced to 4 % of the rate in presence of the inhibitor. For the 3,4-epoxy 2-methyl-1-butene metabolite the effect of epoxide hydrolase inhibition was less dramatic. While hardly any effect was observed in mouse or rat microsomes, in the human microsomes, the rate was reduced to approximately 25 %. The effect of epoxide inhibition on the rate of formation of the diepoxide was not studied. The authors concluded that differences in epoxide hydrolase activity between species may be of crucial importance for the toxicity of 2-methylbuta-1,3-diene in various species (Bogaards et al., 1996).

Following intraperitoneal injection of 64 mg [4-<sup>14</sup>C]-2-methylbuta-1,3-diene per kg to F344 rats, the parent compound was excreted unchanged in the breath (> 50 % of the dose together with < 4 % unidentified material) or via the urine in the form of metabolites ( $\approx$  32 %) over the 24-hours collection period. Only 1.7 % was expired as CO<sub>2</sub> and 0.2 % was eliminated via faeces. 3 % Remained in the carcass. In the urine, 2-methylbuta-1,3-diene was excreted primarily as 2-hydroxy-2-methyl-3-butenic acid (53 % of total urinary metabolites excreted), 2-methyl-3-buten-1,2-diol (23 %), and the C-1 glucuronide conjugate of 2-methyl-3-buten-1,2-diol (13 %). A fraction of 7 % of the radioactivity in the urine was (an) unidentified polar material. The principal urinary acidic metabolite forms via oxidation of the corresponding 1,2-diol. These metabolites indicate a preferential epoxidation of the methyl-substituted vinyl group of 2-methylbuta-1,3-diene (see Figure III.2) in the rat (Buckley et al., 1999).



**Figure III.2.** Major metabolic pathways for 2-methylbuta-1,3-diene (after Del Monte et al. (1985) and Buckley et al. (1999)).

Following intraperitoneal injection of 64 mg [4-<sup>14</sup>C]-2-methylbuta-1,3-diene/kg to B6C3F<sub>1</sub> mice, the parent compound was excreted unchanged in the breath (≈ 44 % of the dose and < 3 % unidentified material) or as urinary metabolites (≈ 33 %) over the 24-hours collection period. Only 1.9 % was expired as CO<sub>2</sub> and 7 % was eliminated via faeces. 2 % remained in the carcass. In comparison with rat urine (see above), the urine of the mice contained several other metabolites, including an unidentified polar fraction which comprised 25 % of the total urinary radioactivity as compared to 7 % in rat urine. The major identified metabolite in mouse urine, 2-hydroxy-2-methyl-3-buten-1-carboxylic acid, accounted for ≈ 15 % of the total urinary radioactivity, whereas 2-methyl-3-buten-1,2-diol and its glucuronide conjugate accounted for ≈ 3.5 and 2.5 %, respectively (Buckley et al., 1999). The authors speculated that the unidentified metabolite in the urine of rats and mice could be related to glutathione conjugates. They concluded that if this were the case, in rats glutathione conjugation of 2-methylbuta-1,3-diene is less important than in mice.

#### Special features of terminal double bond oxidation

Double bonds are usually oxidised by P450 to the corresponding epoxides, which are highly reactive molecules. Due to the large strain associated with the three membered ring structure, epoxides easily react with nucleophilic sites of cellular macromolecules; conversely they are readily detoxified either spontaneously or by the action of epoxide hydrolase to diols or conjugated with reduced glutathione by glutathione-S-transferases. 1-Alkenes are metabolised by cytochrome P450, through double bond oxidation, to the corresponding epoxide or alternatively allylic oxidation (Chiappe et al., 1998). The rates of the two reactions measured with different cytochrome P450 isoforms indicate that epoxide formation is generally favoured (Chiappe et al., 1998) and this may also apply in particular to the candidate substances dodec-1-ene [FL-no: 01.037] and 1-octene [FL-no: 01.070].

Based on this information and the data available for beta-myrcene and 2-methylbuta-1,3-diene, it cannot be excluded that the candidate substances with these terminal double bonds [FL-no: 01.037, 01.064 and 01.070] may be metabolised to epoxides.

#### *Conclusions on the metabolism of subgroup II substances*

The data available on the metabolism of one supporting and one structurally related substance in subgroup II show that metabolic options for the substances in this group are epoxidation of double bonds ultimately resulting in diols, which can be further conjugated. With the supporting substance beta-myrcene also further metabolism of the diols into carboxylic acids has been reported. Both in rats and rabbits, the principal urinary metabolite following gavage administration of beta-myrcene is myrcene-3,10-glycol, formed from the hydration of the epoxide intermediate in both species. Epoxidation of the 3,10-double bond was favoured over epoxidation of the 1,2-double bond.

The studies indicate that the formation of diols from the myrcene-epoxides is very efficient. It is noted, however, that the diols and the related carboxylic acids are all the result of epoxidation of double bonds in which one of the carbon atoms has only hydrogen substituents, but no further carbon chains. In this respect, the candidate substance cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] is most similar to beta-myrcene. With beta-myrcene no diepoxide metabolites or further reaction products thereof have been reported, but it is noted that mass balance data are incomplete, so some metabolites may have been overlooked.

With the structurally related substance 2-methylbuta-1,3-diene, epoxidation of the 1,2-double bond is favoured over epoxidation of the 3,4-double bond. While the 1,2-epoxide is readily hydrolysed, the 3,4-epoxide is far more stable. Both metabolites can be converted to the corresponding diols and at least one hydroxy-carboxylic acid (2-hydroxy-2-methyl-3-butenoic acid) has been identified. In addition, in particular the 3,4-epoxide metabolite can be oxidised a second time, resulting in the formation of a diepoxide metabolite, which is known to be reactive and has been suggested to be responsible for 2-methylbuta-1,3-diene-induced DNA damage. For candidate substance dodec-1-ene [FL-no: 01.037], diol- and alpha-hydroxy-carboxylic acid formation may also be expected. In addition, this substance may also undergo omega and omega-1 or -2 oxidation at the other (saturated) end of the carbon chain (similar to the candidate substances in subgroup I).

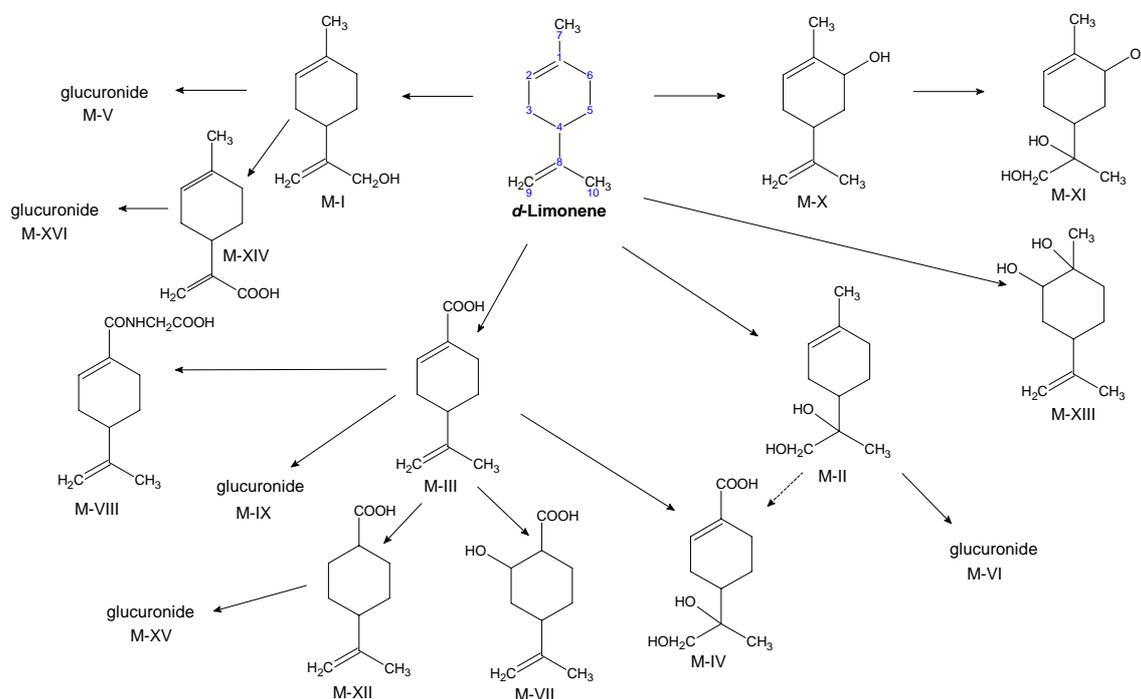
Apart from beta-myrcene and 2-methylbuta-1,3-diene, terminal double bonds appear in candidate substances [FL-no: 01.037, 01.064 and 01.070]. In contrast to corresponding substances in FGE.07 and FGE.18 there is no other functional group in the molecule (e.g. hydroxyl- or keto-group) that could provide a more direct option for detoxication e.g. via conjugation with glucuronide or sulphate. In the two FGEs mentioned above the presence of such groups was an argument to consider that the metabolism of the particular candidate substances would go via innocuous products. It would not be possible to conclude similarly for the candidate substances in FGE.25. The other candidate substances in this subgroup (II) might also be oxidised in the various methyl groups but no data are available to substantiate this. Because of these considerations, it cannot be concluded that the candidate substances in this subgroup [FL-no: 01.032, 01.035, 01.037, 01.064, 01.070 and 01.078] will be metabolised to innocuous products.

#### **Cyclohexene derivatives (Candidate and structurally related supporting substances from subgroup III)**

For the candidate substances in this group, data for one supporting substance (*d*-limonene; [FL-no: 01.045]) were submitted.

More than 10 metabolites were found in the urine of rats given an oral gavage dose of 800 mg/kg bw *d*-limonene (or *p*-mentha-1,8-diene). Four of the metabolites were identified as perillic acid, *p*-menth-1-ene-8,9-diol (= limonene-8,9-diol), perillic acid-8,9-diol and 8-hydroxy-*p*-menth-1-en-9-yl-glucuronic acid. The bile of these rats contained three metabolites, the most important of which was 8-hydroxy-*p*-menth-1-en-9-yl-beta-D-glucuronic acid (Igimi et al., 1974). Six metabolites were identified in the urine of rabbits given

the same oral dose. In addition to the four metabolites identified in rat urine, the rabbit urine contained *p*-mentha-1,8-dien-10-ol (= limonene-10-ol) and *p*-mentha-1,8-dien-10-yl-glucuronic acid (= *p*-mentha-1,8-dien-10-ol-glucuronide). Although not determined quantitatively, perillic acid, perillic acid-8,9-diol and both glucuronic acid conjugates were the major metabolites in rabbit urine, and no unchanged *d*-limonene was detected (Kodama et al., 1974). The same authors identified five additional metabolites in the urine of rats and dogs treated orally with *d*-limonene. These were characterised as 2-hydroxy-*p*-menth-8-en-7-oic acid, perillylglycine, perillyl-glucuronide, *p*-mentha-1,8-dien-6-ol (or limonene-6-ol) and *p*-menth-1-ene-6,8,9-triol. They also found some species differences in the nature of the major metabolites in urine. Perillic acid-8,9-diol was the main metabolite in rats and rabbits, perillyl-glucuronide in hamsters, limonene-8,9-diol in dogs and limonene-8,9-diol-glucuronide in guinea pigs and humans. It should be noted that the fate of only 40 - 65 % of the *d*-limonene dose administered orally to these animals and humans was accounted for (Kodama et al., 1976). The metabolites of *d*-limonene are shown in Figure III.3.



M-I	limonene-10-ol	M-IX	glucuronide conjugate of M-III
M-II	limonene-8,9-diol	M-X	limonene-6-ol
M-III	perillic acid	M-XI	<i>p</i> -menth-1-ene-6,8,9-triol
M-IV	perillic acid-8,9-diol	M-XII	dihydroperillic acid
M-V	glucuronide conjugate of M-I	M-XIII	limonene-1,2-diol
M-VI	glucuronide conjugate of M-II	M-XIV	<i>p</i> -mentha-1,8-diene-10-carboxylic acid
M-VII	2-hydroxy- <i>p</i> -menth-8-en-7-oic acid	M-XV	glucuronide conjugate of M-XII
M-VIII	perillylglycine	M-XVI	glucuronide conjugate of M-XIV

**Figure III. 3.** Metabolism of *d*-limonene.

Perillic acid, dihydroperillic acid and limonene-1,2-diol were the major metabolites identified in the plasma of humans given an oral dose of *d*-limonene. Minor metabolites were the methyl esters of perillic acid and dihydroperillic acid, and *d*-limonene itself (Crowell et al., 1994a). Apart from the parent compound (Poon et al., 1996) and (Vigushin et al., 1998) also identified perillic acid, dihydroperillic acid and limonene-1,2-diol as major metabolites in human plasma. However, they also found two other metabolites, i.e. *p*-mentha-1,8-diene-10-carboxylic acid and limonene-8,9-diol, whereas they did not detect the methyl esters of perillic acid and dihydroperillic acid. Peak plasma levels for all metabolites were achieved four to six hours after administration, with the exception of limonene-8,9-diol which reached its peak level one hour after

administration (Poon et al., 1996). Metabolites in human urine comprised the glucuronic acid conjugates of perillic acid, dihydroperillic acid, *p*-mentha-1,8-diene-10-carboxylic acid, limonene-8,9-diol and limonene-10-ol (Poon et al., 1996).

Experiments with rat liver microsomes have shown that epoxidation of the C8 double bond (in the vinyl substituent) of *d*-limonene is favoured over epoxidation of the C1 double bond (the one in the ring), due to steric hindrance by the 1-methyl group, which was demonstrated by comparison of the metabolism of *d*-limonene with that of 4-vinylcyclohex-1-ene. Upon incubation with rat liver microsomes, the majority of *d*-limonene was converted to the 8,9-epoxide and the 8,9-diol, and to a much lesser extent to the 1,2-epoxide and the 1,2-diol (ratio of 8,9- vs. 1,2-epoxidation = 4:1). In contrast, with 4-vinylcyclohex-1-ene the epoxidation rate of the ring double bond was about four times as fast as the epoxidation rate of the vinyl double bond. Because the 1,2-epoxide of *d*-limonene is a very poor substrate for microsomal epoxide hydrolase, the 1,2-diol could not be found in microsomal incubates, whereas the 8,9-diol could be found (Watabe et al., 1981). Both epoxides of *d*-limonene were tested for mutagenicity in several *Salmonella* strains and showed to be inactive. The mono-epoxides of 4-vinylcyclohex-1-ene were inactive in *Salmonella* strain TA100, but were not tested in the other strains. However, the diepoxide of 4-vinylcyclohex-1-ene was mutagenic in this bacterial strain, but this diepoxide was not detected in the microsomal metabolism studies with 4-vinylcyclohex-1-ene as starting material (Watabe et al., 1981).

Other *in vitro* experiments have shown that male rats can convert *d*- and *l*-limonene into the corresponding *trans*-perillyl alcohol (by hydroxylation of the methyl group at C7) and carveol (or limonene-6-ol; by ring C6-hydroxylation). These reactions are catalysed by CYP2C11 and, when pretreated with phenobarbital, CYP2B1. In female rats, the activity for conversion to either alcohol is much lower. Apparently, the female-specific CYP2C12 has no activity with respect to *d*- and *l*-limonene hydroxylation. In males, the hydroxylation activities were not detectable with foetal liver microsomes, but they increased after birth, closely related to the developmental increase in CYP2C11. This study also investigated whether the *d*- and *l*-limonene enantiomers are differently metabolised by liver microsomes. Both in liver microsomes from untreated and treated with phenobarbital, approximately the same amounts of carveol and perillyl alcohol were formed from the two limonene enantiomers. Ratios over the two routes of metabolism were carveol/perillyl alcohol: 0.87/1.23 for *d*-limonene and 0.61/1.03 for *l*-limonene. Also the rate of formation of carveol and perillyl alcohol from *d*- and *l*-limonene is similar using either liver microsomes or recombinant P450 enzymes (Miyazawa et al., 2002).

In male rats orally administered 3 mmol/kg (408 mg/kg) of [<sup>14</sup>C]-*d*-limonene radioactivity was detected in the renal cytosol. Forty percent of the total cytosolic radioactivity was reversibly associated with the protein fraction and further analysis showed that > 97 % of this activity was associated with one single protein, which was identified as  $\alpha$ -2 $\mu$ -globulin. 1,2-Limonene epoxide, 1,2-limonene-diol and *d*-limonene comprised 82, 5 or 13 %, respectively, of the radioactivity associated with this protein (Lehman-McKeeman et al., 1989).

*d*-Limonene has been shown to induce P450 enzymes of the CYP2B and CYP2C subfamilies and epoxide hydrolase in rats (Austin et al., 1988; Maltzman et al., 1991).

#### *Conclusions on the metabolism of subgroup III substances*

In the subgroup III there are only metabolism data available for one supporting substance, *d*-limonene, which in several animal species and humans has been demonstrated to be oxidised in both side chains and at the cyclohexene ring, resulting in alcohols and/or carboxylic acids. Ring and side chain hydroxylation has also been described for its structural isomer *l*-limonene (a constituent of candidate substance [FL no: 01.001] and a candidate substance on its own [FL-no: 01.046]) in rat liver microsomes. The metabolites of these limonenes are, at least partly, conjugated and eliminated with the urine. It is anticipated that three out of the six candidate substances [FL-no: 01.027, 01.028 and 01.039] in subgroup III are metabolised in a similar way to innocuous products. It cannot be anticipated based on the data available that beta-phellandrene [FL-no: 01.055] with a double bond directly on the ring and in conjugation with double bond in the cyclohexene ring can be metabolised to innocuous products.

## Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup IV)

### *Subgroup IVa: Benzene Hydrocarbons*

No data are available for the candidate substances in this group. Some information was available on the biotransformation of *p*-cymene ([FL-no: 01.002]; *p*-isopropyl toluene). Given the structure of this substance, which deviates considerably from the candidate substances in this subgroup, the information provided was considered to be only of limited relevance for the evaluation of the benzene hydrocarbons in this group. In general, the studies indicate that in various animal species *p*-cymene undergoes extensive oxidation of the methyl and isopropyl side chain to yield polar oxygenated metabolites. These metabolites are either excreted unchanged in the urine or undergo phase II conjugation with glucuronic acid and/or glycine, followed by excretion in the urine (Bakke and Scheline, 1970; Walde et al., 1983; Matsumoto et al., 1992; Boyle et al., 1999). Only in the guinea pig, ring hydroxylation products (carvacrol and hydroxycarvacrol) were found in minute amounts. Ring hydroxylation in guinea pigs only occurred *ortho* to the methyl group (Walde et al., 1983).

