Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission related to

Flavouring Group Evaluation 12:
Primary saturated or unsaturated alicyclic alcohol, aldehyde, and esters from chemical group 7

Adopted 23 February 2005

SUMMARY

The Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food is asked 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 is asked to evaluate four flavouring substances in the Flavouring Group Evaluation FGE.12, using the procedure as referred to in the Commission Regulation EC No 1565/2000. These four flavouring substances belong to chemical group 7, Annex I of the Commission Regulation EC No 1565/2000.

The present Flavouring Group Evaluation deals with two primary alicyclic esters, one alcohol, and one aldehyde.

All four flavouring substances possess one or more chiral centres. In each of these cases, the substances have been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer.

The four flavouring candidate substances are classified into structural class I.

Two of the flavouring substances in the present group have been reported to occur in essential oils.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intakes (MSDIs) 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, the four flavouring substances in this group have intakes in Europe from 0.01 to 0.6 microgram/capita/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day) substances.
The flavouring substances are expected to be metabolised to innocuous products at the estimated levels of use as flavouring substances.

The genotoxic potential of this group of flavouring substances cannot be assessed since information on the candidate and supporting substances is missing, but neither the chemical structures of the candidate substances in this group nor the metabolic data available suggest that reactive metabolites could be generated.

It is considered that on the basis of the default MSDI approach these four flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3700 microgram/person/day for the four flavouring substances from structural class I. Thus, the intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for one flavouring substance [FL-no: 05.183]. This substance is also expected to be metabolised to innocuous products.

Thus for three of the four flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these three substances [FL-no: 02.186, 09.342, and 09.670] more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether this evaluation could be applied to the material of commerce, it is necessary to consider the available specifications.

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for the four flavouring substances, except that information on stereoisomerism is missing for all the substances. Thus, the final evaluation of the materials of commerce cannot be performed for the substances, pending further information.

**KEYWORDS**

Primary alicyclic, saturated, unsaturated, alicyclic, alcohols, aldehydes, esters, flavourings, safety
Primary saturated or unsaturated alicyclic alcohol, aldehyde, and esters from chemical group 7

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) 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 others 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 2004/357/EC (EC, 2004). 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, 2000) which is broadly based on the opinion of the Scientific Committee on Food (SCF, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

After the completion of the evaluation programme the positive 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, 1996).

TERMS OF REFERENCE

EFSA is requested to carry out a risk assessment on flavouring substances prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000).

ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 12

1.1. Description

The present Flavouring Group Evaluation, using the procedure as referred to in the Commission Regulation EC No 1565/2000 (EC, 2000) (The Procedure – shown in schematic form in Annex I), deals with four candidate substances from chemical group 7, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000). The four flavouring substances under consideration, as well as their chemical 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.

The four flavouring substances (candidate substances) are related structurally to 15 flavouring substances (supporting substances) evaluated at the 59th JECFA meeting as “Alicyclic Primary Alcohols, Aldehydes, Acids, and Related Esters” (JECFA, 2003a).

Out of the four substances two are esters with a primary saturated or unsaturated alicyclic alcohol moiety, one is a primary alicyclic saturated alcohol, and one is an alicyclic unsaturated aldehyde.

The four candidate substances under consideration in the present evaluation are listed in Table 1, with their chemical names, FL-, CAS-, CoE-, FEMA-numbers, structures, and specifications.

The summary of evaluation results for the candidate substances are listed in Table 2a.
The hydrolysis products of the candidate esters are listed in Table 2b. The names and structures of the 15 supporting substances are listed in Table 3, together with their evaluation status (JECFA, 2003a).

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 variation of 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.).

All of the four candidate substances possess one or more chiral centres. In each of these cases, the substances have been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer (see Table 1).

1.3. Natural Occurrence in Food

Two of the four candidate substances [FL-no: 02.186 and 09.670] have been reported to occur in essential oils. No quantitative data were reported.

Two of the substances (4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183] and cyclogeranyl acetate [FL-no: 09.342] have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

2. Specifications

Purity criteria for the four candidate substances have been provided by the flavouring industry (EFFA, 2003i).

Judged against the requirements in Annex II of Commission Regulation EC No 1565/2000 (EC, 2000), the information is adequate for the four candidate substances. However, information on chirality is needed for all of the four candidate substances (see section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximized 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, 1999).

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, 1999).

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 in 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, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

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

The Maximised Survey-Derived Daily Intake (MSDI (SCF, 1999)) 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 EU population (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, 1999).

In the present Flavouring Group Evaluation (FGE.12) the total annual volume of production of the four candidate substances for use as flavouring substances in Europe has been reported to be approximately 10 kg (EFFA, 2003j) and for 12 of the 15 supporting substances approximately 62 kg (JECFA, 2003a). The remaining three supporting substances are not reported to be used in Europe.

On the basis of the annual volumes of production reported for the four candidate substances, the daily per capita intakes for each of these flavourings have been estimated (Table 2a).

The estimated daily per capita intakes of these candidate substances from use as a flavouring substance are 0.012 [FL-no: 05.183], 0.37 [FL-no: 02.186], 0.24 [FL-no: 09.342], and 0.58 [FL-no: 09.670] microgram, respectively (Table 2a).
3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

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

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

For the four candidate substances information on food categories and normal and maximum use levels\(^2,3\) were submitted by the Flavour Industry (EFFA, 2003i).

The four candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation 1565/2000 (EC, 2000), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

<table>
<thead>
<tr>
<th>Food category</th>
<th>Description</th>
<th>Flavourings used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>Dairy products, excluding products of category 2</td>
<td>All four</td>
</tr>
<tr>
<td>Category 2</td>
<td>Fats and oils, and fat emulsions (type water-in-oil)</td>
<td>All four</td>
</tr>
<tr>
<td>Category 3</td>
<td>Edible ices, including sherbet and sorbet</td>
<td>All four</td>
</tr>
<tr>
<td>Category 4.1</td>
<td>Processed fruits</td>
<td>All four</td>
</tr>
<tr>
<td>Category 4.2</td>
<td>Processed vegetables (incl. mushrooms &amp; fungi, roots &amp; tubers, pulses and legumes), and nuts &amp; seeds</td>
<td>None</td>
</tr>
<tr>
<td>Category 5</td>
<td>Confectionery</td>
<td>All four</td>
</tr>
<tr>
<td>Category 6</td>
<td>Cereals and cereal products, incl. flours &amp; starches from roots &amp; tubers, pulses &amp; legumes, excluding bakery</td>
<td>All four</td>
</tr>
<tr>
<td>Category 7</td>
<td>Bakery wares</td>
<td>All four</td>
</tr>
<tr>
<td>Category 8</td>
<td>Meat and meat products, including poultry and game</td>
<td>All four</td>
</tr>
<tr>
<td>Category 9</td>
<td>Fish and fish products, including molluscs, crustaceans and echinoderms</td>
<td>All four</td>
</tr>
<tr>
<td>Category 10</td>
<td>Eggs and egg products</td>
<td>None</td>
</tr>
<tr>
<td>Category 11</td>
<td>Sweeteners, including honey</td>
<td>None</td>
</tr>
<tr>
<td>Category 12</td>
<td>Salts, spices, soups, sauces, salads, protein products etc.</td>
<td>All four</td>
</tr>
<tr>
<td>Category 13</td>
<td>Foodstuffs intended for particular nutritional uses.</td>
<td>All four</td>
</tr>
<tr>
<td>Category 14.1</td>
<td>Non-alcoholic (&quot;soft&quot;) beverages, excl. dairy products</td>
<td>All four</td>
</tr>
<tr>
<td>Category 14.2</td>
<td>Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts</td>
<td>None</td>
</tr>
<tr>
<td>Category 15</td>
<td>Ready-to-eat savouries</td>
<td>All four</td>
</tr>
<tr>
<td>Category 16</td>
<td>Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15</td>
<td>All four</td>
</tr>
</tbody>
</table>

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2003i).