### *Subgroup IVb: Naphthalene Hydrocarbons*

#### *Naphthalene [FL-no: 01.053]*

Metabolism of naphthalene (0.031, 0.062, 0.125, 0.25, 0.50 and 1.0 mM) was studied in lung and liver microsomal preparations from Swiss Webster mice, Sprague-Dawley rats, Sprague-Dawley hamsters and rhesus macaque monkeys. Naphthalene metabolism to glutathione conjugates and diols was 4 to 8-fold higher in mouse lung microsomal incubations than in corresponding incubations from hamster and rat lung. Mice lung had a high degree of stereoselectivity in metabolism of naphthalene to naphthalene epoxide, where the ratio of 1R,2S- to 1S,2R-naphthalene epoxide was 11 to 14:1. Little or no stereoselectivity in the metabolism of naphthalene was found in the mouse liver or in the lung and liver from the rat, hamster and monkey. The mouse liver metabolises more of the naphthalene to diol than the mouse lung, 12 % and 5 % of the total metabolites, respectively. Naphthalene is metabolised in lung tissue microsomal incubations of rhesus monkeys at a rate 100-fold less than mice and 10-fold less than rats. Naphthalene is metabolised in lung tissue microsomal incubations of rhesus monkeys at a rate 100-fold less than mice and 10-fold less than rats. In the primate liver microsomes naphthalene diols comprise 80 % of the total metabolites produced (Buckpitt et al., 1992). The olfactory epithelium contained the greatest amount of CYP protein of all tissues investigated in the rat. However, CYP2F expression levels in primate nasal tissues demonstrate that only the nasal ethmoturbinates contained quantifiable amounts of CYP2F with the levels in primates roughly 10- and 20-fold less than the corresponding tissues in rats and mice, respectively (Baldwin et al., 2004). Clearly, humans would produce less naphthalene-1,2-oxide or produce it at a slower rate than rats or mice in respiratory tract tissues. An overview of naphthalene metabolism is presented in Figure III.4.

Three groups of Sprague-Dawley rats were treated orally with a single administration of 2 mg <sup>14</sup>C-naphthalene. The dose/unit body weight was not stated (Bakke et al., 1985). One group of 16 animals were bile duct cannulated, the second group of four germ-free animals were uncannulated, and the third group of 13 animals were standard uncannulated rats. Urine, faeces and bile were collected for 72 hours after administration of naphthalene and the metabolites were identified and quantified. In the standard uncannulated rats the urinary metabolites identified at 24 hours were an N-acetyl cysteinyl naphthalene conjugate (38.1 % of the administered radioactivity), 1,2-dihydro 1,2-dihydroxynaphthalene (dihydrodiol) glucuronide (23.9 %), dihydroxynaphthalene (4.9 %), naphthols and naphthol glucuronides (4.6 %) and 1,2-dihydro-1-hydroxy-2-thionaphthalene glucuronide [CH<sub>3</sub>S-metabolite] (4.6 %). At 24 hours in cannulated rats, 14.4 % of the administered dose was present in the urine as N-acetyl cysteinyl naphthalene conjugate, and 14.5 % as the dihydroxynaphthalene glucuronide conjugate. Naphthols, thionaphthols, CH<sub>3</sub>S-metabolites or their respective glucuronides and sulphates were not detected in either the 24-hours urine or bile. The major urinary metabolites of germ-free rats were N-acetyl cysteinyl naphthalene (89 %) and

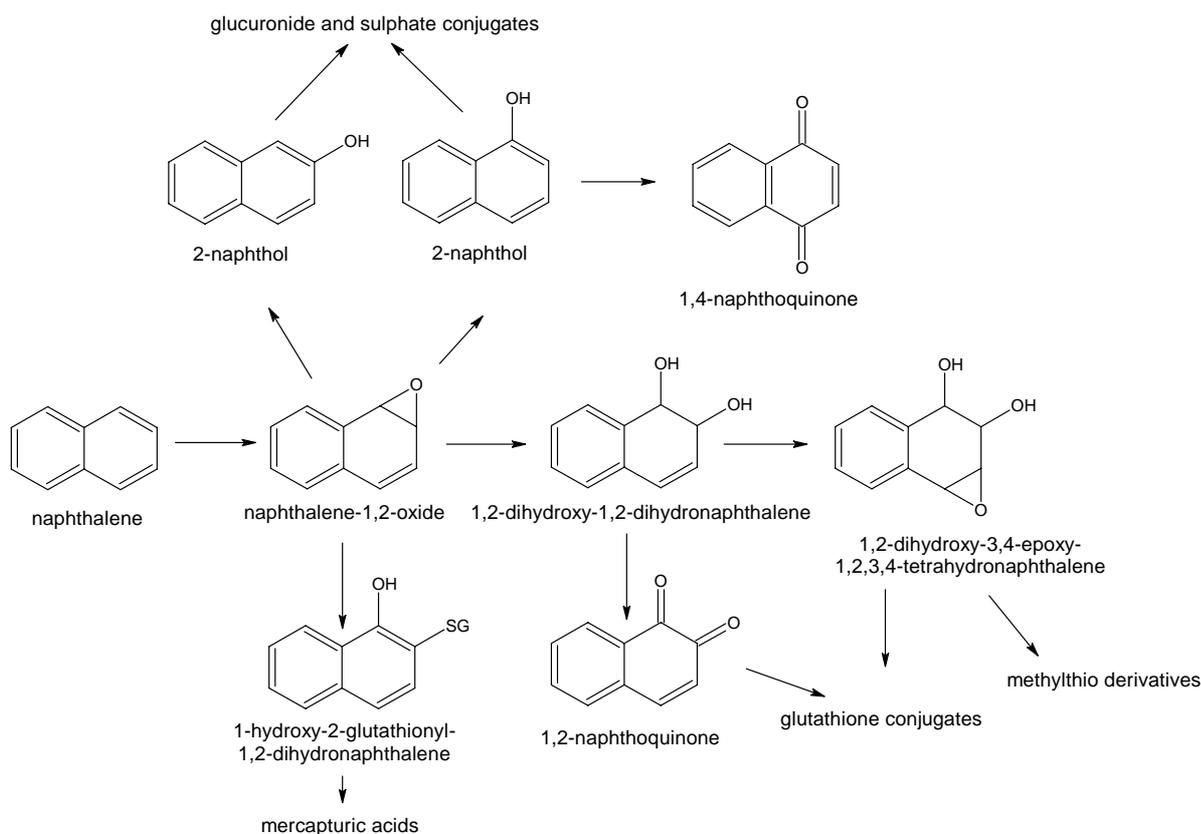
dihydroxynaphthalene glucuronide (4 %). Only trace amounts of naphthols and no CH<sub>3</sub>S-metabolites could be detected indicating that intestinal microflora are probably involved in production of these metabolites followed by enterohepatic recirculation. *In vitro* experiments showed that 1,2-dihydroxy-1,2-dihydronaphthalene may be transformed by isolated rat liver dihydrodiol dehydrogenase to form the 1,2-naphthoquinone, which reacts rapidly with glutathione or cysteine (Smithgall et al., 1986; Smithgall et al., 1988). Naphthalene-1,2-dihydrodiol may be oxidised further by CYP enzymes, resulting in an epoxide, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene. Several urinary metabolites, including a number of trihydroxytetrahydromethylthio-derivatives (Horning et al., 1980) and trihydroxytetrahydromercapturic acid (Pakenham et al., 2002) provide support for the *in vivo* formation of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene. Initially, naphthalene metabolism involves epoxidation of the aromatic ring by cytochrome (CYP) enzymes yielding naphthalene-1,2-oxide. Several studies have shown that a number of CYP isozymes catalyse this reaction, including CYP2E1 (Wilson et al., 1996) and CYP2F (Buckpitt et al., 2002; Shultz et al., 1999).

Limited information is available regarding the metabolism of naphthalene in humans. Incubation of human lung microsomal fractions demonstrated naphthalene metabolism to naphthalene dihydrodiol and glutathione conjugates (Buckpitt and Bahnson, 1986). Human and mouse liver microsomes exposed to naphthalene produced the 1-naphthol and naphthalene-1,2-dihydrodiol metabolites in the ratio 8.6 and 0.4 in human and in phenobarbital-induced mouse microsomes, respectively (Tingle et al., 1993). In human liver microsomes naphthalene-1,2-dihydrodiol is formed following epoxidation and hydrolysis, whereas rearrangement of the 1,2-epoxide leads to the formation of 1-naphthol (Tingle et al., 1993). Preferential formation of the 1S,2R-naphthalene oxide enantiomer in human and rat microsomes provides evidence that human metabolism of naphthalene may be more similar to rats than mice (Lanza et al., 1999). Naphthalene metabolising enzymes have been demonstrated in nasal respiratory epithelial and olfactory epithelial tissue from rodents and humans (Buckpitt et al., 1992; Buckpitt et al., 2002; Plopper et al., 1992; Thornton-Manning & Dahl, 1997).

Waidyanatha *et al.* (2002) have studied covalent binding of naphthalene metabolites to haemoglobin and albumin *in vivo*. They measured cysteinyl adducts of naphthalene-1,2-oxide and 1,2- and 1,4-naphthoquinone in rats, 24 hours after dosing them with 100 - 800 mg naphthalene/kg bw. Cysteinyl adducts with all three metabolites were produced in a dose-dependent manner. Naphthalene-1,2-oxide produced adducts with both proteins and at dose levels > 200 mg/kg bw the formation of adducts became saturated. For 1,2-naphthoquinone-albumin adducts were more abundant than for 1,4-naphthoquinone. Differences for the haemoglobin adducts were much less pronounced. Total naphthoquinone adducts with albumin were also produced in larger amounts than with haemoglobin. Naphthoquinone, but not naphthalene-1,2-oxide adducts, could also be detected in albumin and haemoglobin of control animals (Waidyanatha *et al.*, 2002).

Urine samples were collected from an infant hospitalised because of haemolytic anaemia following ingestion of unknown quantities of naphthalene mothballs. 1- and 2-naphthol and 1,2- and 1,4-naphthoquinone were identified in the samples for up to eight days of hospitalisation. 1-Naphthol was present in the largest, unspecified quantities (Mackell *et al* 1951, as cited in (EU-RAR, 2003)).

It can be concluded that naphthalene [FL-no: 01.053] is metabolised by cytochrome P450 enzymes, yielding naphthalene-1,2-oxide. This epoxide may be metabolised by glutathione S-transferase enzymes to a glutathione conjugate that is subsequently converted into a mercapturic acid conjugate and excreted in the urine. Naphthalene-1,2-oxide may also rearrange to yield 1- or 2-naphthol, which are excreted as glucuronide or sulphate conjugates in the urine. In addition, 1-naphthol may be further metabolised by CYP enzymes to 1,2- or 1,4-naphthoquinone, and 2-naphthol may be further oxidised to give also 1,2-naphthoquinone. Naphthalene-1,2-oxide may be hydrated by epoxide hydrolase, yielding 1,2-dihydroxy-1,2-dihydronaphthalene, which is oxidised by dihydrodiol dehydrogenase also to form 1,2-naphthoquinone. CYP enzymes may oxidise naphthalene-1,2-dihydrodiol to yield 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, which can be further metabolised by a number of conjugation pathways.



**Figure III.4.** Pathways for metabolism of naphthalene (adapted from ATSDR, 2005).

### 2-methylnaphthalene [FL-no: 01.051]

The oxidative metabolism of 2-methylnaphthalene was investigated using rat liver microsome suspensions. One monohydroxylated (2-hydroxymethylnaphthalene) and three isomeric dihydrodiol (3,4-dihydrodiol, 5,6-dihydrodiol and 7,8-dihydrodiol) metabolites of 2-methylnaphthalene were identified (Breger et al., 1983). Melancon *et al.* (1985) confirmed that one 2-hydroxymethyl- and three isomeric dihydrodiol metabolites of 2-methylnaphthalene are formed *in vitro* by rat hepatic microsomes and purified cytochromes (Melancon et al., 1985).

Four female rats were dosed with two subcutaneous injections of 300 mg 2-methyl-[8-<sup>14</sup>C]-naphthalene/kg bw and urine was collected for three days. Of the radioactivity injected, 55 % was found in the urine and only 3 - 5 % (*ca.* 2.2 % of the dose) appeared to be unchanged parent compound. Three isomeric dihydrodiol metabolites of 2-methylnaphthalene represented 6 - 8 % of urine <sup>14</sup>C (*ca.* 3.8 % of the dose). More polar metabolites were identified as 2-naphthoic acid and 2-naphthoyleglycine, the latter of which accounted for 30 - 35 % of urine <sup>14</sup>C (*ca.* 17.5 % of the dose). The remainder of the urinary radioactivity was not fully elucidated but 6 - 8 % were other conjugates of naphthoic acid (*ca.* 3.8 % of the dose), and some were identified as hydrolysable with beta-glucuronidase (not yielding naphthoic acid) (Melancon et al., 1982).

In guinea pigs, when 10 mg/kg bw 2-[1-<sup>3</sup>H]-methylnaphthalene was orally administered the major metabolites excreted in the urine at 24 hours (in total 23 % of the dose) were oxidation products of the methyl group (*i.e.* 2-naphthoic acid (4 %), 2-naphthoyleglucuronic acid (11 %), and 2-naphthoyleglycine (61 %)) which in total accounted for 76 % of the total urinary radioactivity. Dihydrodiols could not be detected, but S-(7-methyl-1-naphthyl)cysteine (10 %), and glucuronic acid and sulphate conjugates of 7-methyl-1-naphthol (each 4 %) were also identified as minor metabolites accounting together for 18 % of the total urinary radioactivity. In incubations of 2-methylnaphthalene with guinea pig liver S9, formation of S-(7-

methyl-1-naphthyl)-glutathione could be demonstrated, and four hours after oral administration of 500 mg 2-methylnaphthalene/kg bw, GSH depletion in the liver was observed, but no mercapturic acids could be found in the urine. It was speculated that the cysteine conjugate might be an artificial break-down product of mercapturic acid derivatives (Teshima et al., 1983). Other authors have also demonstrated that 2-methylnaphthalene can deplete liver GSH stores, e.g. (Griffin et al., 1982). This depletion has been brought into connection with the toxicity of 2-methylnaphthalene (Shelby and Witt, 1995).

Griffin *et al.* (1982) have studied the relationship between 2-methylnaphthalene (400 mg/kg bw intraperitoneally) induced lung toxicity and GSH depletion in the lung, liver and kidneys in the mouse in relationship to 2-methylnaphthalene covalent tissue binding and metabolic conversion into dihydrodiol metabolites, using age at onset modifiers of cytochrome P450 activity. Both phenobarbital (PB) and 3-methylcholanthrene (3MC) reduced lung toxicity of 2-methylnaphthalene, but only PB reduced pulmonary covalent binding. In the liver neither PB nor 3MC had an effect on covalent binding of 2-methylnaphthalene. In liver microsomes, PB increased formation of dihydrodiols, but it had no effect in the lung, whereas 3MC reduced formation of dihydrodiols in lung but not in liver microsomes. Piperonyl butoxide decreased covalent binding in the lung, liver and kidney, but did not affect lung toxicity. SKF-525A had also no effect on pulmonary necrosis. Diethyl maleate had no effect on binding in the liver, but it decreased binding in the lung and kidney. It also did not affect the severity of the pulmonary toxicity, but it enhanced the lethality of 2-methylnaphthalene. Pretreatment of mice with piperonyl butoxide or diethyl maleate did not affect the hepatic or pulmonary microsomal conversion of 2-methylnaphthalene into dihydrodiols. It was also shown that 2-methylnaphthalene strongly decreases liver GSH levels, but not GSH levels in the lung or kidney. The authors concluded that the pulmonary toxicity of 2-methylnaphthalene may be related to metabolism, but that the role of the dihydrodiols or covalent binding as indicators of activity of epoxide intermediates *in vivo* is as yet uncertain (Griffin et al., 1982).

#### ***Subgroup IVc: Diphenylmethane***

The biotransformation of diphenylmethane [FL-no: 01.036] was studied in microsomal fractions prepared from rat liver homogenate. It was determined that diphenylmethane is hydroxylated at both the aromatic (para-) and aliphatic positions of the molecule. If hydroxylation of the methylene group occurs, the resulting diphenylmethanol is further oxidised to diphenylketone (Sípál & Zelingerová, 1978).

Four male rats were administered 900 mg/kg bw of diphenylmethane via intraperitoneal injection. Animals were housed in individual metabolism cages and provided food and water *ad libitum*. Urine and faecal samples were collected after 24 hours and analysed immediately. The urine and faecal samples indicated that both free and conjugated forms of *p*-hydroxydiphenylmethane are eliminated with 88 % of the recovered material (in total *ca.* 6.8 % of the dose) being found in the urine (DeLoach et al., 1978). The major part of the urinary *p*-hydroxyphenylmethane was excreted as unconjugated material. In a subsequent experiment by the same investigators, six male rats were administered 500 mg/kg bw of diphenylmethane via intraperitoneal injection. Animals were housed in individual metabolism cages and provided food and water *ad libitum*. Urine samples were collected after 24 hours and analysed immediately. The urine samples indicated that conjugated benzhydrol (= diphenylmethanol) is eliminated by rats at 0.6 % of the total dose of diphenylmethane administered, free benzhydrol is not eliminated. The authors indicated that others have found that benzhydrol is excreted as glucuronide (Stocklinski et al., 1979).

#### ***Conclusions on the metabolism of subgroup IV substances***

No data are available for the two substances in subgroup IVa [FL-no: 01.031 and 01.058]. Based on information available on *p*-cymene, it may be anticipated that these substances may be oxidised at the ring-substituent methyl groups (resulting in the formation of hydroxymethyl groups). These products may subsequently be excreted after conjugation. Also epoxidation of the 3,4-double bond in [FL-no: 01.031] may be expected. Oxidative attack on the aromatic ring seems less likely. Nevertheless, due to the lack of experimental data it would be impossible to conclude that these substances are metabolised into innocuous products.

Several data are available on the biotransformation of the naphthalene [FL-no: 01.053] and its 2-methyl derivative [FL-no: 01.051] in subgroup IVb. With naphthalene, the primary products resulting from epoxidation can be further metabolised to naphthoquinones and other metabolites, which have been brought into connection with the toxicity of this substance. Whereas epoxidation is the main metabolic option for naphthalene, methyl group oxidation is the major metabolic pathway in the case of 2-methylnaphthalene. However, also with 2-methylnaphthalene, epoxidation may occur to some extent, and 2-methylnaphthalene-induced depletion of glutathione stores, possibly as a result of conjugation, has been suggested to be related to epoxidation reactions. However, although biotransformation does play a role in the toxicity of 2-methylnaphthalene, the exact mechanism of toxicity and the metabolites which are involved have not been clearly identified. Hence, neither for naphthalene [FL-no: 01.053] nor for 2-methylnaphthalene [FL-no: 01.051] can it be concluded that these are metabolised into innocuous substances.

For the one candidate substance in subgroup IVc (diphenylmethane [FL-no: 01.036]) only a very limited amount of metabolites have been identified in the urine. One of these is an aromatic hydroxylation product (i.e. a phenol derivative), which may be generated from epoxidation. Because of lack of further data it cannot be concluded that diphenylmethane [FL-no: 01.036] is metabolised to innocuous products.

### **Bi- and Tricyclic Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup V)**

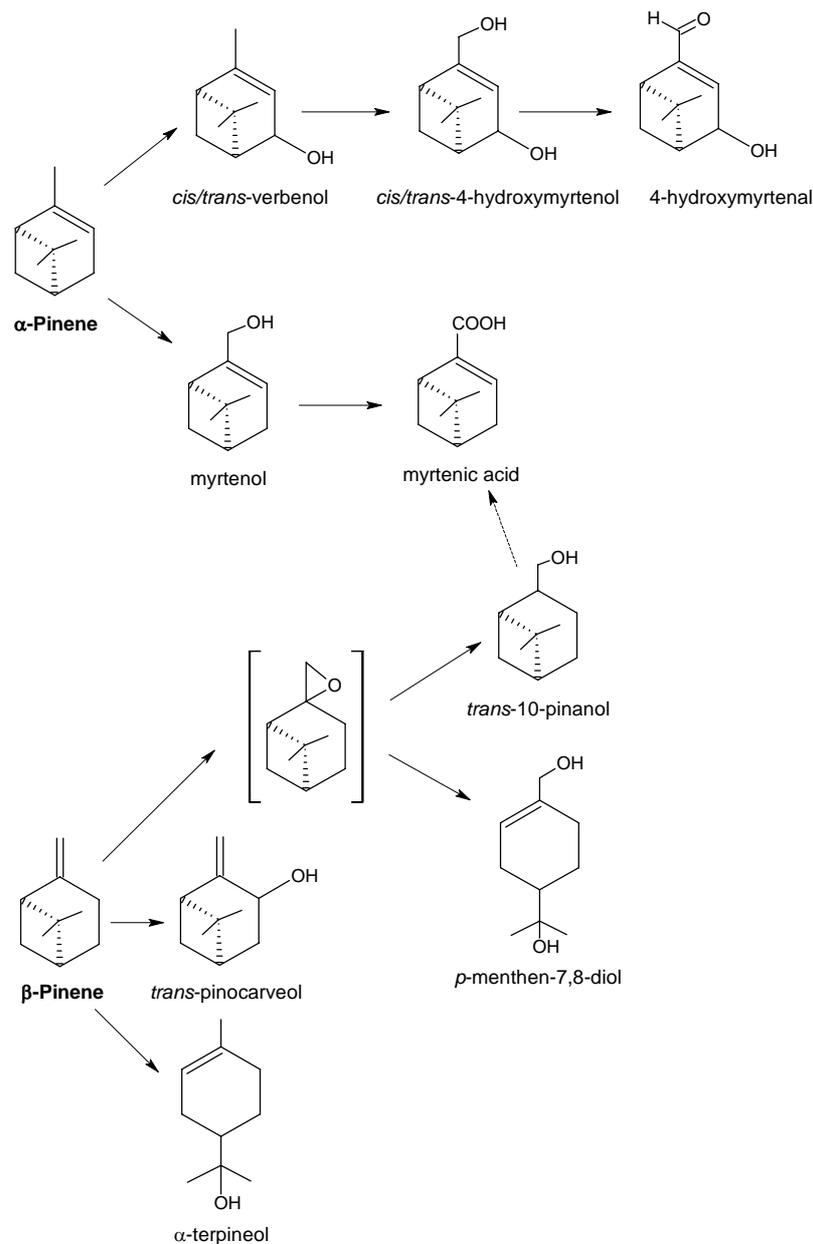
Analysis of urinary metabolites eliminated by human volunteers within four hours following a 2hours inhalation exposure to 10 - 450 mg (+)-alpha-pinene/m<sup>3</sup> [FL-no: 01.004] in a pharmacokinetic study (Falk *et al.*, 1990b) revealed *cis*- and *trans*-verbenol in a ratio of 1:10, with 3.8 and 1.7 % being eliminated at 10 and 450 mg/m<sup>3</sup>, respectively. Most of the verbenols were eliminated within 20 hours. In a more extensive metabolic study, urine was collected from sawmill workers at the end of an 8 - 9 hours work shift or from chamber-exposed individuals. Following hydrolysis of glucuronic acid conjugates, *cis*- and *trans*-verbenol were identified in the urine along with two diols, *cis*- and *trans*-4-hydroxymyrtanol, formed by methyl group hydroxylation of *cis*- and *trans*-verbenol. *trans*-4-Hydroxymyrtanol was also detected (see Figure III.5) (Eriksson and Levin, 1990).

Analysis of the urinary metabolites of a patient attempting suicide with 400 - 500 ml pine oil containing 57 % alpha-pinene showed the presence of myrtanol, verbenol and borneol. Renal excretion reached a peak level five days after ingestion (Koppel *et al.*, 1981).

Male albino rabbits (six/group) administered single gavage doses of 400 - 700 mg/kg bw of (+)-alpha-pinene, (-)-alpha-pinene, (+/-)-alpha-pinene, (-)-beta-pinene or delta-3-carene, excreted bicyclic terpene hydrocarbon metabolites as (glucuronic acid) conjugates or as further oxidised metabolites, notably carboxylic acids. Animals were housed individually and urine was collected daily for three days.

The principal neutral metabolite formed by oxidation at the C4 position in the alicyclic ring of each of the three alpha stereochemical forms of pinene was *trans*-verbenol (see Figure III.5). As a minor pathway, allylic oxidation of the exocyclic methyl group to yield myrtanol was observed for all three alpha-pinene stereoisomers, with also myrtanic acid as minor metabolite (Ishida *et al.*, 1981).

The presence of an exocyclic alkene function in (-)-beta-pinene provided additional metabolic options, and four neutral and one acidic metabolites were identified. Allylic oxidation of the methyl group at the C<sub>2</sub> position yields (+)-*trans*-pinocarveol, while epoxidation of the exocyclic alkene followed by hydration or rearrangement yields (-)-*trans*-10-pinanol and (-)-1-*p*-menthene-7,8-diol, respectively. Ring cleavage yields (-)-alpha-terpineol. These metabolites comprised 11, 39, 30 or 5 % of the total urinary neutral metabolite fraction, respectively. The acidic metabolite identified was identical to the one identified for the alpha-pinenes (i.e. myrtanic acid), which was suggested to be formed via double bond epoxidation and subsequent rearrangement to give myrtanol and further oxidation to the carboxylic acid (Ishida *et al.*, 1981).



**Figure III.5.** Metabolism of  $\alpha$ -pinene and  $\beta$ -pinene in animals.

*Trans*-verbenol and myrtenic acid have also been found in faeces or urine of brushtail possum fed alpha-pinene. When the same species was fed beta-pinene, only myrtenic acid was found in the excreta (Southwell et al., 1980).

It has been stated in a limited review paper that in rabbits, alpha- and beta-pinenes can be excreted as glucuronide conjugates of undetermined nature, which can release cymene upon heating in diluted acids (Williams, 1959 as cited in (Opdyke, 1978c)).

Delta-3-Carene [FL-no: 01.029] undergoes stereoselective hydroxylation at the *gem*-methyl group (yielding 3-carene-9-ol) followed by carboxylation, allylic oxidation of the C10 methyl group followed by carboxylation or, as the main route, allylic ring opening and hydroxylation at a secondary carbon atom, yielding (-)-*m*-mentha-4,6-dien-8-ol (72 % of the total urinary neutral metabolite fraction) and *m*-cymen-8-ol (Ishida et al., 1981).

In addition to the terpenoids pinene and carene, the group of Ishida has also studied the metabolism of the saturated analogues pinane and carane<sup>11</sup> (Ishida *et al.*, 1981). Only a relatively small part of the dose (in total 18 g given to six rabbits) was identified, among which were 3- and 4-pinalol, alpha-terpineol, trans-sobrerol, trans-carveol, and verbenol (some of the structures have been shown in Figure III.5). In short, these products result from hydroxylations of secondary carbon atoms in the 6-membered ring, or cleavage of the 4-membered ring in combination with hydroxylation in the remaining 6-membered ring. In addition, desaturation of the 6-membered ring was also observed and hence, some of the metabolites may be considered as cyclohexene derivatives. No hydroxylation of the primary carbon atom (i.e. the methyl ring substituent) in pinane was observed. With carane, however, the hydroxylation of one of the *gem*-methyl groups and hydroxylation of the C10 exocyclic carbon atom was observed, ultimately resulting in the formation of carane-9,10-dicarboxylic acid.

In rabbits, beta-caryophyllene [FL-no: 01.007] undergoes epoxidation of the endocyclic 5,6-double bond to yield a stable epoxide metabolite and hydroxylation at the *gem*-dimethyl group. The resulting metabolite 14-hydroxycaryophyllene-5,6-epoxide and its C14-acetylated conjugate could be detected in the urine. A second epoxidation of the 5,6-epoxide's exocyclic 2,12-double bond, ultimately resulting in the 14-hydroxycaryophyllene-5,6-epoxide-2,12-diol, was also reported (Asakawa *et al.*, 1981; Asakawa *et al.*, 1986).

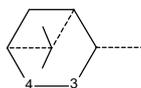
Data were also found on the metabolism of camphene<sup>12</sup>. Ishida *et al.* (1979) administered *dl*-camphene [FL-no: 01.009] to five starved male rabbits at a dose level of 800 mg/kg bw via stomach tube. In total 8 g of the substance was given. Urinary metabolites, collected over three days post-dosing were examined. The following metabolites were identified in urine samples after treatment with beta-glucuronidase/sulphatase: camphene-2,10-glycol (after epoxidation of the double bond), 6-*exo*-hydroxycamphene, 7-hydroxycamphene, 10-hydroxytricyclene and 3-hydroxytricyclene. Quantitative information was provided only for the diol which was found in a total amount of 260 mg, which corresponds to *ca.* 3 % of the dose (Ishida *et al.*, 1979a).

In a human volunteer and in a young pig, camphene was eliminated from the body by exhalation and via bile as unchanged substance or as glucuronide conjugate via the urine (Opdyke, 1975).

(+)-Longifolene [FL-no: 01.047] metabolism was studied following the oral administration of about 2 g/animal as a suspension in 0.02 % Tween80 aqueous solution to six male rabbits. Urine samples were collected daily for three days and treated with beta-glucuronidase / sulphatase. Due to imprecise description of dose and amounts recovered, it is not possible to indicate the extent of metabolism and excretion as percentage of the dose. Deconjugated metabolites were extracted in a neutral and an acidic fraction. In the neutral fraction (10 % of the dose), many peaks were observed but only one was further characterised to be (2S, 7S)-(+)-14-hydroxyisolongifolaldehyde (35 % of the neutral metabolite fraction). It was concluded that (+)-longifolene is metabolised at two sites in two subsequent steps: 1) oxidation of the *exo*-methylene group to form its epoxide with subsequent isomerisation to form a stable aldehyde, and 2) hydroxylation of the *gem*-dimethyl group to form a primary alcohol (Ishida *et al.*, 1982; Asakawa *et al.*, 1986; Ford *et al.*, 1992b).

<sup>11</sup>

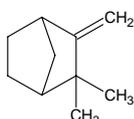
Pinane:



Carane:



<sup>12</sup> camphene: 3,3-dimethyl-2-methylenenorbornane



An *in vitro* study with rat liver microsomes demonstrated the involvement of cytochrome P450 enzymes in the metabolism of alpha-pinene. Metabolites present were beta-pinene and *d*-limonene together with smaller amounts of *trans*-verbenol, myrtenol, verbenone, and pinene oxide (White & Agrosin, 1980).

alpha-Pinene and cadinene, have been shown to induce cytochrome P450 enzymes, especially those from the CYP2B subfamily, and to a lesser extent also CYP3A2 (cadinene) and CYP4A2 (alpha-pinene) (Austin et al., 1988; Hiroi et al., 1995). Based on similarity with beta-pinene it may be speculated that the candidate substance 4(10)-thujene [FL-no: 01.059] may be hydroxylated to thujyl alcohol, which is known to be conjugated with glucuronic acid and eliminated via urine (Hämäläinen, 1912; EFSA, 2009ah). However, based on the same similarity, epoxidation of the exocyclic double bond may also be expected, and it is not clear what other reactions might occur. No indications of the relevance of the various routes are available.

#### *Conclusions on the metabolism of subgroup V substances*

Metabolism data for subgroup V are available for some supporting substances (pinenes, camphene, caryophyllene, delta-3-carene, pinane and carane) and the candidate substance longifolene [Fl no: 01.047]. In general, the metabolic options for these substances include oxidation of methyl ring substituent groups to give the corresponding alcohol and further oxidation products. For the substances studied, double bond epoxidation has also been demonstrated. In addition, ring cleavage has also been observed, e.g. for beta-pinene, resulting in the formation of monocyclic terpenoid derivatives like alpha-terpineol. Hydroxylated metabolites (i.e. alcohols) or further metabolic products may be eliminated as conjugates e.g. with glucuronic acid. However, given the diversity of this group, the lack of data on any of the candidate substances, except longifolene for which the mass balance is incomplete, it cannot be concluded that the candidate substances in this subgroup [FL-no: 01.021, 01.022, 01.023, 01.030, 01.044, 01.047, 01.052, 01.056, 01.059, 01.060, 01.066 and 01.067] can be metabolised to innocuous products.

#### **Macrocyclic, Non-Aromatic Hydrocarbons (Candidate and Structurally Related Supporting Substances from Subgroup VI)**

No data are available for the two substances in subgroup VI [FL-no: 01.042 and 01.043] or for any supporting substance. Due to the lack of experimental data it cannot be concluded that these substances are metabolised to innocuous products.

### **III.5. Summary on Absorption, Distribution, Metabolism and Excretion**

Given the diverse nature of the chemical structures of the candidate flavouring substances in this FGE, it is impossible to draw conclusions which are applicable to all substances. The amount of information on kinetics of either candidate or supporting substances is very limited. For these reasons, the available information is presented here in a subgroup-wise manner.

#### **III.5.1. Absorption, Distribution and Elimination**

##### *Subgroup I*

From the available studies it may be concluded that the candidate alkane substances in subgroup 1 will be absorbed after oral exposure. The extent of absorption is not known, but may be high, given their lipophilic character and their low molecular weight. The ease with which these substances cross the membranes of the respiratory tract further supports the assumption that these substances will also be absorbed after oral intake. Data indicate that straight-chain alkanes will predominantly be broken down to carbon dioxide at low dose levels. At high dose levels, biotransformation becomes saturated and other metabolites will be generated which will be excreted via the urine.

##### *Subgroup II*

For the candidate and supporting substances in subgroup II, the acyclic alkenes, data were only available for beta-myrcene [FL-no: 01.008] and 2-methylbuta-1,3-diene (a structurally related substance). Given the narrow range of molecular weights of the candidate substances (between ~ 68 and 206 D) and their lipophilic nature (estimated or measured Log  $K_{ow}$  values e.g. 2.4 (2-methylbuta-1,3-diene), 6.1 (dodec-1-ene), 4.17 (beta-myrcene) or 4.8 (cis-3,7-dimethyl-1,3,6-octatriene)), it is assumed that these candidate flavouring substances may be absorbed from the gastrointestinal tract. Mass balance data for beta-myrcene are incomplete. For 2-methylbuta-1,3-diene a mass balance of elimination has been presented, which also shows elimination via the exhaled air (~ 50 % of the dose), but as this substance was administered via the intraperitoneal route, the elimination pattern may be different after oral dosing with a lower fraction of the dose exhaled, e.g. because of more efficient first-pass biotransformation after oral dosing.

### *Subgroup III*

For the candidate and supporting substances in subgroup III (cyclohexene derivatives), data were only available for *d*-limonene [FL-no: 01.045]. For this substance, data show a considerable absorption from the gastrointestinal tract. In humans, elimination of the substance followed a triphasic pattern, but still 55 - 83 % of an oral dose could be found in the urine within 72 hours post dosing, with the major part excreted within 24 hours. Also in other species urinary elimination was most important accounting for up to 82 - 96 % of the dose within 72 hours. Hence, it may be anticipated that monocyclic cyclohexene derivatives, such as *d*-limonene, administered orally, are absorbed and distributed throughout the body. Following oral administration to humans, *d*-limonene was distributed preferentially to fatty tissues, as indicated by a high oil-blood partition coefficient and a long half-life during the slow elimination phase. Because of the limited molecular weight of the candidate substances in this group (range ~ 136 - 204 D) and their lipophilic character (e.g. estimated or measured log  $K_{ow}$  values for beta-phellandrene, beta-bisabolene and *d*-limonene are approximately 4.7, 7.1 or 4.6, respectively) it may be assumed that all of the substances in this group may be absorbed, although the extent of absorption for individual substances cannot be accurately estimated from these physico-chemical properties.

### *Subgroup IV*

No data are available on the absorption, distribution and elimination of any candidate or supporting substance in subgroup IVa (benzene hydrocarbons). But there are several data available on the candidate substances in subgroup IVb (naphthalene hydrocarbons). The data show that naphthalene [FL-no: 01.053] will be absorbed after oral administration and will be eliminated predominantly via the urine, in the form of various metabolites, conjugated with e.g. glucuronic acid or with glutathione. In bile duct cannulated animals, considerable elimination into the bile was also observed, but as in intact animals urinary elimination exceeds by far faecal excretion, the material eliminated via the bile is reabsorbed. The glucuronide conjugate of 1-naphthol represents a significant urinary metabolite of naphthalene. Glutathione conjugates of naphthalene are catabolized to (pre)mercapturic and mercapturic acids, particularly in rodents, before excretion in bile or urine. In primates and humans, conjugation with glutathione seems to be less important. Less data are available for 2-methylnaphthalene [FL-no: 01.051], but the available data show that 2-methylnaphthalene is also absorbed from the gastrointestinal tract and eliminated predominantly via the urine. For both substances the data indicate that 85 - 89 % of the dose is excreted within three days after dosing. With respect to the one candidate substance (diphenylmethane [FL-no: 01.036]) in subgroup IVc, it may be concluded that the metabolites of this substance will be eliminated from the body after intraperitoneal injection. No conclusions can be made on absorption from the gastrointestinal tract and the rate of elimination as no oral ADME studies and mass-balance data are available.

### *Subgroup V*

For the substances in subgroup V (bi- and tricyclic, non-aromatic hydrocarbons), the available data from oral studies are incomplete. These studies have only addressed the excretion of some supporting and one candidate substance (longifolene) in the form of metabolites via the urine. No mass-balance data were provided. So, these studies only show that these substances will be absorbed to some extent. The same may be anticipated for the other candidate substances in this subgroup. For the supporting substances alpha- and

beta-pinene and delta-carene information on kinetics is available from humans exposed via inhalation, in occupational settings. These studies show that these substances can be absorbed after inhalation exposure and that metabolites will be excreted into the urine e.g. as glucuronide conjugates. The elimination follows a triphasic pattern with rather long terminal half-lives and the absorbed amount will be eliminated within several days. Based on the lipophilic character of these substances it may be anticipated that they will preferentially distribute in the adipose tissues, which is supported by the slow terminal elimination rates.