The mTAMDI values for the four candidate substances from structural class I (see Section 5) range from 1600 to 3700 microgram/person/day.

\(^2\) "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i)

\(^3\) 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).
For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. Absorption, Distribution, Metabolism and Elimination

The four candidate substances in this group evaluation contain a monocyclic or bicyclic monoterpene moiety, all with a primary oxygenated substituent. The evaluation of the metabolism and other aspects of kinetics of the candidate substances in this Flavouring Group Evaluation depend entirely on information for structurally related substances (see Table 3 and Annex III) and on general knowledge on biochemistry and biotransformation of xenobiotic substances.

It can be expected that the two esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids. It can also be expected that these hydrolysis products may be absorbed, and that any remaining unhydrolysed flavouring substance, after absorption, will be hydrolysed in the liver. Gastro-intestinal absorption can also be expected for the free alcohol and the free aldehyde in the present group.

The metabolic fate of the component alcohols, the free candidate alcohol and the one aldehyde in this Flavouring Group is not completely elucidated. It can be expected that oxidation of the hydroxyl group or aldehyde group will result in the formation of carboxylic acids which can be conjugated and excreted. Alternatively, the component or free alcohols in this group may be conjugated to glucuronide or sulphate without any further oxidation. Further, the two cyclohexene derivatives may undergo allylic hydroxylation of the ring and then possible oxidation to keto groups or conjugation with glucuronic acid. These polar metabolites are expected to be excreted in the urine.

Following absorption, the component acids, can be expected to be metabolised further via beta-oxidation (if applicable). Alternatively, they can be expected to be conjugated and excreted. Neither the chemical structures of the candidate substances in this group nor the metabolic data available suggest that reactive metabolites could be generated. Hence, it may be expected that the candidate substances in this flavouring group are absorbed and metabolised to innocuous products, which are excreted.

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. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the four substances the Procedure (Annex I) was applied. The stepwise evaluation of the four substances is summarised in Table 2a.

Step 1.
All four candidate substances are classified in structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2.
Step 2 requires consideration of whether detoxification pathways are available to safely metabolise substances, at the expected levels of intake, to innocuous products.

It is anticipated that the two esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids, and that the component alcohols as well as the candidate alcohol and the one aldehyde will be metabolised to innocuous products at the estimated levels of intake and accordingly proceed via the A-side of the Procedure.

Step A3.

The four candidate substances, which have all been assigned to structural class I, have estimated European daily per capita intakes from use as flavouring substances ranging from 0.012 to 0.58 microgram. These estimated intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

The substances would accordingly not be expected to be of safety concern at their estimated levels of intake based on the MSDI approach.

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

The MSDI range from 0.012 to 0.58 microgram/capita/day, which is below the threshold of concern for substances belonging to structural class I (1800 microgram/person/day).

The estimated intakes for the four candidate substances in structural class I based on the mTAMDI range from 1600 to 3700 microgram/person/day. For one of the substances [FL-no: 05.183] the mTAMDI is below the threshold of concern of 1800 microgram/person/day. For the three candidate substances exceeding the threshold of concern [FL-no: 02.186, 09.342, and 09.670] more reliable intake data are required. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>MSDI (µg/capita/day)</th>
<th>mTAMDI (µg/person/day)</th>
<th>Structural class</th>
<th>Threshold of concern (µg/person/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.186</td>
<td>Myrtanol</td>
<td>0.37</td>
<td>3700</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>05.183</td>
<td>4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal</td>
<td>0.012</td>
<td>1600</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.342</td>
<td>Cyclogeranyl acetate</td>
<td>0.24</td>
<td>3700</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.670</td>
<td>Myrtyl acetate</td>
<td>0.58</td>
<td>3700</td>
<td>Class I</td>
<td>1800</td>
</tr>
</tbody>
</table>

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 Flavouring Group Evaluation 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 is estimated by summing the MSDIs for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003i), the total estimated daily per capita intake as flavourings of the four candidate flavouring substances assigned to structural class I is 1.2 microgram, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day.

The four candidate substances are structurally related to 15 supporting substances evaluated by JEFCA at its 59th meeting (JECFA, 2003a). The estimated combined intake (in Europe) is approximately 8 microgram/capita/day for 12 of the supporting substances assigned to structural class I. The estimated levels of intake in Europe were not reported for three of the supporting substances [FL-no: 02.141, 09.488, and 09.534]. The total estimated combined intake of candidate and supporting substances (in Europe) is approximately 9 microgram which does not exceed the threshold of concern for the corresponding structural class I (1800 microgram/person/day).

8. Toxicity

8.1. Acute Toxicity

No valid studies were available for any of the four candidate substances (see Annex IV, Table IV.1).

Valid studies were available for three supporting substances, 2-cyclohexylethyl acetate [FL-no: 09.028], campholene acetate [FL-no: 09.289], and 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde [FL-no: 05.119] (see Annex IV, Table IV.1). The oral LD50 in rats range from 2190 mg/kg bw for [FL-no: 09.028] to 4640 – 5270 mg/kg bw for [FL-no: 09.289].

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

No studies were available for any of the four candidate substances.

There was one study available for the supporting substance 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde [FL-no: 05.119]. This is a single dose level, 90 day gavage study. The oral dose of 12 mg/kg bw/day to rats did not induce adverse effects in this study (see Annex IV, Table IV.2).

There are no carcinogenicity studies to be found neither for the four candidate substances nor for any of the 15 supporting substances.

8.3. Developmental / Reproductive Toxicity Studies

There are no studies available on developmental or reproductive toxicity neither for the four candidate substances nor for the 15 supporting substances.

8.4. Genotoxicity Studies

There are no studies available on genotoxicity neither for the four candidate nor for the 15 supporting substances. The genotoxic potential of this group of flavouring substances can therefore not be assessed.
9. Conclusions

Out of the four substances two are esters with a primary saturated or unsaturated alicyclic alcohol moiety, one is a primary alicyclic saturated alcohol, and one is an alicyclic unsaturated aldehyde.

All of the four candidate substances possess one or more chiral centres. In each of these cases the substances have been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer.

All of the four candidate substances belong to structural class I.

Two of the flavouring substances in the present group have been reported to occur naturally in essential oils.

According to the default MSDI approach, the four candidate substances in this group have estimated European daily per capita intakes from use as flavouring substances ranging from 0.01 microgram to 0.6 microgram. These estimated intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the four candidate substances belonging to structural class I would result in a total intake of 1.2 microgram/capita/day. This value is lower than the threshold of concern for structural class I. The combined estimated level of intake of 12 of the 15 supporting substances on which European annual production data are available and of the four candidate substances is approximately 9 microgram/capita/day, which is below the threshold of concern for structural class I (1800 microgram/person/day).