#### *Subgroup VI*

No data were available on candidate or supporting substances in subgroup VI (macrocyclic, non-aromatic hydrocarbons). The two substances in this group have a molecular weight of ~ 204. They are also rather lipophilic (estimated log  $K_{ow}$  are 4.6 and 7.0 for [FL-no: 01.042] and [FL-no: 01.043], respectively). Based on these data it may be assumed that these two candidate chemicals will be absorbed from the gastrointestinal tract. For humulatriene, given the rather high  $K_{ow}$ , the extent of absorption may be relatively low.

### **III.5.2. Metabolism**

#### *Subgroup I*

The substances in subgroup I can be expected to be metabolised through omega-oxidation, which will lead to the formation of alcohols, and after subsequent further oxidation to carboxylic acids, which may be further oxidised via beta-oxidation to yield carbon dioxide. Extensive mass balance data are not available, but in some of the available studies excretion of carboxylic acids and carbon dioxide has been reported. Formation of keto-acids has also been reported, and these may be thought to be formed from omega-oxidation at one end of the molecule and omega-1 or -2 oxidation at the other end. More interest has been put in the identification of metabolites, which did not undergo substantial chain shortening. These metabolites will arise from oxidation of the non-terminal carbon atoms, e.g. via omega-1, -2, -3 or even -4 oxidation. The resulting secondary alcohols can be conjugated with e.g. glucuronic acid and excreted via the urine, or can be further oxidised to yield ketones. A subsequent introduction of another secondary hydroxyl group may result in the formation of diketones. With n-hexane, the resulting 2,5-hexanedione (a gamma-diketone) has been demonstrated to be responsible for hexane-induced neurotoxicity. Neurotoxicity is a common feature of gamma-diketones, and for that reason, candidate substance 3-methylhexane [FL-no: 01.050] cannot be anticipated to be metabolised into innocuous compounds. In addition, it is known that methyl-branching of the carbon chain potentiates the neurotoxicity of the gamma-diketone (Topping et al., 1994; EFSA, 2004e). The two other hexane derivatives in this group [FL-no: 01.033 and 01.034] cannot be oxidised to give a gamma-diketone, due to the presence of methyl groups on the C2 or C5 carbon atoms. Therefore, these substances may be considered as being metabolised to innocuous metabolites. For the longer chain alkanes in this group it would require oxidation of the more central carbon atoms in order to be converted into gamma-diketones. Such oxidations are less favourable than omega, omega-1 or -2 oxidations, and therefore it is concluded that oxidation of these higher alkanes will not result in toxicologically relevant levels of gamma-diketones, also because of rapid conjugation of the precursor alcohols. For heptane it was shown that this substance does not result in neurotoxicity (Bahima et al., 1984), although the gamma-diketone itself (2,5-heptanedione) is known to be neurotoxic (Topping et al., 1994). The remaining three candidate flavouring substances in this group (i.e. [FL-no: 01.038, 01.054 and 01.057]) can be expected to be metabolised to innocuous products.

#### *Subgroup II*

The data available on the metabolism of one supporting and one structurally related substance in subgroup II show that metabolic options for the chemicals in this group are epoxidation of double bonds, ultimately resulting in diols, which can be further conjugated. With the supporting substance beta-myrcene, further metabolism of the diols into carboxylic acids has also been reported. Both in rats and rabbits, the principal urinary metabolite following gavage administration of beta-myrcene is myrcene-3,10-glycol, formed from

the hydration of the epoxide intermediate in both species. Epoxidation of the 3,10-double bond was favoured over epoxidation of the 1,2-double bond.

The studies indicate that the formation of diols from the myrcene-epoxides is very efficient. It is noted, however, that the diols and the related carboxylic acids are all the result of epoxidation of double bonds in which one of the carbon atoms has only hydrogen substituents, but no further carbon chains. In this respect, the candidate substance *cis*-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] is most similar to beta-myrcene. With beta-myrcene, no diepoxide metabolites or further reaction products thereof have been reported, but it is noted that mass balance data are highly incomplete, so some metabolites may have been overlooked.

With the structurally related substance, 2-methylbuta-1,3-diene, epoxidation of the 1,2-double bond is favoured over epoxidation of the 3,4-double bond. While the 1,2-epoxide is readily hydrolysed, the 3,4-epoxide is far more stable. Both metabolites can be converted to the corresponding diols and at least one hydroxy-carboxylic acid (2-hydroxy-2-methyl-3-butenoic acid) has been identified. In addition, in particular the 3,4-epoxide metabolite can be oxidised for a second time, resulting in the formation of a diepoxide metabolite, which is known to be reactive and has been suggested to be responsible for 2-methylbuta-1,3-diene-induced DNA damage. For candidate substance dodec-1-ene [FL-no: 01.037], diol- and alpha-hydroxy-carboxylic acid formation may also be expected. In addition this substance may also undergo omega and omega-1 or -2 oxidation at the other (saturated) end of the carbon chain (similar to the candidate substances in subgroup I).

Apart from beta-myrcene and 2-methylbuta-1,3-diene, terminal double bonds appear in candidate substances [FL-no: 01.037, 01.064 and 01.070]. In contrast to corresponding substances in FGE.07 and FGE.18 there are no other functional groups in the molecule (e.g. hydroxyl- or keto-group) that could provide a more direct option for detoxication e.g. via conjugation with glucuronide or sulphate. In the two FGEs mentioned above, the presence of such groups was an argument to conclude that the metabolism of the particular candidate substances would give rise to innocuous products. It would not be possible to conclude similarly for the candidate substances in FGE.25. The other candidate substances in this subgroup (II) might also be oxidised in the various methyl groups but no data are available to substantiate this. Because of these considerations, it cannot be concluded that the candidate substances in this subgroup [FL-no: 01.032, 01.035, 01.037, 01.064, 01.070 and 01.078] will be metabolised to innocuous products.

### Subgroup III

Hardly any data are available on the metabolism of the candidate substances in subgroup III. Only for *l*-limonene a very limited amount of data was available on biotransformation in incubations with rat liver microsomes. In comparison with the structures of the candidate substances in subgroup III, the structure of the one supporting chemical *d*-limonene is comparatively simple. Major differences between *d*-limonene and the candidate substances are the length of the ring substituents and the number of double bonds. In addition, based on the data available for one of the candidate substances, beta-phellandrene [FL-no: 01.055], with a double bond directly on the ring and in conjugation with double bond in the cyclohexene ring, it cannot be anticipated that it can be metabolised to innocuous products.

Allylic oxidation is by far the major pathway for metabolism of limonene in humans. Minor pathways in limonene metabolism reported for the rat include epoxidation of either the 1,2- or the 8,9-double bond and subsequent hydrolysis to the diol. Given the many metabolic options for this substance, a myriad of metabolites has been found, including conjugates, and for a change mass balance data are available, which show that in various animal species the substance is completely eliminated within three days, predominantly via the urine. No genotoxicity of limonene epoxides could be detected, and it may well be concluded that *d*-limonene is metabolised to innocuous substances. However, although it is very likely that the candidate substances may undergo the same metabolic conversions (allylic oxidations and double bond epoxidation) the conclusion for *d*-limonene cannot be extrapolated to all of the candidate substances because of the structural dissimilarities and the absence of any further data on molecules with closer resemblance to the candidate substances. The data available for *l*-limonene do not indicate that major differences are to be anticipated between the metabolism of *d*-limonene and *l*-limonene. For bisabola-1,8,12-triene [FL-no:

01.027], beta-bisabolene [FL-no: 01.028] and delta-elemene [FL-no: 01.039] it may be concluded that these are metabolised to innocuous products.

It is noted that *d*-limonene (metabolites) causes  $\alpha$ -2 $\mu$ -globulin accumulation in male kidneys, an event known to be associated with male rat specific nephropathy<sup>13</sup>, and irrelevant for human toxicological risk assessment.

#### *Subgroup IV*

No data are available for the two substances in subgroup IVa [FL-no: 01.031 and 01.058]. Based on information available on *p*-cymene, it may be anticipated that these substances may be oxidised at the ring-substituent methyl groups (resulting in the formation of hydroxymethyl groups). These metabolites may subsequently be excreted after conjugation. Also epoxidation of the 3,4-double bond in [FL-no: 01.031] may be expected. Oxidative attack on the aromatic ring seems less likely. Due to the lack of experimental data it cannot be concluded that these substances are metabolised into innocuous products.

Data are available on the biotransformation of naphthalene [FL-no: 01.053] and its 2-methyl derivative [FL-no: 01.051] in subgroup IVb. With naphthalene, the primary products resulting from epoxidation can be further metabolised to naphthoquinones and other metabolites, which have been brought into connection with the toxicity of this substance. Whereas epoxidation is the main metabolic option for naphthalene, methyl group oxidation is the major metabolic pathway in the case of 2-methylnaphthalene. However, also with 2-methylnaphthalene, epoxidation may occur to some extent, and 2-methylnaphthalene-induced depletion of glutathione stores, possibly as a result of conjugation, has been suggested to be related to epoxidation reactions. However, although biotransformation does play a role in the toxicity of 2-methylnaphthalene, the exact mechanism of toxicity and the metabolites which are involved have not been clearly identified. Hence, neither for naphthalene [FL-no: 01.053] nor for 2-methylnaphthalene [FL-no: 01.051] can it be concluded that they are metabolised into innocuous substances.

For the one candidate substance in subgroup IVc (diphenylmethane [FL-no: 01.036]) only a very limited amount of metabolites have been identified in the urine. One of these is an aromatic hydroxylation product, which may be generated from epoxidation. Because of lack of further data it cannot be concluded that diphenylmethane [FL-no: 01.036] is metabolised to innocuous products.

#### *Subgroup V*

Metabolism data for subgroup V are available for the supporting substances pinenes, camphene, caryophyllene, delta-3-carene, pinane and carane and the candidate substance longifolene [FL-no: 01.047]. In general, the metabolic options for these substances include oxidation of methyl ring substituent groups to give the corresponding alcohol and further oxidation products. For the substances studied, double bond epoxidation has also been demonstrated. In addition, ring cleavage has also been observed, e.g. for beta-pinene resulting in the formation of monocyclic terpenoid derivatives like alpha-terpineol. Hydroxylated metabolites or further metabolised products may be eliminated as conjugates e.g. with glucuronic acid. However, given the diversity of this group, the lack of data on any of the candidate substances, except longifolene for which the mass balance is incomplete, it cannot be concluded that the candidate substances in this subgroup [FL-no: 01.021, 01.022, 01.023, 01.030, 01.044, 01.047, 01.052, 01.056, 01.059, 01.060, 01.066 and 01.067] can be metabolised to innocuous products.

Based on similarity with beta-pinene it may be speculated that the candidate substance 4(10)-thujene [FL-no: 01.059] may be hydroxylated to thujyl alcohol, which is known to be conjugated with glucuronic acid and eliminated via urine (Hämäläinen, 1912; EFSA, 2009ah). However, based on the same similarity, epoxidation of the exocyclic double bond may also be expected, and it is not clear what other reactions might occur. No indications of the relevance of these various routes are available. Hence, despite the knowledge of

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<sup>13</sup> For introduction into this area see e.g. the referred paper by Lehman-McKeeman *et al.* (1989)

the fate of thujyl alcohol, also for thujene it cannot be concluded that it will be metabolised to innocuous products.

#### *Subgroup VI*

No data are available for the two candidate substances in subgroup VI [FL-no: 01.042 and 01.043] or for any supporting substance. Due to the lack of experimental data it cannot be concluded that these substances are metabolised to innocuous products.

### **III.1 Overall Conclusion on Absorption, Distribution, Metabolism and Elimination.**

Generally, the available data indicate that the aliphatic and aromatic hydrocarbons participate in similar pathways of metabolic detoxication. Being lipophilic and of low molecular weight, these hydrocarbons may be assumed to be absorbed by the gastrointestinal tract. Subsequently, they are oxidised to more polar oxygenated metabolites e.g. by CYP-450 enzymes. The phase I metabolites are then conjugated and excreted, mainly in the urine. The candidate substances and supporting substances are oxidised either by side chain oxidation or epoxidation of the exocyclic or endocyclic double bond. Oxidation initially yields hydroxylated metabolites that may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites that are also excreted. If a double bond is present, epoxide metabolites may form that are further metabolised either by hydrolysis to yield diols or by conjugation with glutathione to yield mercapturic acid derivatives. The aromatic hydrocarbons that do not contain alkyl substituents may undergo ring hydroxylation to form phenolic metabolites that are subsequently conjugated with sulphate or glucuronic acid and excreted in the urine. The saturated alkanes in this group may be metabolised via omega and omega-1, -2, -3 or -4 oxidation. Whereas omega oxidation would ultimately lead to the formation of carboxylic acids, the other oxidations would give rise to secondary alcohols and ketones. The carboxylic acids may be expected to participate in the endogenous fatty acid metabolism. However, for most of the subgroups the information was incomplete and the similarity between supporting and candidate substances was limited. In addition, proper mass balance data were not available. Some mass balance data available indicated slow elimination. For several subgroups no data were available at all. In Table III.2 the final conclusions for each of the candidate substances has been presented, together with a brief explanatory statement, about the conclusion reached. It is noted that the subgroup III supporting substance *d*-limonene causes  $\alpha$ -2 $\mu$ -globulin accumulation in male kidneys, an event known to be associated with male rat specific nephropathy, and irrelevant for human toxicological risk assessment.

**Table III.2 Can innocuous metabolites be expected to be formed based on available data?**

FL-no:	Substance name	Innocuous metabolites?
<b>Subgroup I: ACYCLIC ALKANES</b>		
01.033	2,2-Dimethylhexane	Yes
01.034	2,4-Dimethylhexane	Yes
01.038	Dodecane	Yes
01.050	3-Methylhexane	No (potential formation of neurotoxic gamma-diketone)
01.054	Pentadecane	Yes
01.057	Tetradecane	Yes
<b>Subgroup II: ACYCLIC ALKENES</b>		
01.032	2,3-Dihydrofarnesene	No (lack of supporting data)
01.035	2,6-Dimethylocta-2,4,6-triene	No (lack of supporting data)
01.037	Dodec-1-ene	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)
Deleted from the Register	2-Methylbuta-1,3-diene	No (known biotransformation to reactive metabolite responsible for toxicity and genotoxicity)
01.064	cis-3,7-Dimethyl-1,3,6-octatriene	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)
01.070	1-Octene	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)
01.078	2,4-Nonadiene	No (lack of supporting data)
<b>Subgroup III: CYCLOHEXENE HYDROCARBONS</b>		
01.055	beta-Phellandrene	No (lack of supporting data)
01.027	Bisabola-1,8,12-triene	Yes
01.028	beta-Bisabolene	Yes
01.039	delta-Elemene	Yes
01.001	Limonene	Yes
01.046	l-Limonene	Yes
<b>Subgroup IV: AROMATIC HYDROCARBONS</b>		
<b>Subgroup IVa: BENZENE HYDROCARBONS</b>		
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene	No (lack of supporting data)
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene	No (lack of supporting dat)
<b>Subgroup IVb: NAPHTHALENE HYDROCARBONS</b>		
01.051	2-Methylnaphthalene	No (known metabolism to toxic metabolites)
01.053	Naphthalene	No (known metabolism to toxic metabolites)
<b>Subgroup IVc: DIPHENYLMETHANE</b>		
01.036	Diphenylmethane	No (lack of supporting data)
<b>Subgroup V: BI- and TRICYCLIC, NON-AROMATIC HYDROCARBONS</b>		
01.021	Delta-Cadinene	No (lack of supporting data)
01.022	alpha-Cedrene	No (lack of supporting data)
01.023	1(5),11-Guaiadiene	No (lack of supporting data)
01.030	beta-Cubebene	No (lack of supporting data)

**Table III.2 Can innocuous metabolites be expected to be formed based on available data?**

FL-no:	Substance name	Innocuous metabolites?
01.044	Isolongifolene	No (lack of supporting data)
01.047	Longifolene	No (lack of supporting data)
01.052	alpha-Muurolene	No (lack of supporting data)
01.056	alpha-Santalene	No (lack of supporting data)
01.059	4(10)-Thujene	No (lack of supporting data)
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane	No (lack of supporting data)
01.066	2-Cedrene	No (lack of supporting data)
01.067	8(14)-Cedrene	No (lack of supporting data)
<b>Subgroup VI: MACROCYCLIC, NON-AROMATIC HYDROCARBONS</b>		
01.042	Germacre-1(10),4(14),5-triene	No (lack of supporting data)
01.043	3,7,10-Humulatriene	No (lack of supporting data)

## ANNEX IV: TOXICITY

Oral acute toxicity data are available for seven candidate substances of the present Flavouring Group Evaluation, and for 16 supporting substances evaluated by the JECFA at the 63<sup>rd</sup> meeting and one structurally related substance (1-methyl-1,3-cyclohexadiene [FL-no: 01.077]). The supporting substances are listed in brackets.

**Table IV.1: ACUTE TOXICITY**

Chemical Name [FL-no]	Species	Sex	Route	LD <sub>50</sub> (mg/kg bw)	Reference	Comments
Tetradecane [01.057]	Rat	NR	Oral	> 5000	(Enichem Augusta Ind., 1987)	Study not available
	Rat	NR	Oral	> 5000	(PETRESA, 19??b)	Study not available
(Undeca-1,3,5-triene [01.061])	Rat	M, F	Oral	> 8000	(Pellmont, 1973c)	Data not possible to interpret
	Mouse	M, F	Oral	2000 - 4000	(Pellmont, 1973c)	Data not possible to interpret
Dodec-1-ene [01.037]	Rat	NR	Oral	> 3200	(Jones, 1969)	No information given on study design
	Rat	NR	Oral	> 1000	(Ethyl Corporation, 1973)	Study not available
(Myrcene [01.008])	Rat	M	Oral	> 5000	(Moreno, 1972j)	Identity of compound not stated as other than code number
(beta-Ocimene [01.018])	Rat	NR	Oral	5000	(Moreno, 1976z)	One dose tested
(d-Limonene [01.045])	Rat	M	Oral	> 5000	(Moreno, 1972m)	Identity of compound not stated as other than code number
	Rat	M, F	Oral	M: 4400 F: 5100	(Tsuji et al., 1975a)	Study is on dogs, rats not mentioned in English text. Study in Japanese
	Mouse	M, F	Oral	M: 5600 F: 6600	(Tsuji et al., 1975a)	Study is on dogs, mice not mentioned in English text. Study in Japanese
(Terpinolene [01.005])	Rat	NR	Oral	4.39 ml/kg (3784)	(Levenstein, 1975o)	Observation period not given.
(alpha-Terpinene [01.019])	Rat	NR	Oral	1680	(Moreno, 1973ah)	
(gamma-Terpinene [01.020])	Rat	M, F	Oral	3650	(Moreno, 1973ag)	
(alpha-Phellandrene [01.006])	Rat	M, F	Oral	> 5700	(Moreno, 1972k)	Submitted study is on dermal toxicity.
	Rat	M, F	Oral	1.87 ml/kg (1590)	(Brownlee, 1940)	Substance mixed with acacia. 6 animals per dose group
beta-Bisabolene [01.028]	Rat	NR	Oral	> 5000	(Moreno, 1974l)	One dose tested
	Mouse	M, F	Oral	> 13,360	(Hoffman-LaRoche, Inc., 1967b)	No of dose levels not given.
(delta-3-Carene [01.029])	Rat	M	Oral	4800	(Moreno, 1972l)	Identity of compound not stated
(Pin-2(3)-ene [01.004])	Rat	M, F	Oral	3700	(Moreno, 1972n)	Identity of compound not stated
(Pin-2(10)-ene [01.003])	Rat	NR	Oral	> 5000	(Moreno, 1975q)	One dose tested.
(Camphene [01.009])	Rat	NR	Oral	> 5000	(Moreno, 1974m)	One dose tested
(Valencene [01.017])	Rat	M	Oral	> 5000	(Moreno, 1980m)	One dose tested
(beta-Caryophyllene [01.007])	Rat	M, F	Oral	> 5000	(Hart and Wong, 1971)	One dose tested
(1(5),7(11)-Guaiaidiene [01.026])	Rat	NR	Oral	> 5000	(Moreno, 1976aa)	One dose tested
Longifolene [01.047]	Rat	NR	Oral	> 5000	(Moreno, 1977ai)	One dose tested
Diphenylmethane [01.036]	Rat	NR	Oral	2250	(Moreno, 1973ai)	
Naphthalene [01.053]	Rat	NR	Oral	> 2000	(Hazleton France, 1990a; Hazleton France, 1990b)	Study not available
	Rat	NR	Oral	> 2000	(Vaughn & Keeler, 1976)	No information on study design
	Rat	M, F	Oral	2400	(Birch, 1978d)	5 animals per dose group

**Table IV.1: ACUTE TOXICITY**

Chemical Name [FL-no]	Species	Sex	Route	LD <sub>50</sub> (mg/kg bw)	Reference	Comments
	Rat	M, F	Oral	2800	(Birch, 1978b)	5 animals per dose group
	Rat	M, F	Oral	1900 <sup>4</sup>	(Birch, 1978c)	5 animals per dose group
	Rat	NR	Oral	2000	(Hazleton France, 1990c)	Study not available
	Rat	F	Gavage	9430	(Carpenter et al., 1949)	
	Mouse	M, F	Gavage	M: 533 F: 710	(Shopp et al., 1984)	
(1-Methylnaphthalene [01.014])	Rat	NR	Oral	1840	(Izmerov et al., 1982b)	Study not available
2-Methylnaphthalene [01.051]	Rat	NR	Oral	1630	(Izmerov et al., 1982b)	Study not available
(1-methyl-1,3-cyclohexadiene [01.077])	Rat	M, F	Oral	> 2000	(Felice, 2005)	Acute toxic class method. 2000 mg/kg is only dose tested, no death was observed.

<sup>1</sup> Administered as a 25 % solution in olive oil.

<sup>2</sup> Dowtherm A, which was a mixture of biphenyl (26 %) and diphenyl oxide (72 %), was tested.

<sup>3</sup> Administered as a 20 % solution in corn oil.

<sup>4</sup> Administered as a 10 % solution in corn oil.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for two candidate substances of the present Flavouring Group Evaluation and for six supporting substances evaluated by the JECFA at the 63<sup>rd</sup> meeting. The supporting substances are listed in brackets.

**Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies**

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Undeca-1,3,5-triene [01.061])	Rat; M, F 10	Diet	Calculated to provide 10 mg Galbelica/kg bw/day corresponding to 2 mg undecatriene, 8 mg pinene and the rest dodecene.	14 days	Corresponding to 2 mg undecatriene <sup>1</sup>	(Shapiro, 1988)	Not valid. See footnote <sup>1</sup> .
<i>(d)</i> -Limonene [01.045])	Rat; M 5	Gavage	0, 75, 150 or 300 mg/kg bw/day 5 days a week	6 or 27 days (5 or 25 doses)	75	(Kanerva et al., 1987)	Study is on kidney toxicity specifically.
	Rat; M 5 – 10	Gavage	0, 2, 5, 10, 30 or 75 mg/kg bw/day, 5 days/week	13 weeks	5	(Webb et al., 1989)	Study is on kidney toxicity specifically.
	Dog; M, F 6	Gavage	0, 0.4, 1.2 or 3.6 ml/kg bw/day corresponding to 0, 340, 1000 or 3000 mg/kg bw/day	180 days	M: 340 F: 340	(Tsuji et al., 1975a)	Study is in Japanese, only summary and tables in English. Quality of study not possible to assess.
	Dog; M, F 10	Gavage (divided doses)	0, 0.12 or 1.2 ml/kg bw/day corresponding to 0, 100 or 1000 mg/kg bw/day.	180 days	100	(Webb et al., 1990)	
	Mouse; M, F 10	Gavage	0, 413, 825, 1650, 3300 or 6600 mg/kg bw/day for 12 days over 16-day period	16 days	1650	(NTP, 1990e)	
	Mouse; M, F 20	Gavage	0, 125, 250, 500, 1000 or 2000 mg/kg bw/day 5 days per week	13 weeks	M: 500 F: 500	(NTP, 1990e)	Clinical signs of rough hair coat and decreased activity were observed at the two highest doses.
	Mouse; M, F M:50 F:50	Gavage	M: 0, 250 or 500 mg/kg bw/day F: 0, 500, 1000 mg/kg bw/day 5 days per week	103 weeks	M: 250 F: 500	(NTP, 1990e)	
	Rat; M, F 10	Gavage	0, 413, 825, 1650, 3300 or 6600 mg/kg bw/day for 12 days over 16-day period	16 days	1650	(NTP, 1990e)	
	Rat; M, F 20	Gavage	0, 150, 300, 600, 1200 or 2400 mg/kg bw/day 5 days per week	13 weeks	M: None F: 1200	(NTP, 1990e)	Dose-dependent nephropathy in all treated males.
Rat; M, F M:50 F:50	Gavage	M: 0, 75 or 150 mg/kg bw/day F: 0, 300 or 600 mg/kg bw/day 5 days per week	103 weeks	M: None F: 300	(NTP, 1990e)		
(Pin-2(10)-ene [01.003])	Rat; M, F 10	Diet	Calculated to provide 10 mg Galbelica/kg bw/day corresponding to 8 mg pin-2(10)-ene	14 days	Corresponding to 8 mg pin-2(10)-ene <sup>1</sup>	(Shapiro, 1988)	Not valid. See footnote <sup>1</sup> .
(Camphene [01.009])	Rat; M, F 10	Gavage	0, 62.5, 250, 1000 mg/ kg bw/day	28 days	M: None F: 250	(Hoechst, 1991b)	Study report very limited in details. Study according to OECD Guideline 407.
Naphthalene [01.053]	Rat; M, F 20	Gavage	0, 25, 50, 100, 200 or 400 mg/kg bw/day, 5 days/week	13 weeks	100 (LOAEL 200)	(Battelle Columbus Laboratory, 1980a)	Unpublished study that is not submitted. It is therefore not possible to assess the quality of the study. Study is referred to in US EPA 1998.
	Rat; NR 28	Diet	10-20 mg/kg bw/day	700 days	42 <sup>2</sup>	(Schmähl, 1955)	Study not considered valid.
	Mouse; M, F 116 – 156	Gavage	0, 5.3, 53, 133 mg/kg bw/day	90 days	53	(Shopp et al., 1984)	No histopathology was conducted, study not considered valid.

**Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies**

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	B6C3F1 Mouse, M,F	Inhalation	0, 10, 30 ppm, 5 days/week estimated to be 0, 3.6-3.9, 10.7-11.4 mg/kg bw/day	105 weeks	None	(NTP, 1992g)	Valid study.
	F344/N rat, M, F	Inhalation	0, 10, 30, 60 ppm, 5 days/week estimated to be 0, 3.6-3.9, 10.7-11.4, 20.1-20.7 mg/kg bw/day	105 weeks	None	(NTP, 2000d)	Valid study.
	Mouse; M, F 20	Gavage	0, 12.5, 25, 50, 100 or 200 mg/kg bw/day, 5 days/week	13 weeks	200 (LOAEL 200)	(Battelle Columbus Laboratory, 1980b)	Unpublished study that is not submitted. It is therefore not possible to assess the quality of the study. Study is referred to in US EPA 1998.
(1-Methylnaphthalene [01.014])	Mouse; M, F 20	Diet	0, 0,0163, 0.049, 0.147, 0.44 or 1.33 % in diet calculated to correspond to 0, 16, 47, 142, 425 or 1259 mg/kg bw/day	13 weeks	0.147% (142)	(Murata et al., 1993)	Dose-finding study.
	Mouse; M, F 100	Diet	0, 0.075 or 0.15 % in diet, average daily doses calculated to correspond to 0, 73 or 142 mg/kg bw/day	81 weeks	None <sup>3</sup>	(Murata et al., 1993)	Exposure related increase in incidence of pulmonary alveolar proteinosis.
2-Methylnaphthalene [01.051]	Mouse; M, F 100	Diet	0, 0.075 or 0.15 %. Average daily doses calculated by the authors to correspond to 50.3-54.3 resp. 107.6-113.8 mg/kg bw/day	81 weeks	No NOAEL derived <sup>4</sup> .	(Murata et al., 1997)	Study considered valid. Pulmonary alveolar proteinosis developed in all treated animals.
Mixture of (1-Methylnaphthalene [01.014]) and 2-Methylnaphthalene [01.051]	B6C3F1 Mouse; F 4, 11, 32	Cutaneous application	0, 29.7, 118.8 mg/kg bw twice a week	61 weeks	No NOAEL derived.	(Emi and Konishi, 1985)	The lesions were diagnosed as endogenous lipid pneumonia. Frequency of animals with these lesions were 0/4 control, 3/11 low dose and 31/32 high dose animals.
(Myrcene [01.008])	Mouse; M,F 5/20	Gavage		3 months	<250	(NTP, 2010b)	As a significant dose-dependent increase in the relative kidney weight was observed for female mice at all treatment doses, no NOAEL for this study could be allocated.
	Mouse; M, F 3/100	Gavage		2 years	250	(NTP, 2010b)	A NOAEL of 250 mg/kg bw for beta-myrcene was allocated, based on the increase in bone marrow atrophy and lymph node atrophy observed in both males and females at 500 mg/kg bw dose.
	Rat; M, F 5/20	Gavage		3 months	<250	(NTP, 2010b)	Based on the presence of renal tubular nephrosis and necrosis in all test groups, a NOAEL could not be assigned.
	Rat; M, F 3/100	Gavage		2 years	<250	(NTP, 2010b)	Due to the observation of renal tubular adenomas and carcinomas in all dose groups in male rats, accompanied by an

**Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies**

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
							increased incidence and severity of nephrosis in both sexes, a NOAEL for beta-myrcene from this study could not be allocated.

<sup>1</sup> Study performed using a dose of 10 mg/kg body weight per day of Galbelica, which is a solution composed of 80 % beta-pinene and 20 % 1,3,5-undecatriene and dodecene.

<sup>2</sup> True NOEL not determined. Only a single dose level was selected for a lifetime carcinogenicity study. Naphthalene was considered not to be carcinogenic.

<sup>3</sup> Study evaluated the carcinogenicity of 1-methylnaphthalene. The results were negative in the females. The males in both treatment groups showed a statistically significant increase in the number of lung adenomas from the control group; however, it may be concluded that because of a lack of tumours in females and at other sites (particularly the liver), it is unlikely that 1-methylnaphthalene is a carcinogen or promoter.

<sup>4</sup> Study evaluated the carcinogenicity of 2-methylnaphthalene. The results were negative, with the exception of the males in the 0.075 % dose group. It was concluded that although the total number of lung adenomas and adenocarcinomas was statistically significant in the 0.075 % dose group, they were in the range of spontaneous tumours.

Developmental and reproductive toxicity data are available for one candidate substance of the present flavouring group evaluation and for three supporting substances evaluated by the JECFA at the 63<sup>rd</sup> meeting. Supporting substances listed in brackets.

**Table IV.3: Developmental and Reproductive Toxicity Studies**

Chemical Name [FL-no]	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(Myrcene [01.008])	Developmental Toxicity: Gestation Days 6 - 15	Rat; F 16 - 29	Gavage	250, 500 or 1200 mg/kg bw/day	Maternal: 500 <sup>2</sup> Foetal: 500	(Delgado et al., 1993a)	Study considered valid.
	Peri- and Postnatal Developmental Toxicity: Gestation Day 15 to Postnatal Day 21	Rat; F 12 - 18	Gavage	0, 250, 500, 1000, 1500 mg/kg bw/day	Maternal: 500 <sup>3</sup> Peri- and Post-natal: 250	(Delgado et al., 1993b)	Study considered valid.
	Reproductive and developmental toxicity: Prior to mating until postnatal day 21	Rat; M, F 60	Gavage	0, 100, 300, 500 mg/kg bw/day	Maternal/paternal: 500 mg/kg bw/day Foetal: 300 mg/kg bw/day	(Paumgarten et al., 1998)	Study considered valid.
(d-Limonene [01.045])	Developmental Toxicity: Gestation Days 9 - 15	Rat; F 20	Oral	0, 591 or 2869 mg/kg bw/day	Maternal: 591 Foetal: 591	(Tsuji et al., 1975b)	Study in Japanese, only summary and tables in English. Quality of study not possible to assess.
	Developmental Toxicity: Gestation Days 7 - 12	Mouse; F 15	Oral	0, 591 or 2363 mg/kg bw/day	Maternal: 591 Foetal: 591	(Kodama et al., 1977b)	Study in Japanese, only summary and tables in English. Quality of study not possible to assess.
	Developmental toxicity: gestation days 6 - 18	Rabbit; F 10, 18 in highest dose group	Oral	0, 250, 500 or 1000 mg/kg bw/day	Maternal: 250 Foetal: 1000	(Kodama et al., 1977c)	Study in Japanese, only summary and tables in English. Quality of study not possible to assess.
(Pin-2(3)-ene [01.004])	Developmental toxicity: Gestation days 9 - 14	Rat; F 12 - 17	Oral	Not relevant	Not relevant	(Hasegawa and Toda, 1978)	Study is not considered valid. Study is on a mixture of menthol, menthone, pinene, borneol cineol, camphene rheochrysin in olive oil. Pin-2(3)-ene content is 17 %. Study in Japanese.
	Developmental toxicity: Gestation Days 6 - 15	Rat; F 20	Gavage		Maternal: 250 <sup>4</sup> Foetal: 1000	(Leuschner, 1992; LPT Research, 1992)	Study not submitted.
Naphthalene [01.053]	Developmental toxicity: Gestation Days 6 - 15	Rat; F 25 - 26	Gavage	0, 50, 150 or 450 mg/kg bw/day	Maternal LOAEL: 50 Foetal NOAEL: 450	(Navarro et al., 1991)	Study considered valid.
	Developmental toxicity: Gestation days 7 - 14	Mouse; F 33	Gavage	0 or 300 mg/kg/day	Not possible to derive a NOAEL <sup>1</sup>	(Booth et al., 1983; Plasterer et al., 1985)	Only one dosage. No assessment of visceral or skeletal foetal development.
	Developmental toxicity: Gestation Days 6 - 18	Rabbit; F 18	Gavage	0, 40, 200 or 400 mg/kg bw/day	Maternal: Not determined Foetal: 400	(Naismith & Matthews, 1986)	Study is considered valid, although only code number is given as identity of test substance.
	Developmental toxicity: Gestation days 6 - 19	Rabbit; F 25 - 27	Gavage	0, 20, 80 or 120 mg/kg bw/day	Maternal: 120 Foetal: 120	(Navarro et al., 1992)	Study considered valid.

<sup>1</sup>NOEL not determined; only a single dose level was tested. Naphthalene decreased the number of surviving dams, maternal body weight, number of live pups and litter weights, but not the weight per pup. However, it was concluded by the authors that maternal toxicity would manifest before high levels of developmental toxicity would occur.

<sup>2</sup>Test substance was beta-myrcene.

<sup>3</sup>Study performed using a dose of 10 mg/kg body weight per day of Galbelica, which is a solution composed of 80 % beta-pinene and 20 % 1,3,5-undecatriene.

<sup>4</sup>Test substance was 78 % pure.

*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 63<sup>rd</sup> meeting and for two separate stereoisomers and for one structurally related non-Register substance (*2-Methylbuta-1,3-diene*). Supporting substances are listed in brackets.