The four candidate substances are expected to be absorbed and metabolised to innocuous products, which will subsequently be excreted. The esters are expected to be hydrolysed to component alcohols and carboxylic acids, and the acids subsequently either oxidised completely or conjugated and excreted. The component alcohols, the candidate alcohol, and the candidate aldehyde are expected to be oxidised to carboxylic acids, conjugated and excreted. The two candidate substances, which are cyclohexene derivatives, may also undergo allylic ringhydroxylation and possible further oxidation or conjugation before excretion. Neither the chemical structures of the candidate substances in this group nor the metabolic data available suggest that reactive metabolites could be generated.

No valid toxicity studies have been provided for any of the candidate substances and only one adequate subchronic study was available on a supporting substance.

The genotoxic potential of this group of candidate substances cannot be assessed since information on the candidate and supporting substances is not available. Nevertheless, this does not preclude applying the Procedure for the flavouring substances (SCF, 1999).

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

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3700 microgram/person/day for the four flavouring substances from structural class I. Thus, the intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for one flavouring substance [FL-no: 05.183]. This substance is also expected to be metabolised to innocuous products.
Thus for three of the four flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these three substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the four candidate substances can be applied to the material of commerce, it is necessary to consider the available specifications of purity.

Provided that information on chirality is submitted by Industry for the four candidate substances then adequate specifications have been provided for the four materials of commerce [FL-no: 02.186, 05.183, 09.342, and 09.670] and these would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.
Flavouring Group Evaluation 12

Primary saturated or unsaturated alicyclic alcohol, aldehyde, and esters from chemical group 7

### Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 12

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Mol.formula</th>
<th>Mol.weight</th>
<th>Solubility 1)</th>
<th>Solubility in ethanol 2)</th>
<th>Boiling point, °C 3)</th>
<th>Melting point, °C 4)</th>
<th>ID test</th>
<th>Refrac. Index 4)</th>
<th>Spec.gravity 5)</th>
<th>Specification comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.186</td>
<td>Myrtanol 6)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>514-99-8</td>
<td>Solid</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;O</td>
<td>154.25</td>
<td>Practically insoluble or insoluble 1 ml in 1 ml</td>
<td>116 (16 kPa) 77 MS 95 %</td>
<td>n.a.</td>
<td>n.a.</td>
<td>(R) or (S) enantiomer not specified by CAS no reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>05.183</td>
<td>4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal 6)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>65405-84-7</td>
<td>Liquid</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;O</td>
<td>210.36</td>
<td>Practically insoluble or insoluble 1 ml in 1 ml</td>
<td>305 MS 95 %</td>
<td>1.468-1.474 0.924-0.930</td>
<td>CAS no reported refers to incompletely defined substance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.342</td>
<td>Cyclogeranyl acetate 6)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>54993-30-5</td>
<td>Liquid</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>196.29</td>
<td>Practically insoluble or insoluble 1 ml in 1 ml</td>
<td>98 (13 kPa) MS 95 %</td>
<td>1.464-1.470 0.958-0.964</td>
<td>CAS has deleted CAS no 54993-30-5 and it is substituted by 69842-11-1. Neither (Z) or (E) isomer nor (R) or (S) enantiomer specified by this CAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.670</td>
<td>Myrtanyl acetate 6)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>29021-36-1</td>
<td>Liquid</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>196.29</td>
<td>Practically insoluble or insoluble 1 ml in 1 ml</td>
<td>106 (9 kPa) MS 95 %</td>
<td>1.470-1.476 0.969-0.975</td>
<td>(R) or (S) enantiomer not specified by CAS no reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) Solubility in water, if not otherwise stated
2) Solubility in 95% ethanol, if not otherwise stated
3) At 1013.25 kPa, if not otherwise stated
4) At 20°C, if not otherwise stated
5) At 25°C, if not otherwise stated
6) Stereoisomeric composition not specified
### TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>MSDI 1) (µg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>Outcome on the named compound [4) or 5])</th>
<th>Outcome on the material of commerce [6), 7), or 8])</th>
<th>Evaluation remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.186</td>
<td>Myrtanol</td>
<td><img src="#" alt="Myrtanol structure" /></td>
<td>0.37</td>
<td>Class I A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
<tr>
<td>05.183</td>
<td>4-(2,6,6-Trimethylcyclohexenyl)-2- methylbutanal</td>
<td><img src="#" alt="Structural formula" /></td>
<td>0.012</td>
<td>Class I A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
<tr>
<td>09.342</td>
<td>Cyclogeranyl acetate</td>
<td><img src="#" alt="Cyclogeranyl acetate structure" /></td>
<td>0.24</td>
<td>Class I A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
<tr>
<td>09.670</td>
<td>Myrtanyl acetate</td>
<td><img src="#" alt="Myrtanyl acetate structure" /></td>
<td>0.58</td>
<td>Class I A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>SCF status 1)</th>
<th>JECFA status 2)</th>
<th>Structural class 4)</th>
<th>Procedure path (JECFA) 5)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclogeraniol</td>
<td></td>
<td>Not evaluated as flavouring substance</td>
<td></td>
<td></td>
<td></td>
<td>Not in EU Register</td>
</tr>
<tr>
<td>02.186</td>
<td>Myrtanol</td>
<td></td>
<td></td>
<td>Class I</td>
<td>A3: Intake below threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>08.002</td>
<td>Acetic acid</td>
<td></td>
<td>Category I a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No safety concern b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Category A c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Class I</td>
<td>A3: Intake above threshold, A4: Endogenous</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) Category 1: Considered safe in use  
2) No safety concern at estimated levels of intake  
3) Category A: Flavouring substance, which may be used in foodstuffs  
4) Category B: Flavouring substance which can be used provisionally in foodstuffs  
5) Threshold of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day  
6) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot  