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Cedrene washed <sup>6</sup> [CAS no 11028-42-5]	Ames test	<i>S. typhimurium</i> TA97, TA98; TA100; TA1535; TA102	8-5000 <sup>3</sup>	Negative <sup>1</sup>	(Gocke, 1999b)	Validity cannot be evaluated as substance is not specified. Cedarwood oil terpenes and terpenoids.
	Ames test	<i>S. typhimurium</i> TA97, TA98; TA100; TA1535; TA102	1.6-1000 <sup>3</sup>	Negative <sup>1</sup>	(Gocke, 1999b)	Validity cannot be evaluated as substance is not specified. Cedarwood oil terpenes and terpenoids.
Longifolene [1.047]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537, TA102	1-5000	Negative <sup>1</sup>	(Sokolowski, 2001)	
Dodecane [01.038]	Ames test	<i>S. typhimurium</i> TA98; TA100	NR	Negative <sup>1</sup>	(Tumme 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 <sup>1</sup>	(Tumme 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 <sup>3</sup>	(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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	50, 150, 500, 1500, 5000 µg/plate	Negative <sup>1</sup>	(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 <sup>3</sup>	(Lankas et al., 1978)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Study designed to evaluate the ability of various alkanes to enhance the mutagenicity induced by the chemical carcinogen methylazoxymethanol acetate. Tetradecane showed no mutagenic activity per se, but increased the mutagenesis induced by pretreatment with the carcinogen.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; UTH8414; UTH8413	0, 50, 100, 500, 1000, 2000 µg/plate	Negative <sup>1</sup>	(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

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Dodec-1-ene [01.037]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538 <i>E. coli</i> WP2uvrA	0.2 to 2000 µg/plate	Negative <sup>1</sup>	(Dean, 1980)	reported. 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	<i>S. typhimurium</i> TA98; TA100; TA1530; TA1535; TA1538	25% atmosphere concentration	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 10000 µg/plate	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> 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)	<i>S. typhimurium</i> TA98; TA100; TA1535 <i>E. coli</i> WP2uvrA/pKM101	0, 500, 1000, 2000, 5000 µg/plate	Negative <sup>1</sup>	(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 <sup>1</sup>	(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 <sup>1</sup>	(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 <sup>1</sup>	(Kauderer et al., 1991)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Mammalian cell gene mutation assay	Chinese hamster ovary V79 cells	100 - 1000 µg/ml	Negative <sup>1</sup>	(Kauderer et al., 1991)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Sister chromatid exchange test	Human lymphocytes	100 - 1000 µg/ml	Negative <sup>1</sup>	(Kauderer et al., 1991)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells and hepatic tumour cell line	100 - 500 µg/ml	Negative <sup>1</sup>	(Röscheisen et al., 1991)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test (plate incorporation method)	<i>S. typhimurium</i> 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 concentrations mentioned. In the first run, concentrations up to cytotoxicity were studied; in a second run only non-toxic concentrations were tested.
	Ames	<i>S. typhimurium</i> TA97; TA98; TA100;	10 – 10 000 µg/plate	Negative <sup>1</sup>	(NTP, 2010b)	

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA1535				
	Reverse mutation	<i>E. coli</i> WP2uvrA/pKM101	50 – 10 000 µg/plate	Negative <sup>1</sup>	(NTP, 2010b)	
	Ames	<i>S. typhimurium</i> TA97a; TA98; TA100; TA1535	10 - 5000	Negative <sup>1</sup>	(Gomes-Carneiro et al., 2005a)	
(d-Limonene [01.045])	Ames	<i>S. typhimurium</i> TA97a; TA98; TA100; TA1535	1 - 1500	Negative <sup>1</sup>	(Gomes-Carneiro et al., 2005a)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/plate)	Negative <sup>1</sup>	(Florin et al., 1980)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Up to 150,000 µg/plate	Negative <sup>1</sup>	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA102	Up to 5000 µg/plate	Negative <sup>2</sup>	(Müller et al., 1993)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	Up to 3333 µg/plate	Negative <sup>1</sup>	(Haworth et al., 1983)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100, UTH8413 and UTH8414	0, 10 to 500 µg/plate (5 concentrations)	Negative <sup>1</sup>	(Conner et al., 1985)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Forward mutation assay	L5178Y Mouse lymphoma	Up to 100 µg/ml	Negative <sup>1</sup>	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Forward mutation assay	L5178Y Mouse Lymphoma	Up to 100 µg/ml	Negative <sup>1</sup>	(Myhr et al., 1990)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Chromosomal aberration assay	Chinese hamster ovary cells	Up to 500 µg/ml	Negative <sup>1</sup>	(Anderson et al., 1990)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells	Up to 162 µg/ml	Negative <sup>1</sup>	(Anderson et al., 1990)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
(gamma-Terpinene [01.020])	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 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Up to 50,000 µg/plate	Negative <sup>1</sup>	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
(alpha-Terpinene [01.019])	Unscheduled DNA synthesis	Rat hepatocytes	Up to 30 µg/ml	Negative	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test (plate incorporation method)	<i>S. typhimurium</i> 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	Up to 1000 µM (136.2 µg/ml)	Negative <sup>3</sup>	(Sasaki et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
(delta-3-Carene [01.029])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA102	Up to 5 µl/plate (up to 4300 µg/plate; 5 concentrations)	Positive <sup>3</sup> Negative <sup>2</sup>	(Kurtio et al., 1990)	Published non-GLP study with insufficiently reported results. Limited validity. Positive without metabolic activation in TA100 and TA102 and at doses of 2.5 µl/plate and higher.
(Pin-2(3)-ene [01.004])	Ames test	<i>S. typhimurium</i> TA98; TA100	Up to 100 µl/plate (Up to 85,800 µg/ plate)	Negative <sup>2</sup>	(Rockwell and Raw, 1979)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/ plate)	Negative <sup>1</sup>	(Florin et al., 1980)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Up to 25000 µg/plate	Negative <sup>1</sup>	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Up to 25 µl/plate (Up to 21,450 µg/ plate)	Negative <sup>1</sup>	(Jagannath, 1984)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; UTH8413; UTH8414	0, 10 to 500 µg/plate (5 concentrations)	Negative <sup>1</sup>	(Conner et al., 1985)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Unscheduled DNA synthesis	Rat hepatocytes	Up to 10000 µg/ml	Negative	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
(+)-alpha-pinene (pin-2(3)-ene) (isomer of [01.004])	Ames test (plate incorporation method)	<i>S. typhimurium</i> 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)	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Up to 5000 µg/plate	Negative <sup>1</sup>	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/plate)	Negative <sup>1</sup>	(Florin et al., 1980)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate (4290 µg/plate)	Negative <sup>1</sup>	(DeGraff, 1983a)	Abstracted by JECFA at their 63 <sup>rd</sup> 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 63 <sup>rd</sup> meeting (JECFA, 2006a).
(Camphene [01.009])	Ames test	<i>S. typhimurium</i> TA98; TA100	0.05 - 100 µl/plate (42.1 - 84,500 µg/ plate)	Negative <sup>2</sup>	(Rockwell and Raw, 1979)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; UTH8414; UTH8413	0, 10 to 1000 µg/plate (5 concentrations)	Negative <sup>1</sup>	(Conner et al., 1985)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells	10 - 1000 µM (1.4 - 136.2 µg/ml)	Negative <sup>3</sup>	(Sasaki et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
(beta-Caryophyllene [01.007])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Up to 150,000 µg/plate	Negative <sup>1</sup>	(Heck et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	0.1 - 150 µl/plate (90.4 – 135 525 µg/plate)	Negative <sup>1</sup>	(Lorillard, 1984)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA102; TA1535; TA1537	Up to 10,000 µg/plate	Negative <sup>1</sup>	(NTP, 2010b)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells	10 - 1000 µM (2.0 - 204.4 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
Naphthalene [01.053]	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (3.9, 39, 385, 3850 g/plate)	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	0, 3, 10, 30, 100, 300 µg/plate	Negative <sup>1</sup>	(Godek et al., 1985)	Unpublished GLP study carried out according to OECD guideline 471.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	0, 3, 10, 30, 100, 300 µg/plate	Negative <sup>1</sup>	(Stankowski, 1987)	Unpublished GLP study carried out according to OECD guideline 471. Repeat confirmation of Ames test by Godek <i>et al.</i> , 1985.
	Ames test	<i>S. typhimurium</i>	0, 1, 2 mM (0, 128, 256	Negative <sup>2</sup>	(Kaden et al., 1979)	Published non-GLP study of limited validity (only

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	(preincubation method)	TM677	µg/ml)			one strain, concentrations used were cytotoxic, insufficient report of experimental details and results).
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	Up to 100 µg/plate	Negative <sup>2</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, (0.3), 1, 3.3, 10, 33, 100 µg/plate	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> 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 <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; UTH8414; UTH8413	0, 100, 500, 1000, 2000 µg/plate	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA97; TA98; TA100	0, 5, 10, 50, 250 µg/plate	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	0, 3.3 to 10,000 µg/plate	Negative <sup>1</sup>	(Longfellow, 1991)	Only summary from CCRIS database available. Validity of the study cannot be evaluated.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537 <i>E. coli</i> WP2uvrA	1 to 100 µg/plate 10 to 10,000 µg/plate	Negative <sup>1</sup>	(Japan Chemical Industry)	Only summary from CCRIS database available. Validity of the study cannot be evaluated.
	Rec assay	<i>E. coli</i> WP2 and WP100 <i>uvrA</i> <i>recA</i>	0 to 2000 µg/plate (≥ 4 concentrations)	Negative <sup>2</sup>	(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).
	Inductest (prophage induction test)	<i>E. coli</i> GY5027 <i>envA</i> <i>uvrB</i> <sup>+</sup> (λ); GY4015 amp <sup>R</sup>	0 to 2000 µg/plate (≥ 4 concentrations)	Negative <sup>2</sup>	(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).

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	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 <sup>1</sup>	(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 <i>et al.</i> , 1987a). 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 <sup>1</sup>	(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 <i>tk<sup>+</sup>/tk<sup>-</sup></i>	0, 22 to 87 µg/ml (-S9) 0, 8 to 30 µg/ml (+S9)	Negative <sup>3</sup> Positive <sup>2</sup>	(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 <sup>3</sup> Positive <sup>2</sup>	(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 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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.3, 43, 427, 4266)	Negative <sup>1</sup>	(Florin et al., 1980)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration µ g/plate	Result	Reference	Comments
	Ames test (preincubation method)	<i>S. typhimurium</i> TM677	0, 0.7, 3.5, 7 mM (0, 498, 995 µg/ml)	Negative <sup>2</sup>	(Kaden et al., 1979)	
	Chromosomal aberration assay	Human lymphocytes	0, 1, 2 mM (0, 142, 284 µg/ml) (-S9)	Negative <sup>1</sup>	(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.
			0, 0.25, 0.5, 1, 2 mM (0, 36, 71, 142, 284 µg/ml) (+S9)			
	Sister chromatid exchange test	Human lymphocytes	0, 1, 2 mM (0, 142, 284 µg/ml) (-S9)	Negative <sup>3</sup>	(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 $\geq 20$ % 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.
			0, 0.25, 0.5, 1, 2 mM (0, 36, 71, 142, 284 µg/ml) (+S9)	Positive <sup>2</sup> (limited evidence)		
2-Methylnaphthalene [01.051]	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.3, 43, 427, 4266 µg/plate)	Negative <sup>1</sup>	(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, 569 microgram/ml) (-S9)	Negative <sup>3</sup>	(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. In the presence of S9 a statistically significant but weak increase (6.5 fold 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
			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 <sup>2</sup> (limited evidence)		

**Table IV.4: GENOTOXICITY (*in vitro*)**

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange test	Human lymphocytes	0, 2.0, 4.0 mM (0, 284, 569 microgram/ml) (-S9)	Negative <sup>3</sup>	(Kulka et al., 1988)	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).
			0, 0.25, 0.5, 1.0, 2.0, 4.0 mM (0, 36, 71, 142, 284 and 569 microgram/ml) (+S9)	Positive <sup>2</sup> (limited evidence)		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 $\geq$ 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.

<sup>1</sup> With and without S9 metabolic activation.

<sup>2</sup> With metabolic activation.

<sup>3</sup> Without metabolic activation.

<sup>4</sup> Plate incorporation.

<sup>5</sup> Pre-incubation..

<sup>6</sup> 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.

*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 63<sup>rd</sup> meeting and for one structurally related *non-Register substance* (2-Methylbuta-1,3-diene). Supporting substances are listed in brackets.

**Table IV.5: GENOTOXICITY (*in vivo*)**

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(2-Methylbuta-1,3-diene)	<i>In vivo</i> 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)	Negative	(Tice et al., 1987; Tice, 1988; Shelby, 1990)	Unpublished study report and published summary report of a valid multiple endpoint cytogenicity study sponsored by NTP, roughly in accordance with OECD guideline 475 (special dosage regimen used).
				0, 70, 220, 700 ppm for 6 hours/day for 12 exposures over a period of 16 days (Trial 2)			
	<i>In vivo</i> 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)	Positive	(Tice et al., 1987; Tice, 1988; Shelby, 1990)	Unpublished study report and published summary report of valid cytogenicity study sponsored 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.
				0, 70, 220, 700 ppm for 6 hours/day for 12 exposures over a period of 16 days (Trial 2)			
	<i>In vivo</i> 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.
	<i>In vivo</i> 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])	<i>In vivo</i> 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 63 <sup>rd</sup> meeting (JECFA, 2006a).
	<i>In vivo</i> Micronucleus test	Mouse (B6C3F1) peripheral blood cells	Gavage	0, 250, 500, 1000, 2000 mg/kg bw (single exposure)	Negative	(NTP, 2004h)	Abstracted by JECFA at their 63 <sup>rd</sup> meeting (JECFA, 2006a).
	Micronucleus assay	Mouse peripheral blood cells	Gavage	250, 500, 1000 mg/kg bw/ day	Negative	(NTP, 2010b)	
(d-Limonene [01.045])	<i>In vivo</i> Comet assay	Mouse (ddY) / Rat (Wistar).	Oral	0, 2000 mg/kg	Negative	(Sekihashi et al., 2002)	
	<i>In vivo</i> 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 63 <sup>rd</sup> meeting (JECFA, 2006a).
	<i>In vivo</i> Comet assay	Rats (Sprague-Dawley) (males) (Kidneys)	Gavage	0, 1000, 2000 mg/kg bw (single exposure)	Negative	(Nesslany et al., 2007)	
	<i>In vivo</i> transgenic mutagenicity assay	Rats (Big blue) (males) (liver,	Diet	0, 525 mg/kg bw/day (10 days)	Negative	(Turner et al., 2001)	The author do not specify whether the tested compound is <i>d-</i> or <i>l-</i> limonene, and the purity of the compound is not stated. However, the

**Table IV.5: GENOTOXICITY (*in vivo*)**

Chemical Name [FL-no]	Test System	Test Object kidney, bladder)	Route	Dose	Result	Reference	Comments
Naphthalene [01.053]	<i>In vivo</i> Unscheduled DNA synthesis	Rat hepatocytes	Gavage	0, 600, 1000, 1600 mg/kg bw	Negative	(Research Toxicology Center, 1999)	stability of the limonene in the diet was measured. 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.
	<i>In vivo</i> 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 10 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.
	<i>In vivo</i> 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.

## REFERENCES

- Anderson BE, Zeiger E, Shelby MD, Resnick MA, Gulati DK, Ivett JL and Loveday KS, 1990. Chromosome aberration and sister chromatid exchange test results with 42 chemicals. *Environ. Mol. Mutag.* 16(Suppl. 18), 55-137.
- Asakawa Y, Taira Z, Takemoto T, Ishida T, Kido M and Ichikawa Y, 1981. X-ray crystal structure analysis of 14-hydroxycaryophyllene oxide, a new metabolite of (-)-caryophyllene, in rabbits. *J. Pharm. Sci.* 70(6), 710-711.
- Asakawa Y, Ishida T, Toyota M and Takemoto T, 1986. Terpenoid biotransformation in mammals IV. Biotransformation of (+)-longifolene, (-)-caryophyllene, (-)-caryophyllene oxide, (-)-cyclocolorenone, (+)-nootkatone, (-)-elemol, (-)-abietic acid and (+)-dehydroabietic acid in rabbits. *Xenobiotica* 16(8), 753-767.
- Ashley DL and Prah JD, 1997. Time dependence of blood concentrations during and after exposure to a mixture of volatile organic compounds. *Arch. Environ. Health* 52(1), 26-33.
- Austin CA, Shephard EA, Pike SF, Rabin BR and Phillips IR, 1988. The effect of terpenoid compounds on cytochrome P-450 levels in rat liver. *Biochem. Pharmacol.* 37(11), 2223-2229.
- 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.
- Bahima J, Cert A and Menéndez-Callego M, 1984. Identification of volatile metabolites of inhaled n-heptane in rat urine. *Toxicol. Appl. Pharmacol.* 76, 473-482.
- Bakke OM and Scheline RR, 1970. Hydroxylation of aromatic hydrocarbons in the rat. *Toxicol. Appl. Pharmacol.* 16(3), 691-700.
- Bakke J, Struble C, Gustafsson J-AA and Gustafsson B, 1985. Catabolism of premercapturic acid pathway metabolites of naphthalene to naphthols and methylthio-containing metabolites in rats. *Proc. Natl. Acad. Sci. USA* 82, 668-671.
- Baldwin RM, Jewell WT, Fanucchi MV, Plopper CG and Buckpitt AR, 2004. Comparison of pulmonary/nasal CYP2F expression levels in rodents and Rhesus Macaque. *J. Pharmacol. Exp. Ther.* 309(1), 127-136.
- 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 EFA to FLAVIS Secretariat.
- Battelle Columbus Laboratory, 1980a. Unpublished subchronic toxicity study: naphthalene (C52904), Fischer 344 rats. Report to U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC, by Battelle's Columbus Laboratories, Columbus, OH, under subcontract no. 76-34-106002. Cited in: U.S. EPA, 1998. Toxicological Review of Naphthalene (Cas No. 91-20-3). August, 1998.

- Battelle Columbus Laboratory, 1980b. Unpublished subchronic toxicity study: naphthalene (C52904), Mice. Report to U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC, by Battelle's Columbus Laboratories, Columbus, OH, under subcontract no. 76-34-106002. Cited in: U.S. EPA, 1998. Toxicological Review of Naphthalene (Cas No. 91-20-3). August, 1998.
- Birch MD, 1978b. Initial Submission: Toxicological investigation of: naphthalene with cover letter dated 08/13/92. Monsanto Co. EPA Doc 88-920007989, microfiche no. OTS0570603. Date 09/11/78. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Birch MD, 1978c. Initial Submission: Toxicological investigation of: naphthalene with cover letter dated 08/13/92. Monsanto Co. EPA Doc 88-920007988, microfiche no. OTS0570602. Date 09/11/78. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Birch MD, 1978d. Initial Submission: Toxicological investigation of: naphthalene with cover letter dated 08/13/92. Monsanto Co. EPA Doc 88-920007987, microfiche no. OTS0570601. Date 09/11/78. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Bock KW, Clausbruch UCV and Winne D, 1979. Absorption and metabolism of naphthalene and benzo[a]pyrene in the rat jejunum in situ. *Med. Biol.* 57, 262-264.
- Bogaards JJP, Venekamp JC and van Bladeren PJ, 1996. The biotransformation of isoprene and the two isoprene monoepoxides by human cytochrome P450 enzymes, compared to mouse and rat liver microsomes. *Chem.-Biol. Interact.* 102(3), 169-182.
- Booth GM, Bradshaw WS and Carter MW, 1983. Screening of priority chemicals for potential reproductive hazard with cover letter dated 05/17/83. Glycol ethers, naphthalene, 2-imidazolidinethione. National Institute for Occupational Safety and Health. EPA Doc FYI-OTS-0583-0240;OTS0000240-0.
- Boylard E and Sims P, 1958. Metabolism of polycyclic compounds: 12. An acid-labile precursor of 1-naphthylmercapturic acid and naphthol: an N-acetyl-S- (1:2-dihydrohydroxynaphthyl)lcysteine. *Biochem. J.* 68, 440-447.
- Boyle R, McLean S, Foley WJ and Davies NW, 1999. Comparative metabolism of dietary terpene, p-cymene, in generalist and specialist folivorous marsupials. *J. Chem. Ecol.* 25, 2109-2126.
- Breger RK, Novak RF, Franklin RB, Rickert D and Lech JJ, 1983. Further structural analysis of rat liver microsomal metabolites of 2-methylnaphthalene. *Drug Metab. Disposition* 11(4), 319-323.
- Brownlee G, 1940. A pharmacological examination of cineole and phellandrene. *Q. J. Pharm. Pharmacol.* 13, 130-137.
- Buckley LA, Coleman DP, Burgess JP, Thomas BF, Burka LT and Jeffcoat AR, 1999. Identification of urinary metabolites of isoprene in rats and comparison with mouse urinary metabolites. *Drug Metab. Disposition* 27(7), 848-854.
- Buckpitt AR and Bahnson LS, 1986. Naphthalene metabolism by human lung microsomal enzymes. *Toxicology* 41, 333-341.
- Buckpitt A, Buonarati M, Avey LB, Chang AM, Morin D and Plopper CG, 1992. Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. II. Comparison of stereoselectivity of naphthalene epoxidation in lung and nasal mucosa of mouse, hamster, rat, and rhesus monkey. *J. Pharmacol. Exp. Ther.* 261(1), 364-372.

- Buckpitt A, Boland B, Isbell M, Morin D, Shultz M, Baldwin R, Chan K, Karlsson A, Lin C, Taff A, West J, Fannucchi M, Van Winkle L and Plopper C, 2002. Naphthalene-induced respiratory tract toxicity: metabolic mechanisms of toxicity. *Drug Metab. Rev.* 34(4), 791-820.
- Carpenter CP, Smyth Jr HF and Pozzani UC, 1949. The assay of acute vapor toxicity, and the grading and interpretation of results on 96 chemical compounds. *J. Ind. Hyg. Toxicol.* 31, 343-346.
- Chen K-C and Dorough HW, 1979. Glutathione and mercapturic acid conjugations in the metabolism of naphthalene and 1-naphthyl N-methylcarbamate (carbaryl). *Drug Chem. Toxicol.* 2, 331-354.
- Chiappe C, De Rubertis A, Amato G and Gervasi PG, 1998. Stereochemistry of the biotransformation of 1-hexene and 2-methyl-1-hexene with rat liver microsomes and purified P450s of rats and humans. *Chem. Res. Toxicol.* 11, 1487-1493.
- CoE, 1992. Flavouring substances and natural sources of flavourings. 4<sup>th</sup> Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.
- Conner 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.
- Crowell PL, Ren Z, Lin S, Vedejs E and Gould MN, 1994a. Structure-activity relationships among monoterpene inhibitors of protein isoprenylation and cell proliferation. *Biochem. Pharmacol.* 47(8), 1405 - 1415.
- Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. *Food Cosmet. Toxicol.* 16(3), 255-276.
- Dahl AR, 1989. The fate of inhaled octane and the nephrotoxicant, isooctane, in rats. *Toxicol. Appl. Pharmacol.* 100, 334-341.
- 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.
- 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.
- Del Monte M, Citti L and Gervasi PG, 1985. Isoprene metabolism by liver microsomal monooxygenases. *Xenobiotica* 15(7), 591-598.
- Delgado IF, Carvalho RR, de Almeida Nogueira ACM, Mattos AP, Figueiredo LH, Oliveira SHP, Chahoud I and Paumgartten FJR, 1993a. Study on embryo-foetotoxicity of beta-myrcene in the rat. *Food Chem. Toxicol.* 31, 31-35.
- Delgado IF, de Almeida Nogueira ACM, Souza CAM, Costa AMN, Figueiredo LH, Mattos AP, Chahoud I and Paumgartten FJR, 1993b. Peri- and postnatal developmental toxicity of beta-myrcene in the rat. *Food Chem. Toxicol.* 31, 623-628.

- Delgado-Rodrigues A, Ortiz-Marttelo R and Graf U, 1995. Genotoxic activity of environmentally important polycyclic aromatic hydrocarbons and their nitro derivatives in the wing spot test of *Drosophila melanogaster*. *Mutat. Res.* 341, 235-247.
- DeLoach GD, Leary HW and Stocklinski AW, 1978. Identification of hydroxydiphenylmethane as a urinary and fecal metabolite of diphenylmethane in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* 22(2), 419-422.
- De-Oliveira A, Ribeiro-Pinto L, Otto S, Goncalves A and Paumgartten F, 1997a. Induction of liver monooxygenase by beta-myrcene. *Toxicology* 124, 135-140.
- 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, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. *Official Journal of the European Communities* 12.4.2002, L 95, 10-11.
- EC, 2008a. Commission Decision 2008/478/EC of 17 June 2008 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. *Official Journal of the European Union* 24.6.2008, L 163, 42.
- 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/](http://www.ecb.jrc.it/classification-labelling/).
- EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
- EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.
- EFFA, 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.

- EFFA, 2005b. 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. FLAVIS/8.38. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat
- EFFA, 2006o. Addendum of 1 flavouring substance (candidate chemical) to the flavouring group evaluation of the chemical group 31 (Annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons [JECFA/WHO FAS 54/63] used as flavouring substances. Addendum to FGE.25 (EFFA submission 2004-3). 21 December 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.106.
- EFFA, 2006p. Addendum of 1 flavouring substance (candidate chemical) to the flavouring group evaluation of the chemical group 31 (Annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons used as flavouring substances. Addendum to FGE.25 (EFFA submission 2004-3). 21 December 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.107
- EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.
- EFFA, 2008b. Poundage data on selected substances. Private communication from EFFA to the FLAVIS secretariat. 19 December 2008. FLAVIS/8.113.
- EFFA, 2009c. Supplement list of EU-only Footnote-10 materials for Commission. Unpublished communication submitted by EFFA to the FLAVIS secretariat. 14 December 2009.
- EFFA, 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
- EFSA, 2004a. Minutes of the 7<sup>th</sup> Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: [http://www.efsa.europa.eu/cs/BlobServer/Event\\_Meeting/afc\\_minutes\\_07\\_en1.pdf?ssbinary=true](http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true)
- 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 carboxylic 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, 2006b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 18: Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical group 6 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 1 March 2006. EFSA-Q-2003-161.
- EFSA, 2006e. 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 20: Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical group 23 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 7 December 2006. EFSA-Q-2003-163.

- EFSA, 2009ah. Opinion of the Scientific Panel on Contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 43: Thujyl alcohol from chemical group 8 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 26 March 2009. EFSA-Q-2008-047.
- EFSA, 2009j. 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 78: Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63<sup>rd</sup> meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 6 March 2008. EFSA-Q-2003-062.
- EFSA, 2011j. Scientific Opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63<sup>rd</sup> meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 19 May 2011. EFSA-Q-2010-01557.
- Emi Y and Konishi Y, 1985. Endogenous lipid pneumonia in female B6C3F1 mice. In: Jones, T.C., Mohr, V., Hunt, R.D. (Eds.). Respiratory System Monographs on the Pathology of Laboratory Animals. International Life Science Institute, Springer-Verlag, Berlin pp. 166-168.
- Enichem Augusta Ind., 1987. Acute oral toxicity (Acceptance N° 7056 22/9/1987). Cited in European Commission. European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 629-59-4, EINECS Name tetradecane. Section 1.0.1-5.11.
- Eriksson K and Levin J-O, 1990. Identification of cis- and trans-verbenol in human urine after occupational exposure to terpenes. *Int. Arch. Occup. Environ. Health* 62, 379-383.
- Eriksson K and Levin JO, 1996. Gas chromatographic mass spectrometric identification of metabolites from  $\alpha$ -pinene in human urine after occupational exposure to sawing fumes. *J. Chromatography* 677(1), 85-98.
- Ethyl Corporation, 1973. EPA/OECD summary data. Report on the acute toxicity of alpha olefin C8. Report from Tulane school of medicine to Ethyl Corp. Summarized by the EPA response to a request from the OECD from available data on HPV chemicals. 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.
- 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.
- Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: [http://epp.eurostat.ec.europa.eu/portal/page?\\_pageid=1090,30070682,1090\\_33076576&\\_dad=portal&\\_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.](http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.)
- Fahrig R, 1984. Genetic mode of action of carcinogens and tumor promoters in yeast and mice. *Mol. General Genet.* 194, 7-14.
- Falk A, Gullstrand E, Löf A and Wigaeus-Hjelm E, 1990a. Liquid/air partition coefficients of four terpenes. *British J. Ind. Med.* 47, 62-64.

- Falk A, Hagberg M, Lof A, Wigaeus-Hjelm E and Zhiping W, 1990b. Uptake, distribution and elimination of alpha-pinene in man after exposure by inhalation. *Scand. J. Work Environ. Health* 16(5), 372-378.
- Falk A, Löf A, Hagberg M, Wigaeus-Hjelm E and Wang Z, 1991. Human exposure to 3-carene by inhalation: toxicokinetics, effects on pulmonary function and occurrence of irritative and CNS symptoms. *Toxicol. Appl. Pharmacol.* 110, 198-205.
- Falk-Fillipsson A, Löf A, Hagberg M, Wigaeus-Hjelm E and Wang Z, 1993. d-Limonene exposure to humans by inhalation: uptake, distribution, elimination, and effects on the pulmonary function. *J. Toxicol. Environ. Health* 38, 77-88.
- Fedtke N and Bolt HM, 1987. The relevance of 4,5-dihydroxy-2-hexanone in the excretion kinetics of nhexane metabolites in rat and man. *Arch. Toxicol.* 61, 131-137.
- Felice B, 2005. Acute oral toxicity test acute toxic class method-OECD. Toxikon final report 05-0262-G1. Toxikon corporation Bedford, MA. Unpublished report submitted by EFA to FLAVIS Secretariat.
- Flavour Industry, 2006t. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-25Rev1.
- Flavour Industry, 2009t. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-25Rev2 [FL-no: 01.001]
- Flavour Industry, 2010a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-78rev1/A-25Rev2 [FL-no: 01.008, 01.022,01.035, 01.047, 01.064].
- EFA, 2011c. E-mail from EFA to FLAVIS Secretariat, Danish Foodinstitute, Technical University of Denmark. Dated 11 January 2011. Re.: FGE.25Rev2: FL-no: 01.021 specifications on isomers. FLAVIS/8.118
- 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.
- Ford RA, Api AM and Letizia CS, 1992b. Fragrance raw materials monographs. Longifolene. *Food Chem. Toxicol.* 30(suppl.), 67S-68S.
- Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpö 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 EFA 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 EFA 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 Paumgarten FJR, 2005a. Evaluation of beta-myrcene, alpha-terpinene and (+)- and (-)-alpha-pinene in the salmonella/microsome assay. *Food Chem. Toxicol.* 43, 247-252.

- Griffin KA, Johnson CB, Breger RK and Franklin RB, 1982. Effects of inducers and inhibitors of cytochrome P-450-linked monooxygenases on the toxicity, *in vitro* metabolism and *in vivo* irreversible binding of 2-methylnaphthalene in mice. *J. Pharmacol. Exp. Ther.* 221, 517-524.
- Hämäläinen J, 1912. [Concerning the behavior of alicyclic compounds with glucuronic acid in organisms]. *Skand. Arch. Physiol.* 27, 141-226. (In German)
- Hard GC, Rodgers IS, Baetchke KP, Richards WL, McGaughy RE and Valcovic LR, 1993. Hazard evaluation of chemicals that cause accumulation of alpha 2 $\mu$ -globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. *Environmental Health Perspectives* 99, 313-349.
- Harper BL, Sadagopa Ramanujam VM, Gad-El-Karim MM and Legator MS, 1984. The influence of simple aromatics on benzene clastogenicity. *Mutat. Res.* 128, 105-114.
- Hart ER and Wong LCK, 1971. Acute oral toxicity studies in rats, acute dermal toxicity and primary skin irritation studies in rabbits of 17 fragrance materials. Bionetics Research Laboratories. July 30, 1971. Report submitted by EFFA to SCF.
- Hasegawa M and Toda T, 1978. Teratological studies on Rowachol, remedy for cholelithiasis. Effect of Rowachol administered to pregnant rats during organogenesis on pre-and post-natal development of their offspring. *Oyo Yakuri* 15(7), 1109-1119. (In Japanese)
- Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutag.* 5 (Suppl. 1) 3-142.
- Hazleton France, 1990a. Hazleton France les Oncins BP 0118, 69593 l'Arbresle Cedex, France. Report No. 008306 of 6 August 1990. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 91-20-3, EINECS Name naphthalene, pure. Section 1.0.1-5.11.
- Hazleton France, 1990b. Hazleton France les Oncins BP 0118, 69593 l'Arbresle Cedex, France. Report No. 008306 of 6 August 1990. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 91-20-3, EINECS Name naphthalene, pure. Section 1.0.1-5.11.
- Hazleton France, 1990c. Hazleton France les Oncins BP 0118, 69593 l'Arbresle Cedex, France. Report No. 008306 of 6 August 1990. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 91-20-3, EINECS Name naphthalene, pure. Section 1.0.1-5.11.
- Heck JD, Vollmuth TA, Cifone MA, Jagannath DR, Myhr B and Curren RD, 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *Toxicologist* 9(1), 257-272.
- Hiroi T, Miyazaki Y, Kobayashi Y, Imaoka S and Funae Y, 1995. Induction of hepatic P450's in rat by essential wood and leaf oils. *Xenobiotica* 25(5), 457-467.
- Hoechst AG, 1991b. Unveroeffentlichte Untersuchung (91.0475). Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 79-92-5, EINECS Name camphene. Section 1.0.1-5.11.
- Hoffman-LaRoche, Inc., 1967b. Acute toxicity, eye and skin irritation tests on aromatic compounds. Roche Chemical Division. Report no. 30642. September 20, 1967. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Holmberg B, Jakobson I and Sigvardsson KA, 1977. study on the distribution of methylchloroform and noctane in the mouse during and after inhalation. *Scand. J. Work Environ. Health* 3, 43-52.

- Horning MG, Stillwell WG, Griffin GW and Tsang W-S, 1980. Epoxide intermediates in the metabolism of naphthalene by the rat. *Drug. Metab. Disposition* 8, 404-414.
- 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.
- Igimi H, Kodama M and Ide H, 1974. Studies on the metabolism of d-limonene (p-mentha-1,8-diene). I. The absorption, distribution and excretion of d-limonene in rats. *Xenobiotica* 4(2), 77-84.
- Imbriani M, Ghittori S, Pezzagno G and Capodaglio E, 1985. Urine/air partition coefficients for some industrially important substances. *G. Ital. Med. Lav* 7, 133-140.
- IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.
- Ishida T, Asakawa Y, Takemoto I and Aratani T, 1979a. Terpene metabolites in rabbit urine. IV. Metabolism of pinan, caran, and myrcene. *Koen Yoshishu-Koryo, Terupen* 23, 39-41. (In Japanese)
- Ishida T, Asakawa Y, Takemoto T and Aratani T, 1981. Terpenoids biotransformation in mammals [I]: biotransformation of alpha-pinene, beta-pinene, Pinane, 3-carene, carene, myrcene and p-cymene in rabbits. *J. Pharmacol. Sci.* 70, 4, 406-415.
- Ishida T, Asakawa Y and Takemoto T, 1982. Hydroxyisolongifolaldehyde: A new metabolite of (+)-longifolene in rabbits. *J. Pharm. Sciences* 71(8), 965-966.
- Izmerov NF et al., 1982b. Toxicometric Parameters of Industrial Toxic Chemicals Under Single Exposure. Moscow, Centre of International Project, GKNT, 1982. Cited in RTECS. Chemical name: Naphtalene, 1-methyl-. Section Toxicity data. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Jagannath DR, 1984. 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.
- Japan Chemical Industry (Date not given) Ecology - Toxicology and Information Center. Mutagenicity of test data of existing chemical substances based on the toxicity investigation of the Industrial Safety and Health Law. Naphthalene. Carcinogenicity studies. Mutagenicity studies. Cited in CCRIS.
- 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, 1993a. 41. Report: Forty-First Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Report: WHO Technical Report Series, no. 837.
- 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 63<sup>rd</sup> 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.
- Jones WH, 1969. Toxicity and Health Hazard Summary. 1-Dodecene. Eastman Kodak Co., 1984. EPA Doc FYI-OTS-0794-1156, microfiche no. OTS0001156. Date 07/04/84. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Kaden DA, Hites RA and Thilly WG, 1979. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. Cancer Res. 39, 4152-4159.
- Kanerva RL, Ridder GM, Lefever FR and Alden CL, 1987. Comparison of short-term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats. Food Chem. Toxicol. 25(5), 345-353.
- Kauderer B, Zamith H, Paumgarten JR and Speit G, 1991. Evaluation of the mutagenicity of beta-myrcene in mammalian cells *in vitro*. Environ. Mol. Mutagen. 18, 28-34.
- Keating JW, 1972a. Acute oral toxicity (rat-5 gms/kg body weight dose). Dermal toxicity (rabbit-5 gms/kg body weight dose). Amyris acetylated, Bois de rose acetylated, Cadinene, Castoreum, Lavandin acetylated, Dihydrojasmane, Trans-2-hexenol, Methyl isoeugenol, Methyl eugenol, Santalyl acetate, Phenyl propyl cinnamate, Phenylacetic acid, 1-Carveol, Santatol, Methyl heptenone. Biological Science Laboratories. Unpublished report submitted by EFFA to SCF.
- 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.
- Kilanowicz A, Czernski B and Sapota A, 1999. The disposition and metabolism of naphthalene in rats. Int. J. Occup. Med. Environ. Health 12(3), 209-219.
- Kimmerle G and Solmecke B, 1972. Isopren-Akute Toxizitätsuntersuchungen. Bayer AG, unveröffentlichter Bericht Nr. 3373. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 78-79-5, EINECS Name isoprene. Section 1.0.1-5.11.
- Kodama R, Noda K and Ide H, 1974. Studies on the metabolism of d-limonene (p-mentha-1,8-diene) - II. The metabolic fate of d-limonene in rabbits. Xenobiotica 4, 85-95.
- Kodama R, Yano T, Furukawa K, Noda K and Ide H, 1976. Studies on the metabolism of d-limonene - IV Isolation and characterization of new metabolites and species differences in metabolism. Xenobiotica 6, 377-389.
- Kodama R, Okubo A, Araki E, Noda K, Ide H and Ikeda T, 1977b. Studies on d-limonene as a gallstone solubilizer (VII): Effects on development of mouse fetuses and offsprings. J. Oyo Yakuri 13(6), 863-873. (In Japanese)

- Kodama R, Okubo A, Sato K, Araki E, Noda K, Ide H and Ikeda T, 1977c. Studies on d-limonene as a gallstone solubilizer (IX): Effects on development of rabbit fetuses and offsprings. *J. Oyo Yakuri* 13(6), 885-898. (In Japanese)
- Koppel C, Tenczer J, Tonnesmann U, Schirop T and Ibe K, 1981. Acute poisoning with pine oil-metabolism of monoterpenes. *Arch. Toxicol.* 49, 73-78.
- Kulka U, Schmid E, Huber R and 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), 263-264.
- Lankas GR, Baxter CS and Christian RT, 1978. Effect of alkane tumor-promoting agents on chemically induced mutagenesis in cultured V79 Chinese hamster cells. *J. Toxicol. Environ. Health* 4, 37-41.
- Lanza DL, Code E, Crespi CL, Gonzalez FJ and Yost GS, 1999. Specific dehydrogenation of 3-methylindole and epoxidation of naphthalene by recombinant human CYP2F1 expressed in lymphoblastoid cells. *Drug Metab. Disposition* 27(7), 798-803.
- 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.
- Lehman-McKeeman LD, Rodriguez PA, Takigiku R, Caudill D and Fey ML, 1989. d-Limonene-induced male rat-specific nephrotoxicity: Evaluation of the association between d-limonene and alpha-2 $\mu$  - globulin. *Toxicol. Appl. Pharmacol.* 99, 250-259.
- Leuschner, 1992. LPT Laboratory of Pharmacology and Toxicology, Report No. 7263/92 (HOE 92.1167). Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 79-92-5, EINECS Name camphene. Section 1.0.1-5.11.
- Levenstein I, 1975o. Acute oral toxicity (rat – 5 gms./kg. body weight dose). Dermal toxicity (rabbit – 5 gms./kg. body weight dose). Terpinolene. Leberco Laboratories. Assay no. 51754. January 21, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Levin JO, Eriksson K, Falk A, Lof A, 1992. Renal elimination of verbenols in man following experimental  $\alpha$ -pinene inhalation exposure. *Int. Arch. Occup. Environ. Health* 63, 571-573.
- Longfellow D, 1991. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute. Naphthalene. Carcinogenicity studies. Mutagenicity studies. Cited in CCRIS.
- Longfellow D, 1998. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute. Beta-caryophyllene. Mutagenicity studies. Cited in CCRIS.
- Longo V, Citti L and Gervasi PG, 1985. Hepatic microsomal metabolism of isoprene in various rodents. *Toxicol. Lett.* 29(1), 33-37.