a) (SCF, 1995)  
b) (JECFA, 1999b)  
c) (CoE, 1992)
### TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>JECFA no</th>
<th>MSDI (EU) 1) (µg/capita/day)</th>
<th>SCF status 2)</th>
<th>JECFA status 3)</th>
<th>CoE status 4)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.114</td>
<td>2-(2,2,3-Trimethylcyclopent-3-enyl)ethan-1-ol</td>
<td><img src="image1" alt="Structural formula" /></td>
<td>3741  1901-38-8</td>
<td>970</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>0.012</td>
<td>-</td>
<td>-</td>
<td>No safety concern a)</td>
</tr>
<tr>
<td>02.141</td>
<td>2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethan-1-ol</td>
<td><img src="image2" alt="Structural formula" /></td>
<td>3938  128-50-7</td>
<td>986</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>No safety concern a)</td>
</tr>
<tr>
<td>05.098</td>
<td>p-Menth-1-en-9-al</td>
<td><img src="image3" alt="Structural formula" /></td>
<td>3178  10347  29548-14-9</td>
<td>971</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>0.12</td>
<td>-</td>
<td>-</td>
<td>No safety concern a)</td>
</tr>
<tr>
<td>05.112</td>
<td>2,6,6-Trimethylcyclohex-1-en-1-acetaldehyde</td>
<td><img src="image4" alt="Structural formula" /></td>
<td>3474  10338  472-66-2</td>
<td>978</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>0.24</td>
<td>-</td>
<td>-</td>
<td>No safety concern a)</td>
</tr>
<tr>
<td>05.119</td>
<td>2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde</td>
<td><img src="image5" alt="Structural formula" /></td>
<td>3592  10325  4501-58-0</td>
<td>967</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>No safety concern a)</td>
</tr>
<tr>
<td>FL-no</td>
<td>EU Register name</td>
<td>Structural formula</td>
<td>FEMA no</td>
<td>CoE no</td>
<td>CAS no</td>
<td>JECFA no</td>
<td>MSDI (EU) 1) (µg/capita/day)</td>
<td>SCF status 2)</td>
<td>JECFA status 3)</td>
</tr>
<tr>
<td>-------</td>
<td>---------------------------------------------------------------------------------</td>
<td>--------------------</td>
<td>---------</td>
<td>--------</td>
<td>--------</td>
<td>----------</td>
<td>-----------------------------</td>
<td>---------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>05.123</td>
<td>5-Isopropenyl-2-methylcyclopentanecarboxaldehyde</td>
<td></td>
<td>3645</td>
<td>55253-28-6</td>
<td></td>
<td>968</td>
<td>0.012</td>
<td>No safety concern a)</td>
<td></td>
</tr>
<tr>
<td>08.034</td>
<td>Cyclohexylacetic acid</td>
<td></td>
<td>2347</td>
<td>5292-21-7</td>
<td></td>
<td>965</td>
<td>0.12</td>
<td>No safety concern a)</td>
<td>Category B b)</td>
</tr>
<tr>
<td>08.060</td>
<td>Cyclohexaneacrylic acid</td>
<td></td>
<td>3531</td>
<td>11911</td>
<td>98-89-5</td>
<td>961</td>
<td>0.061</td>
<td>No safety concern a)</td>
<td>-</td>
</tr>
<tr>
<td>08.067</td>
<td>1,2,5,6-Tetrahydrocuminic acid</td>
<td></td>
<td>3731</td>
<td>71298-42-5</td>
<td></td>
<td>976</td>
<td>0.012</td>
<td>No safety concern a)</td>
<td>-</td>
</tr>
<tr>
<td>09.028</td>
<td>2-Cyclohexyethyl acetate</td>
<td></td>
<td>2348</td>
<td>218</td>
<td>21722-83-8</td>
<td>964</td>
<td>0.97</td>
<td>No safety concern a)</td>
<td>Deleted b)</td>
</tr>
<tr>
<td>09.289</td>
<td>alpha-Campholene acetate</td>
<td></td>
<td>3657</td>
<td>36789-59-0</td>
<td></td>
<td>969</td>
<td>0.061</td>
<td>No safety concern a)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Supporting Substances Summary

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>JECFA no</th>
<th>Specification available</th>
<th>MSDI (EU) 1) (µg/capita/day)</th>
<th>SCF status 2)</th>
<th>JECFA status 3)</th>
<th>CoE status 4)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.488</td>
<td>Ethyl cyclohexanepropionate</td>
<td><img src="image" alt="Structure" /></td>
<td>2431</td>
<td>2095</td>
<td>10094-36-7</td>
<td>966</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>ND</td>
<td>No safety concern a)</td>
<td>Deleted b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.534</td>
<td>Ethyl cyclohexanecarboxylate</td>
<td><img src="image" alt="Structure" /></td>
<td>3544</td>
<td>11916</td>
<td>3289-28-9</td>
<td>963</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>ND</td>
<td>No safety concern a)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.536</td>
<td>Methyl cyclohexanecarboxylate</td>
<td><img src="image" alt="Structure" /></td>
<td>3568</td>
<td>11920</td>
<td>4630-82-4</td>
<td>962</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>0.073</td>
<td>No safety concern a)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.615</td>
<td>p-Menth-1-en-9-yl acetate</td>
<td><img src="image" alt="Structure" /></td>
<td>3566</td>
<td>10748</td>
<td>28839-13-6</td>
<td>972</td>
<td>JECFA specification (JECFA, 2002d)</td>
<td>0.85</td>
<td>No safety concern a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) 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) Deleted: Substances for which CoE Committee of Experts had no information as to their real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992)  
5) a) (JECFA, 2002c)  
6) b) (CoE, 1992)  
ND Not detected
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, 2000), 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, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

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

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\(^4\) (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\(^5\) (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.

---

\(^4\) "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).

\(^5\) "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

Step 1.

Decision tree structural class

Step 2.

Can the substance be predicted to be metabolised to innocuous products?

Step A3.

Yes

Do the conditions of use result in an intake greater than the threshold of concern for the structural class?

No

Step A4.

Yes

Is the substance or are its metabolites endogenous?

No

Step A5.

Does a NOAEL exist for the substance which provides an adequate margin of safety under conditions of intended use, or does a NOAEL exist for structurally related substances which is high enough to accommodate any perceived difference in toxicity between the substance and the related substances?

Yes

No

Step B3.

Yes

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

No

Step B4.

Yes

Substance would not be expected to be of safety concern

No

Step B5.

Yes

Additional data required

No

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, 2000). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

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

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

The “normal and maximum use levels” are provided by Industry (EFFA, 2003i) for the four candidate substances in the present flavouring group (Table II.1.2).

### Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.12 (EFFA, 2003i)

<table>
<thead>
<tr>
<th>Food Categories</th>
<th>Normal use levels (mg/kg)</th>
<th>Maximum use levels (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL-no</td>
<td>01.0</td>
<td>02.0</td>
</tr>
<tr>
<td>02.186</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>05.183</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>09.342</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>09.670</td>
<td>35</td>
<td>25</td>
</tr>
</tbody>
</table>

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.
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, 2000) 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, 2000)
- 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, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum

The mTAMDI values (see Table II.2.3) are presented for each of the four flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003i). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

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

<table>
<thead>
<tr>
<th>Class of product category</th>
<th>Intake estimate (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beverages (non-alcoholic)</td>
<td>324.0</td>
</tr>
<tr>
<td>Foods</td>
<td>133.4</td>
</tr>
<tr>
<td>Exception a: Confectionery</td>
<td>27.0</td>
</tr>
<tr>
<td>Exception b: Condiments, seasonings</td>
<td>20.0</td>
</tr>
<tr>
<td>Exception c: Alcoholic beverages</td>
<td>20.0</td>
</tr>
<tr>
<td>Exception d: Soups, savouries</td>
<td>20.0</td>
</tr>
<tr>
<td>Exception e: Others, e.g. chewing gum</td>
<td>e.g. 2.0 (chewing gum)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Food categories according to Commission Regulation 1565/2000</th>
<th>Distribution of the seven SCF food categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Key</td>
<td>Food</td>
</tr>
<tr>
<td>01 Dairy products, excluding products of category 02.0</td>
<td>Food</td>
</tr>
<tr>
<td>02 Fats and oils, and fat emulsions (type water-in-oil)</td>
<td>Food</td>
</tr>
<tr>
<td>03 Edible ices, including sherbet and sorbet</td>
<td>Food</td>
</tr>
<tr>
<td>04.1 Processed fruit</td>
<td>Food</td>
</tr>
<tr>
<td>04.2 Processed vegetables (incl. mushrooms &amp; fungi, roots &amp; tubers, pulses &amp; legumes), and nuts &amp; seeds</td>
<td>Food</td>
</tr>
<tr>
<td>05 Confectionery</td>
<td>Exception a</td>
</tr>
<tr>
<td>06 Cereals and cereal products, incl. flours &amp; starches from roots &amp; tubers, pulses &amp; legumes, excluding bakery</td>
<td>Food</td>
</tr>
<tr>
<td>07 Bakery wares</td>
<td>Food</td>
</tr>
<tr>
<td>08 Meat and meat products, including poultry and game</td>
<td>Food</td>
</tr>
<tr>
<td>09 Fish and fish products, including molluscs, crustaceans and echinoderms (MCE)</td>
<td>Food</td>
</tr>
<tr>
<td>10 Eggs and egg products</td>
<td>Food</td>
</tr>
<tr>
<td>11 Sweeteners, including honey</td>
<td>Exception a</td>
</tr>
<tr>
<td>12 Salts, spices, soups, sauces, salads, protein products, etc.</td>
<td>Exception d</td>
</tr>
<tr>
<td>13 Foodstuffs intended for particular nutritional uses</td>
<td>Food</td>
</tr>
<tr>
<td>14.1 Non-alcoholic (&quot;soft&quot;) beverages, excl. dairy products</td>
<td>Beverages</td>
</tr>
<tr>
<td>14.2 Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts</td>
<td>Exception c</td>
</tr>
<tr>
<td>15 Ready-to-eat savouries</td>
<td>Exception b</td>
</tr>
<tr>
<td>16 Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0</td>
<td>Food</td>
</tr>
</tbody>
</table>
Table II.2.3 mTAMDI (µg/person/day) and MSDI (µg/capita/day) for substances allocated to structural class I (Threshold of concern for structural class I: 1800 µg/person/day)

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>MSDI (µg/capita/day)</th>
<th>mTAMDI (µg/person/day)</th>
<th>Structural class</th>
<th>Threshold of concern (µg/person/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.186</td>
<td>Myrtanol</td>
<td>0.37</td>
<td>3700</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>05.183</td>
<td>4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal</td>
<td>0.012</td>
<td>1600</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.342</td>
<td>Cyclogeranyl acetate</td>
<td>0.24</td>
<td>3700</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.670</td>
<td>Myrtyl acetate</td>
<td>0.58</td>
<td>3700</td>
<td>Class I</td>
<td>1800</td>
</tr>
</tbody>
</table>
ANNEX III: METABOLISM

III.1. Introduction

The four candidate flavouring substances in this group evaluation are myrtanol and its acetate [FL-no: 02.186 and 09.670, respectively], the acetate of cyclogeraniol [FL-no: 09.342], and an aldehyde: 4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-methylbutanal [FL-no: 05.183]. For none of these candidate substances, absorption, distribution, metabolism or elimination studies were found in the published or available unpublished literature. The assessment of the toxicokinetic properties of this group of substances relies therefore on general knowledge about biotransformation and data for representatives of a group of 15 structurally related (supporting) substances, which have been evaluated during the 59th meeting of JECFA. "Safety evaluations of groups of related flavouring agents: Alicyclic primary alcohols, aldehydes, acids, and related esters" (JECFA, 2003a).

III.2. Absorption, Distribution, Metabolism and Elimination

Candidate Substances

III.2.1. Ester Hydrolysis

Two of the candidate substances in this Flavouring Group Evaluation are esters of alicyclic alcohols and acetic acid: cyclogeranyl acetate [FL-no: 09.342], and myrtanyl acetate [FL-no: 09.670], which can be expected to be subject to hydrolysis.

Ester hydrolysis is catalysed by classes of enzymes known as carboxylesterases (Graffner-Nordberg et al., 1998), the most important of which are the B-esterases. Although these enzymes are present in most mammalian tissues, they predominate in the liver (Heymann, 1980; Graffner-Nordberg et al., 1998). The substrate specificity of B-carboxylesterase isoenzymes has been correlated with the structure of the alcohol and acid components (Heymann, 1980). Aliphatic esters used as flavour ingredients hydrolyse rapidly in liver homogenate, simulated pancreatic fluid, simulated gastric fluid and preparations of intestinal mucosa in vitro (Junge & Heymann, 1979; Leegwater & Straten, 1974a; Leegwater & Straten, 1974b; Longland et al., 1977; Grundschober, 1977; Graffner-Nordberg et al., 1998). Results of in vitro studies indicate that the affinity of the esterases for their substrates increases as the length of the ester increases and that the rate of hydrolyses of the straight-chain esters is approximately 100 times faster than the rate of hydrolysis of the branched-chain esters (Arndt & Krisch, 1973; Butterworth et al., 1975a; Junge & Heymann, 1979).

Cyclohexanecarboxylate methyl ester and cyclohexanecarboxylate ethyl ester were incubated separately with 50 ml simulated gastric fluid at 37° C, for six hours. Results showed approximately 20 % hydrolysis of each ester in the gastric fluid system. After a five-hour incubation in simulated intestinal fluid, approximately 40 and 50 % of cyclohexanecarboxylate methyl- and ethyl esters were hydrolysed, respectively (Moran & Tyburcy, 1979). In an in vitro hydrolysis study, 100 % of cyclohexanepropionate ethyl ester was hydrolysed after two-hours of incubation in 5 % pancreatin (Grundschober, 1977; Leegwater & Straten, 1974a).
The in vitro hydrolysis of the structurally related ester, p-1-(7)8-menthadien-2-yl acetate\(^6\), was investigated in rat liver homogenate. After incubation of this substance in homogenate at 37°C for 15, 30 and 60 minutes, complete (100%) hydrolysis was observed after 60 minutes, with 92% hydrolysis occurring within the first 15 minutes (Salzer, 1998).

These data indicate that after oral exposure, the two candidate esters in this group of flavouring substances [FL-no: 09.342 and 09.670] will be hydrolysed either prior to absorption by enzymes in the gastro-intestinal tract or by esterases in the liver after absorption to yield their component alcohols and carboxylic acids. The component acid (acetic acid) from these two esters has been evaluated previously (e.g. FGE.01 or FGE.02) and will not be further discussed in this FGE.

### III.2.2. Absorption, Distribution and Excretion

For the candidate substances, data on absorption, distribution and excretion are not available. Some data are available on the sodium salt of the supporting substance cyclohexanecarboxylic acid [FL-no: 08.060]\(^6\) and on the related substance perillyl alcohol\(^6\).

**Cyclohexanecarboxylic acid**

Cyclohexanecarboxylate sodium salt, with a \(^{14}C\)-labelled ring was orally administered to male Wistar albino rats at a dose of 100 mg/kg bw. Results showed that > 98% of the original dose was excreted as urinary metabolites within seven hours. Less than 1% was excreted via the faeces or expired air (Brewster et al., 1977b).

Cyclohexanecarboxylic acid and 1-methyl-1-cyclohexanecarboxylate were studied in bile-duct- and urinary tract-cannulated rats. Female Sprague-Dawley rats (four rats per compound) were administered via intravenous infusion a 0.52 mmol/kg bw bolus dose of cyclohexanecarboxylic acid (66 mg/kg bw) or 1-methyl-1-cyclohexanecarboxylate (73 mg/kg bw), followed by a 0.3 ml saline flush for each rat. Hardly any parent substance was excreted into urine or bile. Biliary excretion of base-labile (presumably glucuronide) conjugates accounted for approximately 5 and 59%, and urinary excretion accounted for 12 and 25% of the systemic elimination of cyclohexanecarboxylate and 1-methyl-1-cyclohexanecarboxylate, respectively. The authors concluded that enterohepatic circulation occurs with 1-methyl-1-cyclohexanecarboxylic acid but not with cyclohexanecarboxylic acid itself (Liu et al., 1992).

**Perillyl alcohol**

The kinetics of p-mentha-1,8-dien-7-ol (i.e. perillyl alcohol) have been studied in rats, dogs and in humans. This substance is most closely related to p-mentha-1,8(10)-dien-9-ol and its acetate [FL-no: 02.122 and 09.809, respectively].

Within four hours after a single dose of 1000 mg perillyl alcohol/kg bw, administered to female Wistar-Furth rats via gavage, major plasma metabolites were identified as oxidised metabolites of...
perillyl alcohol (perillic acid and dihydroperillic acid). No trace of perillyl alcohol was detected in
the plasma at any time point, including 15-minutes post-gavage (Haag & Gould, 1994).

Two beagle dogs (male and female) administered 250 mg perillyl alcohol/kg bw by gavage,
exhibited peak plasma levels of oxidised metabolites of perillyl alcohol (i.e. perillic acid and
dihydroperillic acid) at 1 and 5 hours post-administration, respectively. Analysis of blood
specimens collected before dosing and at 19 time points ranging from 10 minutes to 48 hours after
dosing, indicated the presence of the oxidised metabolites 10-minutes post-administration. The
parent substance, perillyl alcohol, was undetectable in the plasma (Phillips et al., 1995).

Patients with various advanced malignancies were treated orally with of 800, 1600 or 2400 mg
perillyl alcohol/m²/dose (equivalent to ca. 32, 64, or 96 mg/kg bw/dose, assuming a body mass
index of 25 kg/m²). On the first day only a single dose was given, but thereafter the treatment was
continued for four weeks but on a three doses per day basis. Kinetics were studied after the first and
last dose. The parent drug was not detected in the plasma. Peak plasma levels for the two main
metabolites of perillyl alcohol occurred at 1.5 – 3.5 hours (perillic acid) and 3 - 5 hours
(dihydroperillic acid) post-administration. Plasma elimination half-lives of the two metabolites
studied were 1 - 6 hours and 2 - 3 hours, respectively. Repeated dosing did not affect Cmaxs, or
AUCs for these two metabolites, but there was a clear “levelling of” of Cmaxs and AUCs for the
metabolites when the dose increased from 1600 to 2400 mg/m². From the patients treated with 2400
mg/m²/dose, urinary metabolites were collected up to 24 hours after the first dose or up to 6 hours
after the last dose. In both cases ~ 1 % of the dose was collected as unchanged perillic alcohol.
Approximately 10 % of the dose was recovered, less than 10 % of which was unchanged parent
substance (Ripple et al., 1998).

As part of a phase I dose-escalation trial, perillyl alcohol was administered p.o. at 800, 1200, or
1600 mg/ m²/dose (equivalent to ca. 32, 48, or 64 mg/kg bw/dose, assuming a body mass index of
25 kg/m²) to sixteen patients with advanced refractory malignancies on a four times per day
continuous basis for four weeks to characterise its kinetic profile, maximum tolerated dose, toxicity
and antitumour activity. There appeared to be a dose-dependent increase in the plasma levels of the
two main metabolites, perillic acid and dihydroperillic acid (see below). There was a trend toward
decreasing metabolite levels on day 29 as compared to days 1 and 2. Peak metabolite levels were
seen 1 - 3 hours post-administration and metabolite half-lives were about 2 hours. No indication of
dose-related effects on the kinetics was obtained. Approximately 9 % of the total dose was
recovered in the urine in the first 24 hours,. Only ~ 0.1 % of the dose was recovered as parent
substance (Ripple et al., 2000).

From the above mentioned studies it can be concluded that in humans, dogs and rats orally
administered perillyl alcohol is rapidly absorbed and metabolised after ingestion.

III.2.3.  Biotransformation

**Cyclohexyl Derivatives**

Metabolism studies conducted on representative flavouring agents indicate that these substances are
metabolised primarily by oxidation of the primary alcohol or aldehyde function to yield the
corresponding carboxylic acid or oxidation of the alkyl ring substituents to yield polyoxygenated
polar metabolites that are readily excreted.

The metabolic options available to alicyclic substances increase as the number and types of
functional groups and ring substituents in the molecule increase. If a primary alcohol, aldehyde or
carboxylic acid function is present on an alkyl side-chain of the ring, the substance may undergo
beta-oxidation at the side chain. For the present group of flavouring substances, this seems in
particular important for [FL-no: 05.183], because this is the only one with a side chain may be shortened by beta-oxidation. If the number of carbons present in the side-chain is odd, beta-oxidative cleavage cannot continue beyond the point of side-chain attachment but the resulting carboxylic acids may be conjugated with glucuronic acid or glycine (Bernhard & Caflisch-Weill, 1945; Brewster et al., 1977b; Williams, 1959).

**Terpenoid Primary Alcohols, and Aldehydes**

An indication about the metabolic fate of the monocyclic and bicyclic terpenoid aldehydes and alcohols (e.g. candidate substance 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal, [FL-no: 05.183], and candidate substance myrtanol [FL-no: 02.186] and supporting substances) can be obtained from the biotransformations of representative supporting substance aldehydes p-mentha-1,8-dien-7-al (i.e. perillaldehyde) and 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (i.e. myrtenal), which have been described below. In addition, for the metabolism of the flavouring substances cyclogeranyl acetate [FL-no: 09.342] and 4-(2,6,6-trimethylcyclohexenyl) –2-methylbutenal [FL-no: 05.183], in which multiple and cycloalkene methyl side-chains occur, the metabolism of isophorone (3,5,5-trimethylcyclohex-2-ene-1-one (FL-no: 07.126)) might be used as an example.

**Isophorone**

When isophorone was given to rabbits in an oral dose of 1 g/kg bw, in the urine glucuronic acid conjugates could be detected, and after treatment of the urine with hydrochloric acid, the metabolite 5,5-dimethyl-cyclohex-1-ene-3-one-1-carboxylic acid was found. This shows that for these substances oxidation of the methyl side chain is a possible metabolic pathway, which, probably via alcohol and aldehyde intermediates, leads to formation of free or conjugated carboxylic acid end products (Truhaut et al., 1970).

**Alpha- and beta-ionone**

The two candidate substances 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183] and cyclogeranyl acetate [FL-no: 09.342] are structurally related to beta-ionone [FL-no: 07.008] and alpha-ionone [FL-no: 07.007], respectively. Available metabolic data on these two ionones indicate that they may undergo allylic ring hydroxylation and possible further oxidation to keto groups. These reactions result in the formation of polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acid (JECFA, 1999a). It is anticipated the the two candidate substances [FL-no: 05.183 and 09.342], at least partially, may form similar polar metabolites and be excreted with the urine.

**Perillyl alcohol and perillaldehyde**

The metabolism of perillyl alcohol, perillaldehyde and perillic acid was determined after intravenous injection in male Wistar rats and in exposed isolated rat hepatocytes. Although perillaldehyde can react spontaneously with glutathione, no indication of the formation of GSH conjugates was found either in vivo or in hepatocytes. After dosing with perillaldehyde about 50 % of the dose were recovered as glucuronides in bile and urine. From perillic acid only the acyl
glucuronide was generated, whereas perillyl alcohol and perillaldehyde formed both acyl and ether glucuronides. The results, together with those of studies in which alcohol dehydrogenase or aldehyde dehydrogenase were inhibited, indicate that perillaldehyde is a major intermediary metabolite of perillyl alcohol in the rat \textit{in vivo} and in rat hepatocytes \textit{in vitro} (Boon et al., 2000).

To six male rabbits \textit{p}-mentha-1,8-dien-7-al (perillaldehyde) was administered orally at a dose level of 2000 mg per animal. Urine was collected for three consecutive days, pooled and treated with glucuronidase/aryl sulphatase. The neutral urinary fraction contained two metabolites comprising 7 % of the totally administered amount of parent substance. These metabolites were identified as \((-\)-perillyl alcohol and \((-\)-cis-shisool (i.e. \textit{para}-menth-8-en-7-ol), representing 46 and 39 % of the neutral metabolites, respectively. The acidic fraction comprised 39 % of the administered amount of perillaldehyde and the two major metabolites in this fraction were perillic acid, which represented 57 % of the acidic urinary metabolites and \(p\)-isopropylbenzoic acid (amount not specified). These results indicate that perillaldehyde was oxidised to \textit{p}-mentha-1,8-dien-7-carboxylic acid (i.e. perillic acid). Aromatisation of the cyclohexene ring and reduction of the isopropenyl double bond converted perillic acid in part to \(p\)-isopropylbenzoic acid. To a lesser extent, \textit{p}-mentha-1,8-dien-7-al was reduced to perillyl alcohol, which can be selectively hydrogenated to yield \textit{p}-mentha-8-en-7-ol (see Figure III.1) (Ishida et al., 1989b). Only a fairly low part of the administered dose was recovered. Other metabolites were not mentioned.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FigureIII1.png}
\caption{Metabolism of perillaldehyde in rabbits}
\end{figure}

Female Wistar-Furth rats were given a single oral dose of 100 mg perillyl alcohol/kg bw by gavage or were given a diet of 2 % perillyl alcohol for a period of 3, 5, or 10 weeks (nominally approximately 1.5 g/kg bw/d). Perillic acid and dihydroperillic acid were detected as major plasma metabolites and perillic acid methyl ester and dihydroperillic acid methyl ester were identified as minor metabolites. The authors concluded that the methyl esters were artifacts formed during
processing of urine. Unchanged perillyl alcohol was not detected, neither after the gavage dose, not even at 15 minutes post gavage, and also not after sub-chronic feeding. These results indicate that perillyl alcohol is rapidly absorbed from the gastrointestinal tract and metabolised. The presence of dihydroperillic acid indicates that the endocyclic double bond was hydrogenated. After acute exposure the ratio perillic acid / dihydroperillic acid amounted to > 10, while after 3 – 10 weeks of exposure via the diet this ratio had dropped to 2 – 3 (Haag & Gould, 1994).

An in vivo study conducted in male Wistar rats confirmed that the oxidation of perillyl alcohol to perillic acid involved perillaldehyde as an intermediate. Rats were administered intravenously perillyl alcohol, perillaldehyde or perillic acid at a dose of 80 micromol/kg bw (approximately 12.2, 12.0, or 13.3 mg/kg bw, respectively). Urine and bile were collected for two consecutive hours post administration. In all cases, the glucuronic acid conjugate of perillic acid was the predominant metabolite detected in the urine (10 % of the dose) and bile (46 % of the dose). The glucuronic acid conjugate of perillyl alcohol was also a major biliary metabolite following the intravenous administration of perillyl alcohol (5 %), while urinary excretion of this conjugate amounted to 1 % of the dose. Based on the results, the authors concluded that within two hours, approximately 56 % of the original dose had been oxidised to perillic acid through perillaldehyde, and eventually excretion as a glucuronic acid conjugate (Boon et al., 2000).

Patients with various advanced malignancies were treated orally with one dose, followed by three daily doses on the following 29 days, of 2400 mg perillyl alcohol/m² (equivalent to ca. 96 mg/kg bw, assuming a body mass index of 25 kg/m²). Urinary metabolites were collected up to 24 hours after the first dose or up to 6 hours after the last dose. In both cases ~ 1 % of the dose was collected as unchanged perillic alcohol. Two metabolites were found, which comprised approximately 9 % of the dose of which ~ 90 % perillic acid and 10 % dihydroperillic acid. Other metabolites were not monitored (Ripple et al., 1998).

As part of a phase I dose-escalation trial, perillyl alcohol was administered p.o. at 1200 or 1600 mg/m²/dose (equivalent to ca. 48 or 64 mg/kg bw/dose, assuming a body mass index of 25 kg/m²) to sixteen patients with advanced refractory malignancies on a four times per day continuous basis for four weeks. Approximately 9 % of the total dose was recovered in the urine in the first 24 hours on the first day of treatment and slightly more was recovered on day 15 or 29 during 6 hours post dosing. At all time points, approximately 80 to 85 % of the recovered metabolites were perillic acid and 10 to 17 % was dihydroperillic acid. Only about 1 % of the dose was recovered as parent substance. Other metabolites were not monitored (Ripple et al., 2000).

Myrtenal

Six male rabbits received an oral dose of 2000 mg of 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (= (-)-myrtenal) per animal. In the urine of these animals myrtenol, dihydromyrtenol, myrtenic acid and perillic acid could be detected. Myrtenol and dihydromyrtenol comprised together 99 % of the neutral metabolite fraction (5 % of the dose). Myrtenic acid represented 76 % of the acid metabolites detected in the urine, but the amount of perillic acid was not specified. The total acid fraction of urinary metabolites comprised 24 % of the dose. These results indicate that myrtenal can be metabolised to the corresponding carboxylic acid (myrtenic acid). The presence of perillic acid indicates some cleavage of the strained bicyclic ring. To a lesser extent, the aldehyde can either be reduced to myrtenol, which may be conjugated with glucuronic acid and excreted or it may undergo hydrogenation of the double bond to yield dihydromyrtenol (myrtanol) see Figure III.2; which is one of the candidate substances [FL-no: 02.186], is shown to be the major neutral metabolite and is excreted unchanged with the urine (Ishida et al., 1989b). Urine was collected during 3 days post dosing. Only a fairly low part of the administered dose was recovered. Other metabolites were not mentioned.
Humans exposed to sawmill dust excreted the glucuronic acid conjugate of myrtenol (2-hydroxymethyl-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene; [FL-no: 02.091]; the component alcohol in candidate flavouring substances [FL-no: 09.899 and 09.900]) in the urine (Eriksson & Levin, 1990). The myrtenol was not detected in the sawdust (Eriksson & Levin, 1990), but could have originated from side-chain oxidation of alpha-pinene (= 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene; [FL-no: 01.004]; (Ishida et al., 1981).

![Figure III.2 Metabolism of myrtenal in rabbits](image)

**Figure III.2 Metabolism of myrtenal in rabbits**

In summary, in mammals, monocyclic or bicyclic terpenoid primary alcohols (e.g. candidate substances cyclogeraniol [from FL-no: 09.342] and myrtanol [FL-no: 02.186], and the structurally related substance perillyl alcohol) are generally oxidised to the corresponding carboxylic acid, conjugated with glucuronic acid, and are excreted as urinary metabolites. The same is true for the monocyclic aldehyde (candidate substance 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183]) and structurally related substances perilaldehyde and isophorone), which contain alkyl ring substituents. In a minor pathway, the aldehyde may be reduced to the alcohol and excreted as the glucuronide (Ishida et al., 1989b; Haag & Gould, 1994). If an endocyclic double bond is present, reduction of this double bond may occur.

**III.3. Conclusions**

The four candidate substances in this group evaluation contain a monocyclic or bicyclic terpenoid moiety, all with a primary oxygenated substituent. The evaluation of the metabolism and other aspects of kinetics of the candidate substances in this Flavouring Group Evaluation depend entirely on information for structurally related substances and on general knowledge on biochemistry and biotransformation of xenobiotic substances.

It can be expected that the esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids. It can also be expected that these hydrolysis products may be absorbed, and that any remaining unhydrolysed flavouring substance after absorption will be hydrolysed in the
liver. Gastro-intestinal absorption can also be expected for the free alcohol and the free aldehyde in this group.

The metabolic fate of the component alcohols, the free candidate alcohols and the one aldehyde in this Flavouring Group is not completely elucidated. It can be expected that oxidation of the hydroxyl group or aldehyde group will result in the formation of carboxylic acids which can be conjugated and excreted. Alternatively, the component or free alcohols in this group may be conjugated to glucuronide or sulphate without any further oxidation. Further, the two cyclohexene derivatives may undergo allylic hydroxylation of the ring and then possible oxidation to keto groups or conjugation with glucuronic acid. These polar metabolites are expected to be excreted in the urine.

Following absorption, the component acids, can be expected to be metabolised further via beta-oxidation (if applicable). Alternatively, they can be expected to be conjugated and excreted via the urine.

Neither the chemical structures of the candidate substances in this group nor the metabolic data available suggest that reactive metabolites could be generated. Hence, it may be expected that the candidate substances in this flavouring group are absorbed and metabolised to innocuous products, which are excreted.
ANNEX IV: TOXICITY

Oral acute toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 7, and for 9 supporting substances evaluated by JECFA at the 59th meeting. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Species</th>
<th>Sex</th>
<th>Route</th>
<th>LD50 (mg/kg bw)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cyclohexancarboxylic acid [08.060])</td>
<td>Rat</td>
<td>M, F</td>
<td>Gavage</td>
<td>3265</td>
<td>(Moran et al., 1980)</td>
<td>Study acceptable but number of dosage groups, and thus number of animals tested, has not been referred.</td>
</tr>
<tr>
<td>(Methyl cyclohexancarboxylate [09.536])</td>
<td>Rat</td>
<td>M, F</td>
<td>Gavage</td>
<td>3881</td>
<td>(Moran et al., 1980)</td>
<td>Study acceptable but number of dosage groups has not been referred.</td>
</tr>
<tr>
<td>(Ethyl cyclohexancarboxylate [09.534])</td>
<td>Rat</td>
<td>M, F</td>
<td>Gavage</td>
<td>3962</td>
<td>(Moran et al., 1980)</td>
<td>Study acceptable but number of dosage groups has not been referred.</td>
</tr>
<tr>
<td>(Cyclohexaneethyl acetate [09.028])</td>
<td>Rat</td>
<td>NR</td>
<td>Oral</td>
<td>3200</td>
<td>(Wohl, 1974c)</td>
<td>Not adequate LD50 study.</td>
</tr>
<tr>
<td>(2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde [05.119])</td>
<td>Rat</td>
<td>NR</td>
<td>Oral</td>
<td>4300</td>
<td>(BIBRA, 1976)</td>
<td>The LD50 value cited is deduced according to Litchfield &amp; Wilcoxon, 1949. Another LD50 value is also cited in the BIBRA study, 3900 mg/kg, deduced according to Weill, 1952.</td>
</tr>
<tr>
<td>(Campholene acetate [09.289])</td>
<td>Rat</td>
<td>M, F</td>
<td>Gavage</td>
<td>M: 4640 – 5270 F: 3000</td>
<td>(Piccirillo et al., 1979)</td>
<td>The study is considered valid.</td>
</tr>
<tr>
<td>(alpha-Campholenic alcohol [02.114])</td>
<td>Rat</td>
<td>NR</td>
<td>Oral</td>
<td>1000 – 2000</td>
<td>(Levenstein, 1982)</td>
<td>Study is inadequate for determination of LD50. Also substance name is only given as code.</td>
</tr>
<tr>
<td>(1,2,5,6-Tetrahydrocuminic acid [08.067])</td>
<td>Rat</td>
<td>NR</td>
<td>Gavage</td>
<td>&gt; 2500</td>
<td>(Levenstein, 1981)</td>
<td>Study inadequate for derivation of LD50. Also only code name given for substance.</td>
</tr>
<tr>
<td>(4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal [05.183])</td>
<td>Rat</td>
<td>NR</td>
<td>Oral</td>
<td>&gt; 5000</td>
<td>(Moreno, 1977i)</td>
<td>Study inadequate for derivation of LD50. Also substance name given as ‘catonal’. It has not been possible to confirm that this is the same substance.</td>
</tr>
<tr>
<td>(10-Hydroxymethylene-2-pinene [02.141])</td>
<td>Rat</td>
<td>NR</td>
<td>Oral</td>
<td>890</td>
<td>(Moreno, 1977a)</td>
<td>Study acceptable, but substance name given as Nopol. It has not been possible to confirm that this is the same substance.</td>
</tr>
</tbody>
</table>

NR = Not reported.
M = Male; F = Female.
Subacute / subchronic / chronic / Carcinogenic toxicity data are available for none of the candidate substance of the present flavouring group evaluation from chemical group 7 but for one supporting substances evaluated by JECFA at the 59th meeting.

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

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Species; Sex No./Group</th>
<th>Route</th>
<th>Dose levels</th>
<th>Duration</th>
<th>NOAEL (mg/kg/day)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2,2,3-Trimethylcyclopent-3-en-1-yl)acetaldehyde [05.119]</td>
<td>Rat; M, F 8</td>
<td>Gavage</td>
<td>12 mg/kg bw/day</td>
<td>90</td>
<td>12</td>
<td>(BIBRA, 1976)</td>
<td>This is a single dose study.</td>
</tr>
</tbody>
</table>

*M = Male; F = Female.

1 This study was performed at either a single dose or multiple dose levels that produced no adverse effects.
**TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES**

No developmental and reproductive toxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 7 or for the supporting substances evaluated by JECFA at the 59th meeting.

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

No *in vitro* mutagenicity/genotoxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 7 or for supporting substances evaluated by JECFA at the 59th meeting.

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

No *in vivo* mutagenicity/genotoxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 7 or for supporting substances evaluated by JECFA at the 59th meeting.
REFERENCES:


Brewster, D., Jones, R.S., Parke, D.V., 1977b. The metabolism of cyclohexanecarboxylic acid in the isolated perfused rat liver. Xenobiotica 7(10), 601-609.


Ishida, T., Toyota, M., Asakawa, Y., 1989b. Terpenoid biotransformation in mammals. V. Metabolism of (+)-citronellal,(-)-7-hydroxycitronellal, citral, (-)-perillaldehyde, (-)-myrtenal, cuminaldehyde, thujone, and (+-)-carvone in rabbits. Xenobiotica 19(8), 843-855.


SCIENTIFIC PANEL MEMBERS


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