- Lorillard (Lorillard Tobacco Company), 1984. Mutagenicity evaluation of beta-caryophyllene in the Ames Salmonella/microsome plate test. Final report. Litton Bionetics, Inc. LBI Project no. 20988. January, 1984. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Low LK, Meeks JR and Mackerer CR, 1987. n-Octane. In: Snyder, R. (Ed.). Ethel Browning's toxicity and metabolism of industrial solvents. 2<sup>nd</sup> Ed. Elsevier, Amsterdam pp. 307-311.
- LPT Research, 1992. Report No. 7114/91. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 79-92-5, EINECS Name camphene. Section 1.0.1-5.11.
- 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-pentadiene-3-ol, isoprene, 2,4-hexadiene, cis- and trans-piperlylene. J. Health Sci. 48(1), 73-78.
- Madyastha KM and Srivatsan V, 1987. Metabolism of beta-myrcene *in vivo* and *in vitro*: its effects on rat-liver microsomal enzymes. Xenobiotica 17, 539-549.
- Maltzman TH, Christou M, Gould MN and Jefcoate CR, 1991. Effects of monoterpenoids on *in vivo* DMBA-DNA adduct formation and on phase I hepatic metabolising enzymes. Carcinogenesis 12(11), 2081-2087.
- 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.
- Manini P, Andreoli R, Bergamaschi E and Franchini I, 1999. Determination of free and glucuronated hexane metabolites without prior hydrolysis by liquid- and gas-chromatography coupled with mass spectrometry. Toxicol Lett. 108(2-3), 225-31.
- Matsumoto T, Ishida T, Yoshida T, Terao H, Takeda Y and Asakawa Y, 1992. The enantioselective metabolism of p-cymene in rabbits. Chem. Pharm. Bull. 40(7), 1721-1726.
- 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.
- Melancon MJ, Rickert DE and Lech JJ, 1982. Metabolism of 2-methylnaphthalene in the rat *in vivo*. I. Identification of 2-naphthoylglycine. Drug Metab. Disposition 10(2), 128-133.
- Melancon MJ, Williams DE, Buhler DR and Lech JJ, 1985. Metabolism of 2-methylnaphthalene by rat and Rainbow trout hepatic microsomes and purified cytochromes P-450. Drug. Metab. Disposition 13(5), 542-547.
- Miyazawa M, Shindo M and Shimada T, 2002. Sex differences in the metabolism of (+)- and (-)-limonene enantiomers to carveol and perillyl alcohol derivatives by cytochrome P450 enzymes in rat liver microsomes. Chem. Res. Toxicol. 15(1), 15-20.
- Moreno OM, 1972j. Acute oral toxicity in rats. Myrcene. Toxicological Resources. Project no. 812-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1972k. Acute dermal toxicity in rabbits. alpha-Phellandrene. Toxicological Resources. Project no. 854-72. May 1, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1972l. Acute oral toxicity in rats. 3-Carene. Toxicological Resources. Project no. 843-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1972m. Acute oral toxicity in rats. d-Limonene. Toxicological Resources. Project no. 818-72. May 5, 1970. Unpublished data submitted by EFFA to FLAVIS Secretariat.

- Moreno OM, 1972n. Acute oral toxicity in rats. alpha-Pinene. Toxicological Resources. Project no. 840-72. May 5, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1973ag. Acute oral toxicity in rats. Dermal toxicity in rabbits. gamma-Terpinene. MB Research Laboratories, Inc. Project no. MB 73-203. July 23, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1973ah. Acute oral toxicity in rats. alpha-Terpinene. MB Research Laboratories, Inc. Project no. MB 73-206. July 25, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1973ai. Acute oral toxicity in rats. Dermal toxicity in rabbits. Diphenyl methane. MB Research Laboratories, Inc. Project no. MB 72-14. Date 2/1/73. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1974l. Acute oral toxicity in rats. Dermal toxicity in rabbits. Bisabolene. MB Research Laboratories, Inc. Project no. MB 74-598. August 23, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1974m. Acute oral toxicity in rats. Dermal toxicity in rabbits. Camphene. MB Research Laboratories, Inc. Project no. MB 74-570. June 28, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1975q. Acute dermal toxicity in rats. Dermal toxicity in rabbits. beta-Pinene. MB Research Laboratories, Inc. Project no. MB 75-822. June 25, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1976aa. Acute dermal toxicity in rats. Dermal toxicity in rabbits. Guaiene. Project no. MB 76-1222. July 31, 1976. MB Research Laboratories, Inc. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1976z. Acute oral toxicity in rats. Dermal toxicity in rabbits. Ocimene. MB Research Laboratories, Inc. Project no. MB 76-1033. March 13, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1977ai. Acute oral toxicity in rats. Dermal toxicity in rabbits. Longifolene. MB Research Laboratories, Inc. Project no. MB 77-1706. July 25, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1980m. Oral toxicity in rats. Dermal toxicity in rabbits. Valencene. MB Research Laboratories, Inc. Project no. MB 80-4726. August 30, 1980. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- 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.
- Murata Y, Denda A, Maruyama H and Konishi Y, 1993. Chronic toxicity and carcinogenicity studies of 1-methylnaphthalene in B6C3F1 mice. Fundam. Appl. Toxicol. 21, 44-51.
- Murata Y, Denda A, Maruyama H, Nakae D, Tsutsumi M, Tsujiuchi T and Konishi Y, 1997. Short communication. Chronic toxicity and carcinogenicity studies of 2-methylnaphthalene in B6C3F1 Mice. Fundam. Appl. Toxicol. 36(1), 90-93.

- 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.
- Naismith RW and Matthews RJ, 1986. Developmental toxicity study in rabbits using test article 5601-56-1. Naphthalene. Texaco Inc. EPA Doc 86-870000563, microfiche no. OTS0513641. Date 2/17/86. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Navarro HA, Price CJ, Marr MC, Myers CB, Heindel JJ and Schwetz BA, 1991. Developmental toxicity evaluation of naphthalene (CAS no. 91-20-3) in Sprague-Dawley (CD) rats on gestational days 6 through 15. Chemistry and Life Sciences Research Triangle Institute. NTIS Technical Report (NTIS/PB92-135623). December 1991.
- Navarro HA, Price CJ, Marr MC, Myers CB, Heindel JJ and Schwetz BA, 1992. Final report on the developmental toxicity of naphthalene (CAS no. 91-20-3) in New Zealand White rabbits on gestational days 6 through 19. Chemistry and Life Sciences Research Triangle Institute. NTIS Technical Report (NTIS/PB92-219831). July, 1992.
- 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.
- Nilsen OG, Haugen OA, Zahlsten K, Halgunset J, Helseth A, Aarset H and Eide I, 1988. Toxicity of n-C9 to n-C13 alkanes in the rat on short term inhalation. *Pharmacol. Toxicol.* 62, 259-266.
- 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, 2000d. NTP technical report on the toxicology and carcinogenesis studies of naphthalene (CAS no. 91-20-3) in F344/N rats (inhalation studies). December 2000. NTP-TR 500. NIH Publication no. 01-4434.
- NTP, 2003c. Initial study results from a 90-day toxicity study on beta-myrcene in mice. Study number C99023.
- 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](http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=123%2D35%2D3). 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/>.
- Olson CT, Yu KO, Hobson DW and Serve P, 1986. The metabolism of n-octane in Fischer 344 rats. *Toxicol. Lett.* 31, 147-150.
- Opdyke DLJ, 1975. Fragrance raw materials monographs: 1-Hexanol. Alcohol C-6. Camphene. *Food Cosmet. Toxicol.* 13, 695-696 and 735-738.

- Opdyke DLJ, 1978c. Fragrance raw materials monographs: beta-Pinene. Food Cosmet. Toxicol. 16 (suppl. 1), 859-861.
- Pakenham G, Lango J, Buonarati M, Morin D and Buckpitt A, 2002. Urinary naphthalene mercapturates as biomarkers of exposure and stereoselectivity of naphthalene epoxidation. Drug Metab. Disposition 30(3), 247-253.
- Paumgarten FJ, De-Carvalho RR, Souza CC, Madi K and Chahoud I, 1998. Study of the effects of beta-myrcene on rat fertility and general reproductive performance. Braz. J. Med. Biol. Res. 31(7), 955-965.
- Pellmont B, 1973. Determination of LD50 on mouse and rat. 1,3,5-Undecatriene. June 7, 1973. Unpublished data submitted by EFTA to FLAVIS Secretariat.
- Perbellini L, Amantini MC, Brugnone F and Frontali N, 1982. Urinary excretion of n-hexane metabolites. A comparative study in rat, rabbit and monkey. Arch. Toxicol. 50, 203-215.
- Perbellini L, Brugnone F, Cocheo V, De Rosa E and Bartolucci GB, 1986. Identification of the n-heptane metabolites in rat and human urine. Arch. Toxicol. 58, 229-234.
- Peter H, Wiegand HJ, Bolt HM, Greim H, Walter G, Berg M and Filser JG, 1987. Pharmacokinetics of isoprene in mice and rats. Toxicol. Lett. 36(1), 9-14.
- 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.
- Plasterer MR, Bradshaw WS, Booth GM, Carter MW, Schuler RL and Hardin BD, 1985. Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: naphthalene, p-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea, and four glycol ether derivatives. J. Toxicol. Environ. Health 15, 25-38.
- Plopper CG, Suverkropp C, Morin D, Nishio S and Buckpitt A, 1992. Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. J. Pharmacol. Exp. Ther. 261(1), 353-363.
- Poon GK, Vigushin D, Griggs LJ, Rowlands MG, Coombes RC and Jarman M, 1996. Identification and characterization of limonene metabolites in patients with advanced cancer by liquid chromatography/mass spectrometry. Drug Metab. Disposition 24, 565-571.
- 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.
- 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, Paumgarten 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.
- Rozman K, Summer KH, Rozman T and Greim H, 1982. Elimination of thioethers following administration of naphthalene and diethylmaleate to the rhesus monkey. Drug Chem. Toxicol. 5(3), 265-275.
- 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, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.
- SCF, 1999a. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.
- Schmähl D, 1955. Prüfung von Naphthalin und Anthracen auf cancerogene Wirkung an Ratten. *Z. Krebsforsch.* 60, 697-710. (In German)
- 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.
- Serve MP, Bombick DD, Baughman TM, Jarnot BM, Ketcha M and Mattie DR, 1995. The metabolism of n-nonane in male Fischer 344 rats. *Chemosphere* 31(2), 2661-2668.
- Shapiro, R., 1988. Acute toxicity feeding study in rats in af 20% solution of 1,3,5-undecatriene in 80% pinene supra vehicle. Product Safety Labs. Report no. T-8221. August 29, 1988. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Shelby MD and Witt KL, 1995. Comparision of results from mouse bone marrow chromosome aberration and micronucleus test. *Environ. Mol. Mutag.* 25, 302-313.
- Shelby MD, 1990. Results of NTP-sponsered mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. *Environ. Health Perspect.* 86, 71-73.
- Shopp GM, White KL Jr, Holsapple MP, Barnes DW, Duke SS, Anderson AC, Condie LW Jr, Hayes JR and Borzelleca JF, 1984. Naphthalene toxicity in CD-1 mice: general toxicity and immunotoxicity. *Fundam. Appl. Toxicol.* 4, 406-419.
- Shultz MA, Choudary PV and Buckpitt AR, 1999. Role of murine cytochrome P-450 2F2 in metabolic activation of naphthalene and metabolism of other xenobiotics. *J. Pharmacol. Exp. Ther.* 290(1), 281-288.
- 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.
- Sípal Z and Zelingerová J, 1978. Hydroxylation of diphenylmethane and diphenylethane in rat liver microsomes. In: Fouts, J.R., I., Gut (Eds.). *Industrial and Environmental Xenobiotics. In vitro versus in vivo. Biotransformation and Toxicity.* Excerpta Medica, Amsterdam, pp. 180-183.
- Smithgall TE, Harvey RG and Penning TM, 1986. Regio- and stereospecificity of homogeneous 3alpha-hydroxysteroid-dihydrodiol dehydrogenase for trans-dihydrodiol metabolites of polycyclic aromatic hydrocarbons. *J. Biol. Chem.* 261(14), 6184-6191.

- Smithgall TE, Harvey RG and Penning TM, 1988. Spectroscopic identification of ortho-quinones as the products of polycyclic aromatic transdihydrodiol oxidation catalyzed by dihydrodiol dehydrogenase. *J. Biol. Chem.* 263(4), 1814-1820.
- 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.
- Southwell IAI, Flynn TM and Degrabrielle R, 1980. Metabolism of alpha- and beta-pinene, p-cymene and 1,8-cineole in the brushtail possum. *Xenobiotica*, 10, 17-23.
- 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.
- Stillwell WG, Bouwsma OJ, Thenot J-P and Horning MG, 1978. Methylthio metabolites of naphthalene excreted by the rat. *Res. Commun. Chem. Pathol. Pharmacol.* 20(3), 509-530.
- Stocklinski AW, DeLoach GD and Williams H, 1979. Identification of benzhydrol as a urinary metabolite of diphenylmethane in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* 24(2), 397-400.
- Summer KH, Rozman K, Coulston F and Greim H, 1979b. Urinary excretion of mercapturic acids in chimpanzees and rats. *Toxicol. Appl. Pharmacol.* 50, 207-212.
- Teshima R, Nagamatsu K, Ikebuchi H and Kido Y, 1983. *In vivo* and *in vitro* metabolism of 2-methylnaphthalene in the guinea pig. *Drug Metab. Disposition* 11, 152-157.
- Thornton-Manning JR and Dahl AR, 1997. Metabolic capacity of nasal tissue interspecies comparisons of xenobiotic-metabolizing enzymes. *Mutat. Res.* 380, 43-59.
- 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 naphthalene by human liver microsomes. *Biochem. Pharmacol.* 46, 1529-1538.
- TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- TNO, 2010. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- TNO, 2011. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

- Topping DC, Morgott DA, David RM and O'Donoghue JL, 1994. Ketones. In: Clayton, G.D., Clayton, F.E. (Eds.). *Patty's Industrial Hygiene and Toxicology*, 4<sup>th</sup> Ed. vol. 2C. John Wiley & Sons, Inc., pp. 1739-1878.
- Tsuji M, Fujisaki Y, Arikawa Y, Masuda S, Tanaka T, Sato K, Noda K, Ide H and Kikuchi M, 1975a. Studies on d-limonene, as gallstone solubilizer (IV): Chronic toxicity in dogs. *J. Oyo Yakuri* 10, 775-808.
- Tsuji M, Fujisaki Y, Okubo A, Arikawa Y, Noda K, Ide H and Ikeda T, 1975b. Studies on d-limonene as a gallstone solubilizer (V) effects on development of rat fetuses and offsprings. *J. Oyo Yakuri* 10, 179-186.
- Tummeley 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>
- Vaughn C and Keeler PA, 1976. Toxicological properties and industrial handling hazards of naphthalene concentrate with cover letter dated 08/31/87. Dow Chemical Co. EPA Doc 86-870002059, microfiche no. OTS0517148. Date 3/09/76. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Vigushin DM, Poon GK, Boddy A, English J, Halbert GW, Pagonis C, Jarman M and Coombes RC, 1998. Phase I and pharmacokinetic study of d-limonene in patients with advanced cancer. *Cancer Chemother. Pharmacol.* 42, 111-117.
- Waidyanatha S, Troester MA, Lindstrom AB and Rappaport SM, 2002. Measurement of hemoglobin and albumin adducts of naphthalene-1,2-oxide, 1,2-naphthoquinone and 1,4-naphthoquinone after administration of naphthalene to F344 rats. *Chem. -Biol. Int.* 141, 189-210.
- Walde A, Ve B, Scheline RR and Monge P, 1983. p-Cymene metabolism in rats and guinea pigs. *Xenobiotica* 13, 503-512.
- Watabe T, Hiratsuka A, Ozawa N and Isobe M, 1981. A comparative study on the metabolism of d-limonene and 4-vinylcyclohex-1-ene by hepatic microsomes. *Xenobiotica* 11, 333-344.
- Webb DR, Ridder M and Alden CL, 1989. Acute and subchronic nephrotoxicity of d-limonene in Fischer 344 rats. *Food Chem. Toxicol.* 27(10), 639-649.
- Webb DR, Kanerva RL, Hysell DK, Alden CL and Lehman-McKeeman LD, 1990. Assessment of the subchronic oral toxicity of d-limonene in dogs. *Food Chem. Toxicol.* 28, 669-675.
- White Jr RA and Agosin M, 1980. Metabolism of alpha-pinene by rat liver reconstituted cytochrome P-450 systems. *Development in Biochemistry* 13, 85-88.
- 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 Paumgarten 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.

**ABBREVIATIONS**

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
CPN	Chronic progressive nephropathy
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EC	European Commission
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
GSH	Glutathione
ID	Identity
IOFI	International Organization of the Flavor Industry
Ip	Intraperitoneal
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest Observed Adverse Effect Level

MC	Methylcholanthrene
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PB	Phenobarbital
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA Synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation