European Food Safety Authority

EFSA Journal 2012;10(12):2993

## SCIENTIFIC OPINION

# Scientific Opinion on Flavouring Group Evaluation 12, Revision 3 (FGE.12Rev3): Primary saturated or unsaturated alicyclic alcohol, aldehyde, acid, and esters from chemical group 7[1]

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

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 10 flavouring substances in the Flavouring Group Evaluation 12 (FGE.12), including an additional substance in revision 3, using the Procedure in Commission Regulation (EC) No 1565/2000. This revision is made due to inclusion of one additional flavouring substance, 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182]. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that all 10 substances [FL-no: 02.134, 02.186, 05.157, 05.182, 05.183, 05.198, 08.135, 09.342, 09.670 and 09.829] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all 10 candidate substances.



## KEY WORDS

Primary alicyclic, saturated, unsaturated, alicyclic, alcohols, aldehydes, esters, flavourings, safety.

---

1 On request from the European Commission, Question No EFSA-Q-2012-00682, adopted on 20 November 2012.

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

3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Leon Brimer, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific Opinion.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 12, Revision 3 (FGE.12Rev3): Primary saturated or unsaturated alicyclic alcohol, aldehyde, acid, and esters from chemical group 7. EFSA Journal 2012;10(12):2993. [45 pp.]. doi:10.2903/j.efsa.2012.2993. Available online: www.efsa.europa.eu/efsajournal.htm

## SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 10 flavouring substances in the Flavouring Group Evaluation 12, Revision 3 (FGE.12Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 10 primary saturated or unsaturated alicyclic alcohol, aldehyde, acid and esters belong to chemical group 7, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.12, FGE.12Rev3, includes the assessment of one additional flavouring substance, 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], compared to FGE.12Rev2.

Eight flavouring substances possess one or more chiral centres and additionally, due to the presence of a double bond, one of these substances can exist as geometric isomer. For all eight substances, the stereoisomeric composition has been specified sufficiently.

The 10 flavouring substances are classified into structural class I according to the decision tree approach presented by Cramer et al., 1978.

Four of the flavouring substances in the present group have been reported to occur in essential oils and in a few 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, the 10 flavouring substances in this group have intakes in Europe from 0.011 to 43 microgram*/capita/*day, which are below the threshold of concern value for structural class I (1800 microgram/person/day) substances.

The genotoxic potential of this group of flavouring substances cannot be assessed since information on the flavouring substances or on structurally related substances is missing. However, this does not preclude evaluation of the flavouring substances in the present group using the Procedure.

The flavouring substances are expected to be metabolised to innocuous products at the estimated levels of intake as flavouring substances.

It is considered that on the basis of the default MSDI approach these 10 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 2 to 5000 microgram/person/day for the 10 flavouring substances from structural class I. For six of the substances the intakes were above the threshold of concern for structural class I of 1800 microgram/person/day. Thus, for six 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 six substances [FL-no: 02.134, 02.186, 08.135, 09.342, 09.670 and 09.829] 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. The four substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

In order to determine whether this evaluation could be applied to the material of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity tests for the materials of commerce have been provided for each of the 10 flavouring substances.

For these 10 flavouring substances [FL-no: 02.134, 02.186, 05.157, 05.182, 05.183, 05.198, 08.135, 09.342, 09.670 and 09.829], the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

## TABLE OF CONTENTS

## BACKGROUND

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

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

The 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 Union list of flavourings and source materials is established in Commission Regulation (EC) No 872/2012 (EC, 2012a).

## HISTORY OF THE EVALUATION

The first version of the Flavouring Group Evaluation 12 (FGE.12) dealt with four primary saturated or unsaturated alicyclic alcohol, aldehyde, acid and esters.

The first Revision of FGE.12 (FGE.12Rev1) included the assessment of three additional candidate substances [FL-no: 02.134, 05.137 and 05.198]. Additional information on two substances [FL-no: 05.183 and 09.342] was made available since FGE.12 was published.

The second Revision of FGE.12, FGE.12Rev2, includes the assessment of two additional candidate substances [FL-no: 08.135 and 09.829]. No toxicity and/or metabolism data were provided for these substances. Furthermore, for four substances [FL-no: 02.186, 05.157, 05.198 and 09.670], information from Industry (EFFA, 2010a) on stereoisomeric composition and missing specifications, received after publication of the last revision, was included in Revision 2.

| FGE | Opinion adopted by EFSA | Link | No. of candidate substances |
|---|---|---|---|
| FGE.12 | 23 February 2005 | http://www.efsa.europa.eu/en/scdocs/scdoc/208.htm | 4 |
| FGE.12Rev 1 | 28 August 2008 | http://www.efsa.europa.eu/en/scdocs/scdoc/791.htm | 7 |
| FGE.12Rev 2 | 30 September 2010 | http://www.efsa.europa.eu/en/efsajournal/doc/1846.pdf | 9 |
| FGE.12Rev 3 | 20 Novembre 2012 | | 10 |

The present Revision of FGE.12, FGE.12Rev3, includes the assessment of one additional candidate substance, 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182]. No toxicity or metabolism data were provided for the substance. A search in open literature did not provide any further data on toxicity or metabolism for the substance.

Furthermore, additional information on stereoisomeric composition has been submitted for four substances [FL-no: 02.186, 05.157, 05.198 and 09.670] (EFFA, 2012v).

## TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

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 the 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. The evaluation programme was finalised at the end of 2009.

In addition, the Commission has asked EFSA to reflect newly submitted information on specifications in the revisions of FGEs.

## ASSESSMENT

### 1. Presentation of the Substances in Flavouring Group Evaluation 12, Revision 3

#### 1.1. Description

The present Flavouring Group Evaluation 12, Revision 3 (FGE.12Rev3), 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), deals with 10 candidate substances from chemical group 7, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 10 flavouring substances under consideration, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

Out of the 10 substances, one is a primary saturated alicyclic acid [FL-no: 08.135], three are esters, two [FL-no: 09.342 and 09.670] with a primary saturated or unsaturated alicyclic alcohol moiety and one [FL-no: 09.829] is an ethyl ester of a saturated alicyclic carboxylic acid, two substances [FL-no: 02.134 and 02.186] are primary alicyclic saturated alcohols and four are alicyclic unsaturated aldehydes [FL-no: 05.157, 05.182, 05.183 and 05.198].

A summary of the safety evaluation is summarised in Table 2a.

The 10 flavouring substances (candidate substances) are related structurally to 15 flavouring substances (supporting substances) evaluated at the 59$^{th}$ JECFA meeting as "Alicyclic Primary Alcohols, Aldehydes, Acids, and Related Esters" (JECFA, 2003a). The supporting substances, with the respective structural formulas, FEMA, CoE, and CAS register numbers, evaluation status by Scientific committee on Food (SCF), JECFA, and by CoE and the European Maximised Survey-derived Daily Intake (MSDI) values, are listed in Table 3.

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

#### 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.).

Eight of the 10 flavouring substances possess one or more chiral centres and additionally, due to the presence of a double bond, one of these substances [FL-no: 05.198] can exist as geometric isomer. The stereoisomeric composition has been specified for all eight substances (see Table 1).

#### 1.3. Natural Occurrence in Food

Three of the 10 candidate substances 2-cyclohexylethan-1-ol [FL-no: 02.134], myrtanol [FL-no: 02.186] and myrtanyl acetate [FL-no: 09.670] have been reported to occur in essential oils. One

substance, 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], has been reported to occur naturally in grape brandy and tomato (TNO, 2012). No quantitative data were reported.

According to TNO, six of the substances isocyclocitral [FL-no: 05.157], 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183], alpha-methyl ional [FL-no: 05.198], 4-(2,2,3-trimethylcyclopentyl)butanoic acid [FL-no: 08.135], cyclogeranyl acetate [FL-no: 09.342] and ethyl cyclohexyl acetate [FL-no: 09.829] have not been reported to occur naturally in any food items (TNO, 2000; TNO, 2009).

## 2. Specifications

Purity criteria for the 10 candidate substances have been provided by the Flavouring Industry (EFFA, 2003i; EFFA, 2004z; Flavour Industry, 2009i).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the information is adequate for all 10 candidate substances (see Section 1.2 and Table 1).

## 3. Intake Data

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

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

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

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

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

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

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

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995a). 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[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present Flavouring Group Evaluation (FGE.12Rev3) the total annual volume of production of the 10 candidate substances for use as flavouring substances in Europe has been reported to be approximately 370 kg (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; EFFA, 2011e; Flavour Industry, 2009i). Three hundred fifty kg of this amount is accounted for by one of these flavouring substances, 4-(2,2,3-trimethylcyclopentyl)butanoic acid [FL-no: 08.135]. For the 15 supporting substances the total annual volume of production is approximately 370 kg (JECFA, 2003a).

On the basis of the annual volumes of production reported for the 10 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a).

The estimated daily *per capita* intake of 4-(2,2,3-trimethylcyclopentyl)butanoic acid [FL-no: 08.135] from use as a flavouring substance is 43 microgram. For the remaining substances, the estimated daily *per capita* intake is less than 1 microgram each (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 10 candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; EFFA, 2012q; Flavour Industry, 2009i).

The 10 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

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

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

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

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

**Table 3.1 Use of Candidate Substances in Various Food Categories**

| Food category | Description | Flavourings used |
|---|---|---|
| 01.0 | Dairy products, excluding products of category 2 | All except [FL-no: 05.182] |
| 02.0 | Fats and oils, and fat emulsions (type water-in-oil) | All except [FL-no: 05.182, 08.135] |
| 03.0 | Edible ices, including sherbet and sorbet | All except [FL-no: 08.135] |
| 04.1 | Processed fruits | All except [FL-no: 08.135] |
| 04.2 | Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds | None |
| 05.0 | Confectionery | All |
| 06.0 | Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery | All except [FL-no: 05.182, 08.135] |
| 07.0 | Bakery wares | All except [FL-no: 08.135] |
| 08.0 | Meat and meat products, including poultry and game | All except [FL-no: 05.182, 08.135] |
| 09.0 | Fish and fish products, including molluscs, crustaceans and echinoderms | All except [FL-no: 05.182, 08.135] |
| 10.0 | Eggs and egg products | None |
| 11.0 | Sweeteners, including honey | Only [FL-no: 08.135] |
| 12.0 | Salts, spices, soups, sauces, salads, protein products etc. | All except [FL-no: 05.182, 08.135] |
| 13.0 | Foodstuffs intended for particular nutritional uses | All except [FL-no: 05.182, 08.135] |
| 14.1 | Non-alcoholic ("soft") beverages, excl. dairy products | All |
| 14.2 | Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts | All except [FL-no: 05.182] |
| 15.0 | Ready-to-eat savouries | All except [FL-no: 05.182, 08.135] |
| 16.0 | Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15 | All except [FL-no: 05.182] |

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 0.002 - 20 mg/kg food, and the maximum use levels are in the range of 0.02 - 100 mg/kg (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; EFFA, 2012q; Flavour Industry, 2009i).

The mTAMDI values for the 10 candidate substances from structural class I (see Section 5) range from 2 to 5000 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

All 10 candidate substances in this group evaluation contain a monocyclic or bicyclic alicyclic moiety with substituents containing a primary alcohol, aldehyde, carboxylic acid or ester function. 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 is expected that the three esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids. It is also anticipated that these hydrolysis products may be absorbed and that any remaining unhydrolysed flavouring esters, after absorption, will be hydrolysed in the liver. Gastro-intestinal absorption can also be expected for the alcohols, carboxylic acids and the aldehydes in the present group.

The metabolic fate of the three component alcohols, the two candidate alcohols and the four aldehydes 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 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. One substance [FL-no: 05.198] has a double bond in the side chain. This is not anticipated to alter the major metabolic pathways outlined above.

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.

For more detailed information, see Annex III.

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

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

For the safety evaluation of the 10 candidate substances from chemical group 7 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2a.

Step 1

All 10 candidate substances are classified into structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

It is anticipated that the three esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids, and that the component alcohols and carboxylic acids as well as the two candidate alcohols, the four aldehydes and the carboxylic acid 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 10 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 0.011 to 43 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 0.011 to 43 microgram/capita/day. These figures are below the threshold of concern value for substances belonging to structural class I (1800 microgram/person/day).

The estimated intakes for the 10 candidate substances in structural class I based on the mTAMDI range from 2 to 5000 microgram/person/day. For four of the substances [FL-no: 05.157, 05.182, 05.183 and 05.198], the mTAMDI is below the threshold of concern of 1800 microgram/person/day. For six candidate substances [FL-no: 02.134, 02.186, 08.135, 09.342, 09.670 and 09.829], the mTAMDI is exceeding the threshold of concern.

Thus, for six substances [FL-no: 02.134, 02.186, 08.135, 09.342, 09.670 and 09.829] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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

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

| FL-no | EU Register name | MSDI (µg/*capita*/day) | mTAMDI (µg/person/day) | Structural class | Threshold of concern (µg/person/day) |
|---|---|---|---|---|---|
| 02.134 | 2-Cyclohexylethan-1-ol | 0.011 | 3900 | Class I | 1800 |
| 02.186 | Myrtanol | 0.37 | 3900 | Class I | 1800 |
| 05.157 | Isocyclocitral | 0.011 | 1600 | Class I | 1800 |
| 05.182 | 2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde | 0.061 | 2.1 | Class I | 1800 |
| 05.183 | 4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal | 0.012 | 1600 | Class I | 1800 |
| 05.198 | alpha-Methyl ional | 0.011 | 1600 | Class I | 1800 |
| 08.135 | 4-(2,2,3-Trimethylcyclopentyl)butanoic acid | 43 | 5000 | Class I | 1800 |
| 09.342 | Cyclogeranyl acetate | 0.24 | 3900 | Class I | 1800 |
| 09.670 | Myrtanyl acetate | 0.58 | 3900 | Class I | 1800 |
| 09.829 | Ethyl cyclohexyl acetate | 0.61 | 3900 | Class I | 1800 |

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

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

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

On the basis of the reported annual production volumes in Europe (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; EFFA, 2011e; Flavour Industry, 2009i), the combined estimated daily *per capita* intake as flavourings of the 10 candidate flavouring substances assigned to structural class I is 45 microgram,

which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day.

The 10 candidate substances are structurally related to 15 supporting substances evaluated by the JEFCA at its 59th meeting (JECFA, 2003a). The estimated combined intake (in Europe) is approximately 41 microgram/*capita*/day for the 15 of the supporting substances assigned to structural class I. The total estimated combined intake of candidate and supporting substances (in Europe) would be approximately 86 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

Studies were available for three of the 10 candidate substances and for nine supporting substances. The oral $LD_{50}$ in rats range from 890 to 5270 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

No studies were available for any of the 10 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 days gavage study. The oral dose of 12 mg/kg bw/day to rats did not induce adverse effects in this study.

There are no carcinogenicity studies to be found neither for the 10 candidate substances nor for any of the 15 supporting substances.

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

### 8.3. Developmental / Reproductive Toxicity Studies

There are no studies available on developmental or reproductive toxicity neither for the 10 candidate substances nor for the 15 supporting substances.

### 8.4. Genotoxicity Studies

There are no studies available on genotoxicity neither for the 10 candidate nor for the 15 supporting substances. The genotoxic potential of this group of flavouring substances can therefore not be assessed properly. However, this does not preclude evaluation of the candidate substances in the present group using the Procedure (SCF, 1999a).

## CONCLUSIONS

The present Revision of FGE.12, FGE.12Rev3, includes the assessment of one additional candidate substance, 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], compared to FGE.12Rev2.

Out of the total 10 substances, one is an alicyclic saturated acid, two are primary alicyclic saturated alcohols, four are alicyclic unsaturated aldehydes and three are esters, two with a primary saturated or unsaturated alicyclic alcohol moiety and one is an ethyl ester of a saturated alicyclic carboxylic acid.

Eight of the 10 flavouring substances possess one or more chiral centres and additionally, due to the presence of a double bond, one of these substances can exist as geometric isomer. For all eight substances, the stereoisomeric composition has been specified sufficiently.

All of the 10 candidate substances belong to structural class I according to the decision tree approach presented by Cramer et al., 1978.

Four of the flavouring substances in the present group have been reported to occur naturally in essential oils and in a few foods.

According to the default MSDI approach, the 10 candidate substances in this group have estimated European daily per capita intakes from use as flavouring substances ranging from 0.011 microgram to 43 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 10 candidate substances, belonging to structural class I, would result in a total intake of 45 microgram/capita/day. This value is below the threshold of concern for structural class I. The total combined intakes of the 15 supporting substances and the 10 candidate substances is approximately 86 microgram/capita/day, which is below the threshold of concern for structural class I (1800 microgram/person/day).

The genotoxic potential of this group of flavouring substances cannot be assessed since information on the candidate and supporting substances is missing. However, this does not preclude evaluation of the flavouring substances in the present group using the Procedure.

The 10 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 alcohols, and the candidate aldehydes are expected to be oxidised to carboxylic acids, conjugated and excreted. The candidate substances, which are cyclohexene derivatives, may also undergo allylic ring hydroxylation 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.

It is considered that on the basis of the default MSDI approach the 10 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 2 to 5000 microgram/person/day for the 10 flavouring substances from structural class I. The intakes were above the threshold of concern for structural class I of 1800 microgram/person/day for six flavouring substances [FL-no: 02.134, 02.186, 08.135, 09.342, 09.670 and 09.829] and below the threshold for four flavouring substances [FL-no: 05.157, 05.182, 05.183 and 05.198]. Thus, for six of the 10 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 six 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. The four substances, which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 10 candidate substances can be applied to the material 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 each of the 10 flavouring substances.

For these 10 flavouring substances [FL-no: 02.134, 02.186, 05.157, 05.182, 05.183, 05.198, 08.135, 09.342, 09.670 and 09.829], the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

**Table 1:** Specification Summary of the Substances in the Flavouring Group Evaluation 12, Revision 3

**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 12, Revision 3**

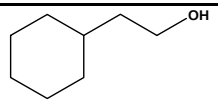
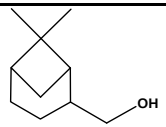
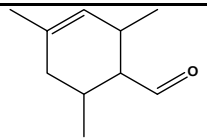
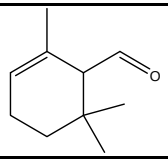
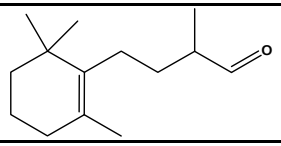
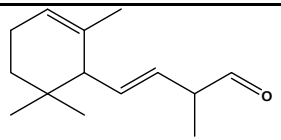
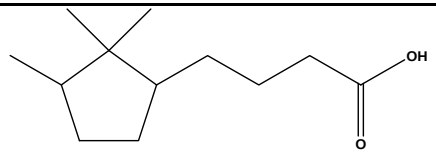
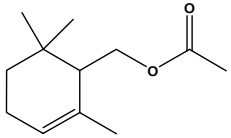
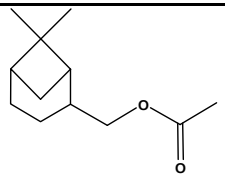
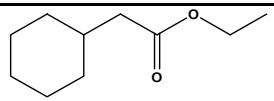
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility 1)<br>Solubility in ethanol 2) | Boiling point, °C 3)<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index 4)<br>Spec.gravity 5) | Specification comments |
|---|---|---|---|---|---|---|---|---|
| 02.134 | 2-Cyclohexylethan-1-ol |  | <br><br>4442-79-9 | Liquid<br>$C_8H_{16}O$<br>128.21 | Slightly soluble<br>Freely soluble | 222<br><br>MS<br>95 % | 1.463-1.469<br>0.918-0.924 | |
| 02.186 | Myrtanol |  | <br><br>514-99-8 | Solid<br>$C_{10}H_{18}O$<br>154.25 | Practically insoluble or insoluble<br>Freely soluble | 116 (16 hPa)<br>77<br>MS<br>95 % | n.a.<br>n.a. | Mixture of four diastereoisomers (EFFA, 2010a).<br>Four diastereoisomers, 20 - 30 % each, with a higher likelyhood for the trans forms (EFFA, 2012v). |
| 05.157 | Isocyclocitral |  | <br><br>1335-66-6 | Liquid<br>$C_{10}H_{16}O$<br>152.23 | Practically insoluble or insoluble<br>Freely soluble | 214<br>-55<br>MS<br>95 % | 1.484-1.490<br>0.885-0.891 | Mixture of two positional isomers (95 % sum of isomers, mainly 2,4,6-trimethyl-cyclohex-3-ene-1-carbaldehyde) (EFFA, 2010a). CASrn in Register refers to "Incomplet defined structure" (positions of two methyl groups not assigned).<br>Mixture of 8 diastereoisomers (approximately 12.5 % each) (EFFA, 2012v). |
| 05.182 | 2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde |  | 3639<br>10326<br>432-24-6 | Liquid<br>$C_{10}H_{16}O$<br>152.23 | Insoluble<br>Soluble | 62 (0.4 hPa)<br><br>MS<br>99 % | 1.476-1.483<br>0.950-0.957 | Racemate (EFFA, 2012v). |
| 05.183 | 4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal |  | <br><br>65405-84-7 | Liquid<br>$C_{14}H_{24}O$<br>210.36 | Practically insoluble or insoluble<br>Freely soluble | 305<br><br>MS<br>95 % | 1.468-1.474<br>0.924-0.930 | Racemate. CASrn in Register to be changed to 73398-85-3.<br>New CASrn refers to the racemate. |

**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 12, Revision 3**

| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight | Solubility 1)<br>Solubility in ethanol 2) | Boiling point, °C 3)<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index 4)<br>Spec.gravity 5) | Specification comments |
|---|---|---|---|---|---|---|---|---|
| 05.198 | alpha-Methyl ional |  | 58102-02-6 | Liquid<br>$C_{14}H_{22}O$<br>206.33 | Practically insoluble or insoluble<br>Freely soluble | 90 (0.1 hPa)<br><br>MS<br>95 % | 1.485-1.491<br>0.911-0.917 | Mixture of (Z)- & (E)-isomers. Name to be changed to 3-Butenal, 2-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl) (EFFA, 2010a). E-form (60 - 90 %); Z-form (10 - 40 %). In each case racemate (EFFA, 2012v). |
| 08.135 | 4-(2,2,3-Trimethylcyclopentyl)butanoic acid |  | 4529<br><br>957136-80-0 | Liquid<br>$C_{12}H_{22}O_2$<br>198 | Slightly soluble<br>Partially soluble | 140 - 143<br><br>NMR MS<br>99 % | 1.461-1.467<br>0.955-0.961 | Composition of mixture: 50 - 60 % 4-((1S,3R)-2,2,3-trimethylcyclopentyl)butanoic acid;<br>32 - 40 % 4-((1R,3S)- ;<br>0 - 6 % 4-((1S,3S)- ;<br>0 - 4 % 4-((1R,3R)- |
| 09.342 | Cyclogeranyl acetate |  | 54993-30-5 | Liquid<br>$C_{12}H_{20}O_2$<br>196.29 | Practically insoluble or insoluble<br>Freely soluble | 98 (13 hPa)<br><br>MS<br>95 % | 1.464-1.470<br>0.958-0.964 | Racemate. CASrn in Register to be changed to 69842-11-1. New CASrn refers to the racemate. |
| 09.670 | Myrtanyl acetate |  | 29021-36-1 | Liquid<br>$C_{12}H_{20}O_2$<br>196.29 | Practically insoluble or insoluble<br>Freely soluble | 106 (9 hPa)<br><br>MS<br>95 % | 1.470-1.476<br>0.969-0.975 | Mixture of R,S-enantiomers, i.e. the (+)- and (-)- (cis)- and (trans)-isomers, mixture of all diastereoisomers (EFFA, 2010a). Four diastereoisomers, 20 - 30 % each, with a higher likelyhood for the trans forms. (EFFA, 2012v). |
| 09.829 | Ethyl cyclohexyl acetate |  | 2348<br>218<br>5452-75-5 | Liquid<br>$C_{10}H_{18}O_2$<br>170.25 | Practically insoluble or insoluble<br>Freely soluble | 211<br><br>NMR MS<br>95 % | 1.442-1.450<br>0.945-0.948 | |

*1) Solubility in water, if not otherwise stated.*

*2) Solubility in 95 % ethanol, if not otherwise stated.*

*3) At 1013.25 hPa, if not otherwise stated.*

*4) At 20°C, if not otherwise stated.*

*5) At 25°C, if not otherwise stated.*

*6) Stereoisomeric composition not specified.*

**Table 2a:** Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

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

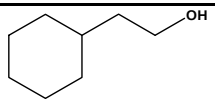
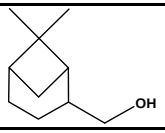
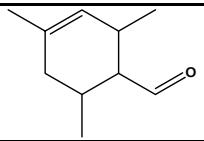
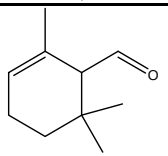
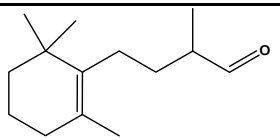
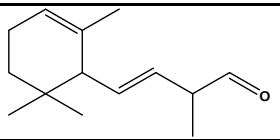
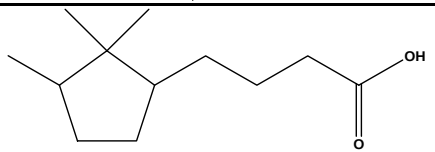
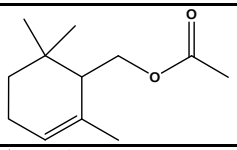
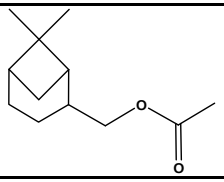
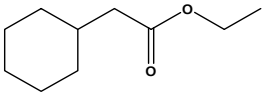
| FL-no | EU Register name | Structural formula | MSDI 1) (µg/capita/day) | Class 2) Evaluation procedure path 3) | Outcome on the named compound [ 4) or 5] | Outcome on the material of commerce [6), 7), or 8)] | Evaluation remarks |
|---|---|---|---|---|---|---|---|
| 02.134 | 2-Cyclohexylethan-1-ol |  | 0.011 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 02.186 | Myrtanol |  | 0.37 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 05.157 | Isocyclocitral |  | 0.011 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 05.182 | 2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde |  | 0.061 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 05.183 | 4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal |  | 0.012 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 05.198 | alpha-Methyl ional |  | 0.011 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 08.135 | 4-(2,2,3-Trimethylcyclopentyl)butanoic acid |  | 43 | Class I<br>A3: Intake below threshold | 4) | 6) | |

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

| FL-no | EU Register name | Structural formula | MSDI 1) (µg/capita/day) | Class 2) Evaluation procedure path 3) | Outcome on the named compound [ 4) or 5] | Outcome on the material of commerce [6), 7), or 8)] | Evaluation remarks |
|---|---|---|---|---|---|---|---|
| 09.342 | Cyclogeranyl acetate |  | 0.24 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 09.670 | Myrtanyl acetate |  | 0.58 | Class I<br>A3: Intake below threshold | 4) | 6) | |
| 09.829 | Ethyl cyclohexyl acetate |  | 0.61 | Class I<br>A3: Intake below threshold | 4) | 6) | |

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

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

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

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

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

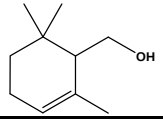
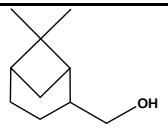
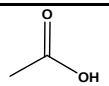
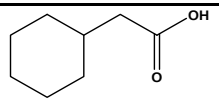
6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).*

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

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

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

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

| FL-no | EU Register name<br>JECFA no | Structural formula | SCF status 1)<br>JECFA status 2)<br>CoE status 3)<br>EFSA status | Structural class 4)<br>Procedure path (JECFA) 5) | Comments |
|---|---|---|---|---|---|
| | Cyclogeraniol |  | Not evaluated as flavouring substance | | Not in EU-Register. |
| 02.078 | Ethanol<br>41 |  | Category 1 a)<br>No safety concern b) | No evaluation | At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents. |
| 02.186 | Myrtanol |  | FGE.12 | Class I<br>A3: Intake below threshold | |
| 08.002 | Acetic acid<br>81 |  | Category 1 a)<br>No safety concern c)<br>Category A d) | Class I<br>A3: Intake above threshold,<br>A4: Endogenous | |
| 08.034 | Cyclohexylacetic acid<br>965 |  | No safety concern e)<br>Category B d) | Class I<br>A3: Intake below threshold | |

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

*2) No safety concern at estimated levels of intake.*

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

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

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

*a) (SCF, 1995).*

*b) (JECFA, 1997a).*

*c) (JECFA, 1999b).*

*d) (CoE, 1992).*

*e) (JECFA, 2002c).*

*ND: Not detected.*

**Table 3:** Supporting Substances Summary

**Table 3: Supporting Substances Summary**

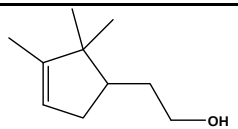
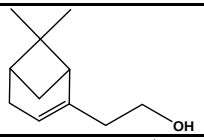
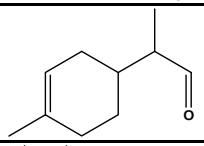
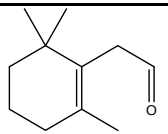
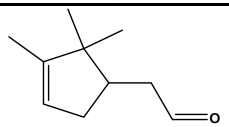
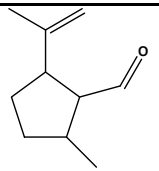
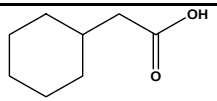
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | JECFA no<br>Specification available | MSDI (EU) 1)<br>(µg/capita/day) | SCF status 2)<br>JECFA status 3)<br>CoE status 4) | Comments |
|---|---|---|---|---|---|---|---|
| 02.114 | 2-(2,2,3-Trimethylcyclopent-3-enyl)ethan-1-ol |  | 3741<br><br>1901-38-8 | 970<br>JECFA specification (JECFA, 2002d). | 0.012 | No safety concern a) | |
| 02.141 | 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethan-1-ol |  | 3938<br><br>128-50-7 | 986<br>JECFA specification (JECFA, 2002d). | 33 | No safety concern a) | |
| 05.098 | p-Menth-1-en-9-al |  | 3178<br>10347<br>29548-14-9 | 971<br>JECFA specification (JECFA, 2002d). | 0.12 | No safety concern a) | |
| 05.112 | 2,6,6-Trimethylcyclohex-1-en-1-acetaldehyde |  | 3474<br>10338<br>472-66-2 | 978<br>JECFA specification (JECFA, 2002d). | 0.24 | No safety concern a) | |
| 05.119 | 2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde |  | 3592<br>10325<br>4501-58-0 | 967<br>JECFA specification (JECFA, 2002d). | 5.0 | No safety concern a) | |
| 05.123 | 5-Isopropenyl-2-methylcyclopentanecarboxaldehyde |  | 3645<br><br>55253-28-6 | 968<br>JECFA specification (JECFA, 2002d). | 0.012 | No safety concern a) | JECFA evaluated cis-5-isopropenyl-cis-methylcyclopentan-1-carboxaldehyde (CASrn as in Register). CASrn in Register refers to the (Z,Z)-isomer. |
| 08.034 | Cyclohexylacetic acid |  | 2347<br>34<br>5292-21-7 | 965<br>JECFA specification (JECFA, 2002d). | 0.12 | No safety concern a)<br>Category B b) | |

**Table 3: Supporting Substances Summary**

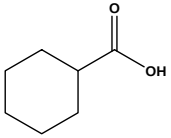
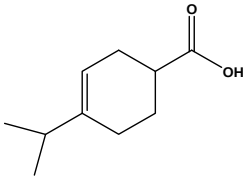
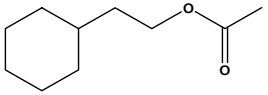
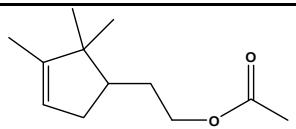
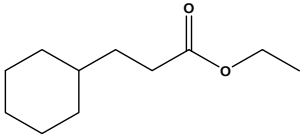
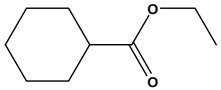
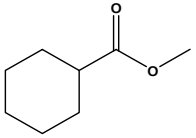
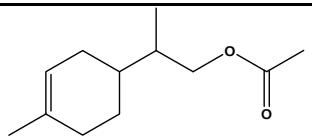
| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | JECFA no<br>Specification available | MSDI (EU) 1)<br>(μg/capita/day) | SCF status 2)<br>JECFA status 3)<br>CoE status 4) | Comments |
|---|---|---|---|---|---|---|---|
| 08.060 | Cyclohexanecarboxylic acid |  | 3531<br>11911<br>98-89-5 | 961<br>JECFA specification (JECFA, 2002d). | 0.061 | No safety concern a) | |
| 08.067 | 1,2,5,6-Tetrahydrocuminic acid |  | 3731<br><br>71298-42-5 | 976<br>JECFA specification (JECFA, 2002d). | 0.012 | No safety concern a) | |
| 09.028 | 2-Cyclohexylethyl acetate |  | 2348<br>218<br>21722-83-8 | 964<br>JECFA specification (JECFA, 2002d). | 0.97 | No safety concern a)<br>Deleted b) | |
| 09.289 | alpha-Campholene acetate |  | 3657<br><br>36789-59-0 | 969<br>JECFA specification (JECFA, 2002d). | 0.061 | No safety concern a) | JECFA evaluated campholene acetate (CASrn as in Register). CASrn in Register refers to the (S)-enantiomer. |
| 09.488 | Ethyl cyclohexanepropionate |  | 2431<br>2095<br>10094-36-7 | 966<br>JECFA specification (JECFA, 2002d). | 0.12 | No safety concern a)<br>Deleted b) | |
| 09.534 | Ethyl cyclohexanecarboxylate |  | 3544<br>11916<br>3289-28-9 | 963<br>JECFA specification (JECFA, 2002d). | 0.24 | No safety concern a) | |
| 09.536 | Methyl cyclohexanecarboxylate |  | 3568<br>11920<br>4630-82-4 | 962<br>JECFA specification (JECFA, 2002d). | 0.073 | No safety concern a) | |

**Table 3: Supporting Substances Summary**

| FL-no | EU Register name | Structural formula | FEMA no<br>CoE no<br>CAS no | JECFA no<br>Specification available | MSDI (EU) 1)<br>(µg/capita/day) | SCF status 2)<br>JECFA status 3)<br>CoE status 4) | Comments |
|---|---|---|---|---|---|---|---|
| 09.615 | p-Menth-1-en-9-yl acetate |  | 3566<br>10748<br>28839-13-6 | 972<br>JECFA specification (JECFA, 2002d). | 0.85 | No safety concern a) | |

*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, 2002c).*

*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 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

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

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

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

- can the flavourings be predicted to be metabolised to innocuous products[8] (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[9] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

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

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

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

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

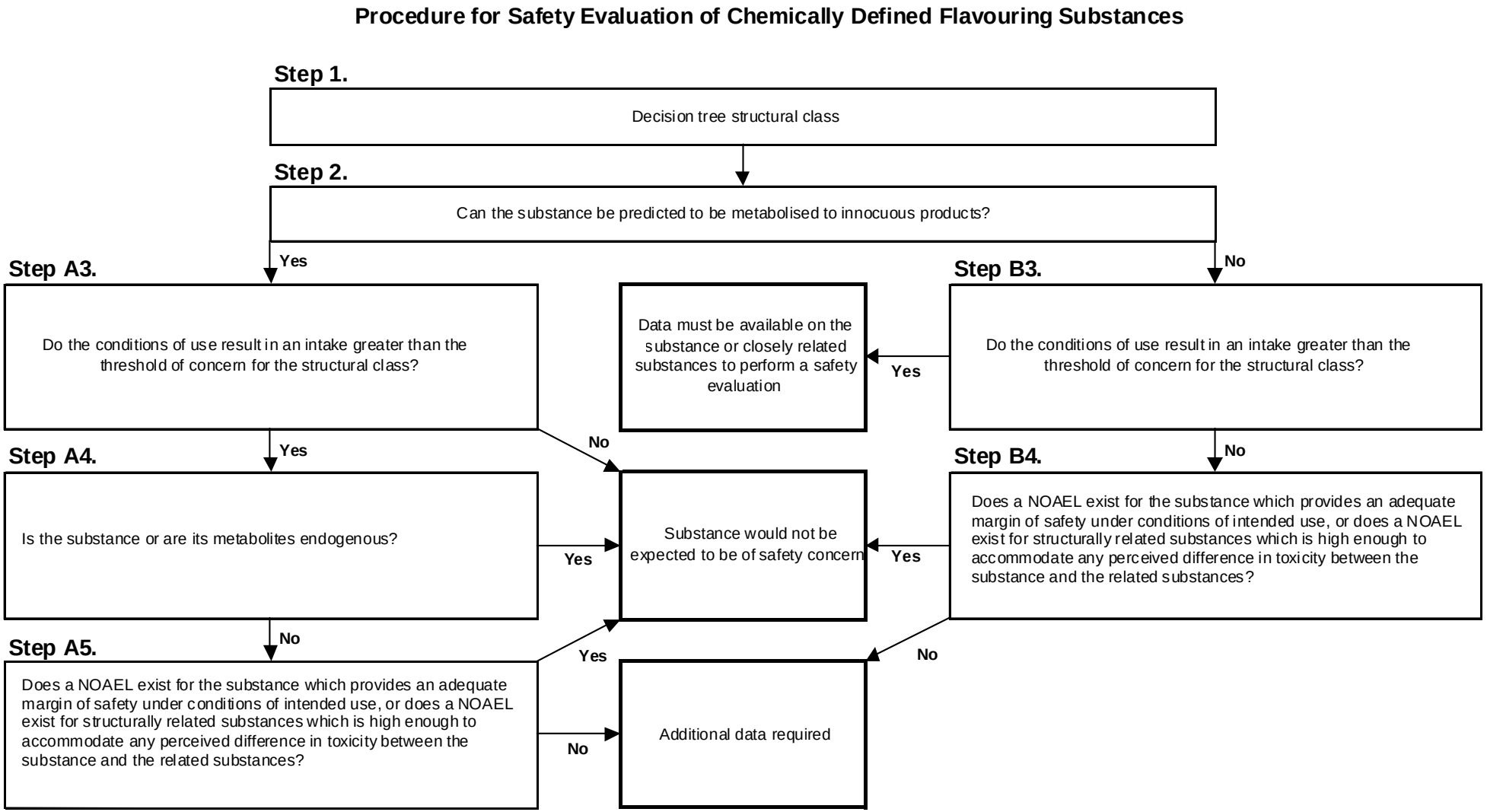



**Figure I.1** *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

# 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$^{th}$ percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

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

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

The "normal and maximum use levels" are provided by Industry for the 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.12Rev2 (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; EFFA, 2012q; Flavour Industry, 2009i).**

| FL-no | Food Categories<br>Normal use levels (mg/kg)<br>Maximum use levels (mg/kg) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **01.0** | **02.0** | **03.0** | **04.1** | **04.2** | **05.0** | **06.0** | **07.0** | **08.0** | **09.0** | **10.0** | **11.0** | **12.0** | **13.0** | **14.1** | **14.2** | **15.0** | **16.0** |
| 02.134 | 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 |
| 02.186 | 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 |
| 05.157 | 3 | 2 | 3 | 2 | - | 4 | 2 | 5 | 1 | 1 | - | - | 2 | 3 | 2 | 4 | 5 | 2 |
| | 15 | 10 | 15 | 10 | - | 20 | 10 | 25 | 5 | 5 | - | - | 10 | 15 | 10 | 20 | 25 | 10 |
| 05.182 | - | - | 0,01 | 0,00 | - | 0,00 | - | 0,00 | - | - | - | - | - | - | 0,00 | 0 | - | - |
| | - | - | 0,1 | 5 | - | 5 | - | 6 | - | - | - | - | - | - | 2 | 0 | - | - |
| | | | | 0,05 | | 0,05 | | 0,06 | | | | | | | 0,02 | | | |
| 05.183 | 3 | 2 | 3 | 2 | - | 4 | 2 | 5 | 1 | 1 | - | - | 2 | 3 | 2 | 4 | 5 | 2 |
| | 15 | 10 | 15 | 10 | - | 20 | 10 | 25 | 5 | 5 | - | - | 10 | 15 | 10 | 20 | 25 | 10 |
| 05.198 | 3 | 2 | 3 | 2 | - | 4 | 2 | 5 | 1 | 1 | - | - | 2 | 3 | 2 | 4 | 5 | 2 |
| | 15 | 10 | 15 | 10 | - | 20 | 10 | 25 | 5 | 5 | - | - | 10 | 15 | 10 | 20 | 25 | 10 |
| 08.135 | 10 | - | - | - | - | 10 | - | - | - | - | - | 10 | - | - | 10 | 10 | - | 10 |
| | 30 | - | - | - | - | 40 | - | - | - | - | - | 40 | - | - | 30 | 40 | - | 40 |
| 09.342 | 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 |
| 09.670 | 7 | 5 | 10 | 7 | - | 10 | 5 | 10 | 2 | 2 | - | - | 5 | 10 | 5 | 10 | 20 | 5 |

**Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.12Rev2 (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; EFFA, 2012q; Flavour Industry, 2009i).**

| FL-no | Food Categories | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normal use levels (mg/kg)<br>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 |
| 09.829 | 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 placed in categories 01.0 - 15.0 | Food | | |

The mTAMDI values (see Table II.2.3) are presented for the 10 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; EFFA, 2012q; Flavour Industry, 2009i). The mTAMDI values are only given for the highest reported normal use levels.

**TableII.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) |
|---|---|---|---|---|
| 02.134 | 2-Cyclohexylethan-1-ol | 3900 | Class I | 1800 |
| 02.186 | Myrtanol | 3900 | Class I | 1800 |
| 05.157 | Isocyclocitral | 1600 | Class I | 1800 |
| 05.182 | 2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde | 2.1 | Class I | 1800 |
| 05.183 | 4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal | 1600 | Class I | 1800 |
| 05.198 | alpha-Methyl ional | 1600 | Class I | 1800 |
| 08.135 | 4-(2,2,3-Trimethylcyclopentyl)butanoic acid | 5000 | Class I | 1800 |
| 09.342 | Cyclogeranyl acetate | 3900 | Class I | 1800 |
| 09.670 | Myrtanyl acetate | 3900 | Class I | 1800 |
| 09.829 | Ethyl cyclohexyl acetate | 3900 | Class I | 1800 |

## ANNEX III: METABOLISM

### III.1. Introduction

The 10 candidate flavouring substances in this group evaluation are 2-cyclohexylethan-1-ol [FL-no: 02.134], myrtanol and its acetate [FL-no: 02.186 and 09.670, respectively], four aldehydes, isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183] and alpha-methyl ional [FL-no: 05.198], one acid, 4-(2,2,3-trimethylcyclopentyl)butanoic acid [FL-no: 08.135] and ethyl cyclohexyl acetate [FL-no: 09.829] as well as the acetate of cyclogeraniol [FL-no: 09.342]. For none of these candidate substances, absorption, distribution, metabolism or elimination studies were available. 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 59$^{th}$ meeting of the 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

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], and one is an ester of alicyclic carboxylic acid and ethanol, ethyl cyclohexyl acetate [FL-no: 09.829], which can be expected to be subject to hydrolysis.

Ester hydrolysis is catalysed by classes of enzymes known as carboxyl-esterases (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). The aliphatic esters hydrolyse rapidly in liver homogenate, simulated pancreatic fluid, simulated gastric fluid and preparations of intestinal mucosa *in vitro* (Junge and Heymann, 1979; Leegwater and van Straten, 1974a; Leegwater and van 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 and Krisch, 1973; Butterworth et al., 1975a; Junge and 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 and 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 and Straten, 1974a).

The *in vitro* hydrolysis of the structurally related ester *p*-1-(7)8-menthadien-2-yl acetate[10] 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 three candidate esters in this group of flavouring substances [FL-no: 09.342, 09.670 and 09.829] 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 two of these esters [FL-no: 09.342 and 09.670] has been evaluated previously (e.g. FGE.01 or FGE.02) and the component ethanol from [FL-no: 09.829] (the JECFA had concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents (JECFA, 1997a)) 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][10] and on the related substance perillyl alcohol[10].

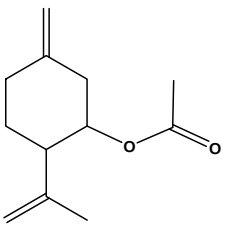
*Cyclohexanecarboxylic acid*

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).

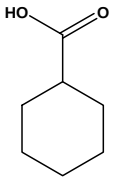
Cyclohexanecarboxylic acid and 1-methyl-1-cyclohexanecarboxylate were studied in bile-duct- and urinary tract-cannulated rats. Female Sprague-Dawley rats (four rats per compound) were administered *via* intravenous infusion a 0.52 mmol/kg bw bolus dose of cyclohexanecarboxylic acid (66 mg/kg bw) or 1-methyl-1-cyclohexanecarboxylate (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*

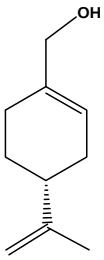
The kinetics of *p*-mentha-1,8-dien-7-ol (i.e. perillyl alcohol) have been studied in rats, dogs and in humans. This substance is most closely related to *p*-mentha-1,8(10)-dien-9-ol and its acetate [FL-no: 02.122 and 09.809, respectively] (Subgroup 2.1 of FGE.19 (EFSA, 2008b)).




[10] p-1-(7)8-menthadien-2-yl acetate




cyclohexanecarboxylic acid




perillyl alcohol

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 and 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 doses of 800, 1600 or 2400 mg perillyl alcohol/$m^2$/dose (equivalent to *ca.* 32, 64 or 96 mg/kg bw/dose, assuming a body mass index of 25 kg/$m^2$). 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 $C_{maxs}$ or AUCs for these two metabolites, but there was a clear "levelling of" of $C_{maxs}$ and AUCs for the metabolites when the dose increased from 1600 to 2400 mg/$m^2$. From the patients treated with 2400 mg/$m^2$/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/$m^2$/dose (equivalent to *ca.* 32, 48, or 64 mg/kg bw/dose, assuming a body mass index of 25 kg/$m^2$) 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 and 08.135], because these are the only ones with a side chain which might 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 and Caflisch-Weill, 1945; Brewster et al., 1977b; Williams, 1959a).

*Terpenoid Primary Alcohols and Aldehydes*

An indication about the metabolic fate of the monocyclic and bicyclic terpenoid aldehydes and alcohols (e.g. candidate substances myrtanol [FL-no: 02.186], isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], cyclogeranyl acetate [FL-no: 09.342] and myrtanyl acetate [FL-no: 09.670] 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 isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183], alpha-methyl ional [FL-no: 05.198] and cyclogeranyl acetate [FL-no: 09.342], in which multiple and cycloalkene methyl side-chains occur, the metabolism of isophorone (3,5,5-trimethylcylohex-2-ene-1-one [FL-no: 07.126][11], alpha-ionone [FL-no: 07.007][11] and beta-ionone and [FL-no: 07.008][11]might be used as an example.

*Isophorone*

When isophorone[11] was given to rabbits in an oral dose of 1 g/kg bw, glucuronic acid conjugates could be detected in the urine, 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 candidate substances 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], alpha-methyl ional [FL-no: 05.198] and cyclogeranyl acetate [FL-no: 09.342] are structurally related to alpha-ionone [FL-no: 07.007][11] and 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183] is structurally related to beta-ionone [FL-no: 07.008][11]. 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 that the four candidate substances [FL-no: 05.182, 05.183, 05.198 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 doses 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 *in vivo* and in rat hepatocytes *in vitro* (Boon et al., 2000).

To six male rabbits, *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. *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 *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, *p*-mentha-1,8-dien-7-al was reduced to perillyl alcohol, which can be selectively hydrogenated to yield *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.

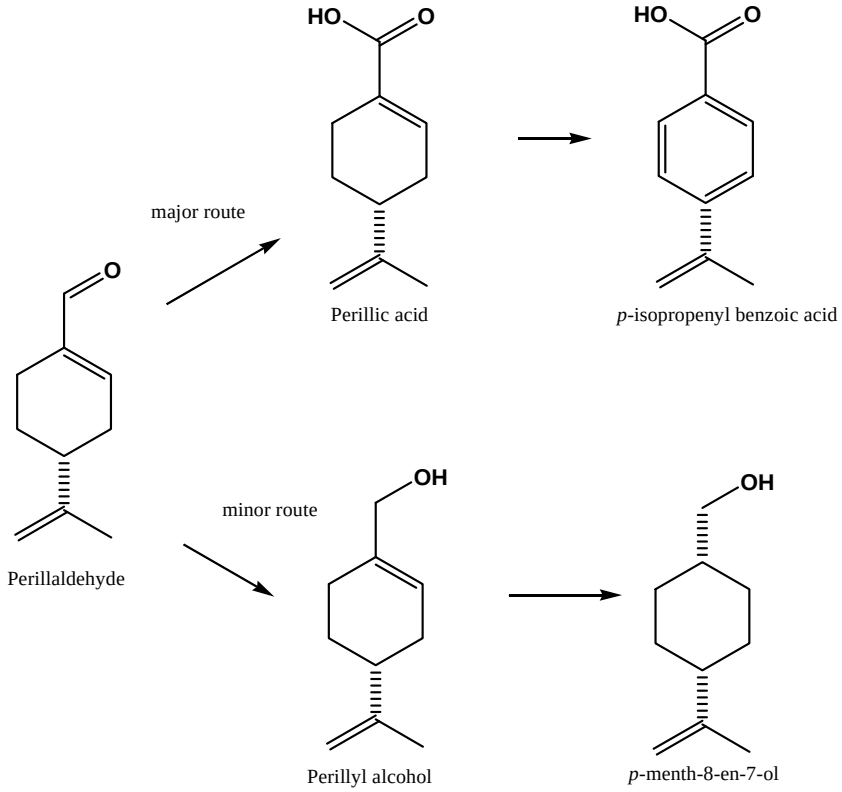



**Figure III.1** *Metabolism of perillaldehyde in rabbits*

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/day). 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 after the gavage dose, not even at 15 minutes post gavage, nor 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 and 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/$m^2$ (equivalent to ca. 96 mg/kg bw, assuming a body mass index of 25 kg/$m^2$). 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/ $m^2$/dose (equivalent to ca. 48 or 64 mg/kg bw/dose, assuming a body mass index of 25 kg/$m^2$) 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-dimethyl-bicyclo[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 acidic 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], shown to be the major neutral metabolite and 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 in the urine 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])) (Subgroup 2.2 of FGE.19 (EFSA, 2008b)) (Eriksson and Levin, 1990). The myrtenol was not detected in the sawdust (Eriksson and Levin, 1990), but could have originated from side-chain oxidation of alpha-pinene (= 2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene [FL-no: 01.004] (FGE, 78Rev1 (EFSA, 2011j)) (Ishida et al., 1981).

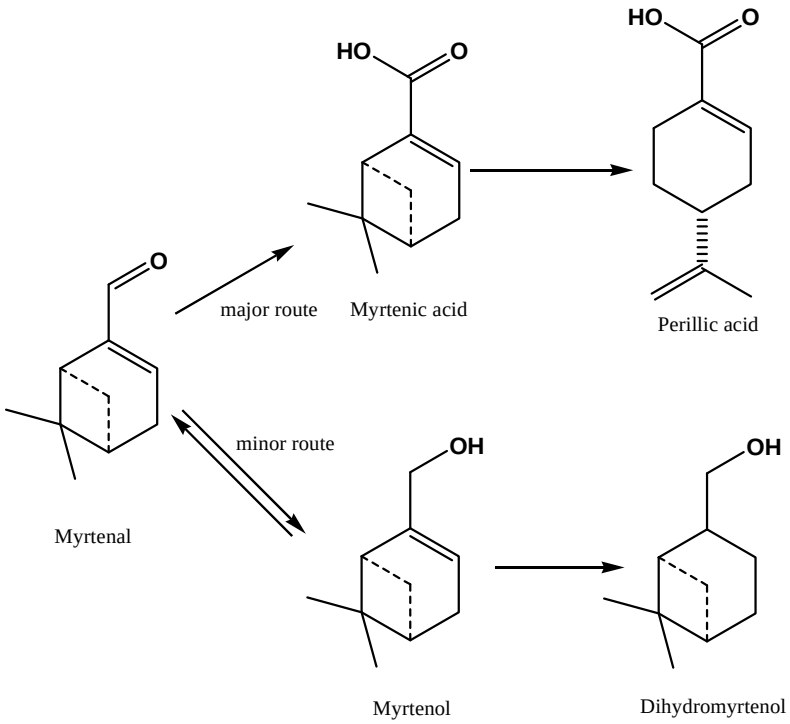



**Figure III.2** *Metabolism of myrtenal in rabbits*

In summary, in mammals, monocyclic or bicyclic terpenoid primary alcohols (e.g. cyclogeraniol [from FL-no: 09.342] and myrtanol [FL-no: 02.186] (and from [FL-no: 09.670]), 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 aldehydes (candidate substances isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182] and 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183]) and structurally related substances perillaldehyde 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 and Gould, 1994). If an endocyclic double bond is present, reduction of this double bond may occur

## III.3. Summary and Conclusions

The 10 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 four aldehydes 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 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 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 three candidate substances of the present Flavouring Group Evaluation, and for nine supporting substances evaluated by the JECFA at the 59th meeting (JECFA, 2003a). The supporting substances are listed in brackets.

### TABLE IV.1: ACUTE TOXICITY

| Chemical Name [FL-no] | Species | Sex | Route | $LD_{50}$ (mg/kg bw) | Reference | Comments |
|---|---|---|---|---|---|---|
| (Cyclohexanecarboxylic acid [08.060]) | Rat | M, F | Gavage | 3265 | (Moran et al., 1980) | Study acceptable but number of dosage groups, and thus number of animals tested, has not been referred. |
| (Methyl cyclohexanecarboxylate [09.536]) | Rat | M, F | Gavage | 3881 | (Moran et al., 1980) | Study acceptable but number of dosage groups has not been referred. |
| (Ethyl cyclohexanecarboxylate [09.534]) | Rat | M, F | Gavage | 3962 | (Moran et al., 1980) | Study acceptable but number of dosage groups has not been referred. |
| (Cyclohexaneethyl acetate [09.028]) | Rat | NR | Oral | 3200 | (Wohl, 1974c) | Not adequate $LD_{50}$ study. |
| | Rat | NR | Oral | 2190 | (Moreno, 1978h) | The study is considered valid. |
| (2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde [05.119]) | Rat | NR | Oral | 4300 | (BIBRA, 1976) | The $LD_{50}$ value cited is deduced according to Litchfield & Wilcoxon, 1949. Another $LD_{50}$ value is also cited in the BIBRA study, 3900 mg/kg, deduced according to Weill, 1952. |
| | Rat | NR | Oral | 4100 | (Moreno, 1978h) | Study acceptable. Substance name is given as 'aldehyde campholenique'. |
| (Campholene acetate [09.289]) | Rat | M, F | Gavage | M: 4640 – 5270<br>F: 3000 | (Piccirillo et al., 1979) | The study is considered valid. |
| (*alpha*-Campholenic alcohol [02.114]) | Rat | NR | Gavage | 1000 – 2000 | (Levenstein, 1982a) | Study is inadequate for determination of $LD_{50}$.<br>Also substance name is only given as code. |
| (1,2,5,6-Tetrahydrocuminic acid [08.067]) | Rat | NR | Gavage | > 2500 | (Levenstein, 1981a) | Study inadequate for derivation of $LD_{50}$.<br>Also only code name given for substance. |
| 4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal [05.183] | Rat | NR | Oral | > 5000 | (Moreno, 1977l) | Study inadequate for derivation of $LD_{50}$.<br>Also substance name given as 'cetonal'. It has not been possible to confirm that this is the same substance. |
| (10-Hydroxymethylene-2-pinene [02.141]) | Rat | NR | Oral | 890 | (Moreno, 1977u) | Study acceptable, but substance name given as Nopol. It has not been possible to confirm that this is the same substance. |
| 2-Cyclohexylethan-1-ol [02.134] | Rat | NR | Oral | 0.94 | (Wohl, 1974h) | |
| Isocyclocitral [05.157] | Rat | NR | Oral | 4.5 ml/kg bw | (Levenstein, 1973f) | |

*NR = Not reported.*

*M = Male; F = Female.*

**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 |
|---|---|---|---|---|---|---|---|
| (2,2,3-Trimethylcyclopent-3-en-1-yl)acetaldehyde [05.119]) | Rat; M, F 8 | Gavage | 12 mg/kg bw/day | 90 | 12 | (BIBRA, 1976) | Single dose study. |

*M = Male; F = Female.*

**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 the JECFA at the 59$^{th}$ meeting (JECFA, 2003a).

**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 the supporting substances evaluated by the JECFA at the 59$^{th}$ meeting (JECFA, 2003a).

**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 the supporting substances evaluated by the JECFA at the 59$^{th}$ meeting (JECFA, 2003a).

## REFERENCES

Arndt R and Krisch K, 1973. Catalytic properties of an unspecific carboxylesterase (E1) from rat-liver microsomes. European Journal of Biochemistry 36, 129-134.

Bernhard K and Caflisch-Weill H, 1945. [On the dehydrogenation of hexahydro-benzoic acid in the animal body]. Helvetica Chimica Acta 28, 1697-1707. (In German)

BIBRA, 1976. The acute toxicity of samples TT171-TT174 in rats. Short term toxicity of samples TT171, TT172, TT173, TT174 in rats. Campholenic aldehyde, 6-hydroxy-dihydrotheaspirane, 2-methyl-4-propyl-1,3-oxathiane, 2,5-dimethyl-1,3,4-trithiolane. September 1976. Unpublished report submitted by EFFA to SCF.

Boon PJM, van der Boon D and Mulder GJ, 2000. Cytotoxicity and biotransformation of the anticancer drug perillyl alcohol in PC12 cells and in the rat. Toxicology and Applied Pharmacology 167, 55-62.

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

Butterworth KR, Carpanini GB, Gaunt IF, Grasso P and Lloyd AG, 1975a. Proceedings: A new approach to the evaluation of the safety of flavouring esters. British Journal of Pharmacology 54(2), 268p.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

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

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

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

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

EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

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

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

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, 2003i. Submission 2003-2. Flavouring group evaluation of 8 flavouring substances (candidate chemicals) of the chemical group 7 (Annex I of 1565/2000/EC) structurally related to alicyclic primary alcohols, aldehydes, acids, and related esters [JECFA/WHO FAS 50/59] used as flavouring substances. 1 April 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.20.

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, 2004z. Submission 2003-2 supplement. Supplement of three flavouring substances (candidate chemicals) to the flavouring group of chemical group 7 (Annex I of 1565/2000/EC) structurally related to alicyclic primary alcohols, aldehydes, acids, and related esters [JECFA/WHO FAS 50/59] used as flavouring substances. 3 June 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.69.

EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages. FLAVIS/8.70.

EFFA, 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

EFFA, 2011e. Specifications and poundage data for 42 Register substances submitted by EFFA/Industry to FLAVIS Secretariat. August 2011. FLAVIS/8.124

EFFA, 2012q. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark . Dated 3 September 2012. Use levels and structural classes for six substances from FGE.06Rev4 [FL-no: 02.229, 05.137, 05.170, 05.188, 09.562 and 09.854], one substance from FGE.12Rev3 [FL-no: 05.182], four substances from FGE.20Rev4 [FL-no: 05.026, 05.028, 05.029 and 09.858] and one substance from FGE.23Rev4 [FL-no: 13.170]. FLAVIS/8.160.

EFFA, 2012v. E-mails from EFFA to EFSA/CEF Secretariat, dated 17 September and 29 October 2012. Information on five substances evaluated in FGE.12Rev3 [FL-no: 02.186, 05.157, 05.182, 05.198, 09.670].

EFSA, 2004a. Minutes of the 7th Plenary Meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true

EFSA, 2008b. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Parma on 27 - 29 November 2007. Parma, 7 January 2008. [Online]. Available: http://www.efsa.europa.eu/EFSA/Event_Meeting/afc_minutes_26thplen_en.pdf

EFSA, 2011j. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commision related to Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 18 May 2011. EFSA-Q-2010-01557.

Eriksson K and Levin J-O, 1990. Identification of cis- and trans- verbenol in human urine after occupational exposure to terpenes. International Archives of Occupational and Environmental Health 62, 379-383.

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.

Flavour Industry, 2009i. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-12Rev2

Graffner-Nordberg M, Sjödin K, Tunek A and Hallberg A, 1998. Synthesis and enzymatic hydrolysis of esters, constituting simple models of soft drugs. Chemical & Pharmaceutical Bulletin 46(4), 591-601.

Grundschober F, 1977. Toxicological assessment of flavouring esters. Toxicology 8, 387-390.

Haag JD and Gould MN, 1994. Mammary carcinoma regression induced by perillyl alcohol, a hydroxylated analog of limonene. Cancer Chemotherapy and Pharmacology 34(6), 477-483.

Heymann E, 1980. Carboxylesterases and amidases. In: Jakoby WB (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

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. Journal of Pharmacological Sciences 70, 4, 406-415.

Ishida T, Toyota M and 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.

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. 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, 1999a. Safety evaluation of certain food additives. Fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 42. IPCS, WHO, Geneva.

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

JECFA, 2002c. Evaluation of certain food additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 913. Geneva, 4-13 June 2002.

JECFA, 2002d. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Add. 10.

JECFA, 2003a. Safety evaluation of certain food additives. Fifty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.

Junge W and Heymann E, 1979. Characterization of the isoenzymes of pig liver esterase. II. Kinetic studies. European Journal of Biochemistry 95, 519-525.

Leegwater DC and van Straten S, 1974a. *In vitro* study of the hydrolysis of twenty-six organic esters by pancreatin. Central Institute for Nutrition and Food Research. Report no. R 4319. Project no. 8.33.01. February, 1974.

Leegwater DC and van Straten S, 1974b. *In vitro* study on the hydrolysis of eight carboxylic esters by intestinal and liver enzymes. Central Institute for Nutrition and Food Research. Report no. R 4414. Project no. 8.33.06. August, 1974.

Levenstein I, 1973f. Acute toxicity, oral LD50 in rats. Report to RIFM, 9 January. As cited in Opdyke DLJ, 1976. Fragrance raw materials monographs. Isocyclocitral. Food and Cosmetics Toxicology 14, 313.

Levenstein I, 1981a. Oral toxicity testing of 1,2,5,6-tetrahydrocuminic acid in rats. Assay no. 17107. August 18, 1981. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levenstein I, 1982a. Acute oral, LD50 of alpha-campholenic alcohol in rats. Assay no. 22178. February 5, 1982. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Liu M-J, Brouwer KLR and Pollack GM, 1992. Pharmacokinetics and pharmacodynamics of valproate analogs in rats. III. Pharmacokinetics of valproic acid, cyclohexanecarboxylic acid, and 1-methyl-1-cyclohexanecarboxylic acid in the bile-exteriorized rat. Drug Metabolism and Disposition 20(6), 810-815.

Longland RC, Shilling WH and Gangolli SD, 1977. The hydrolysis of flavouring esters by artificial gastrointestinal juices and rat tissue preparations. Toxicology 8, 197-204.

Moran EJ and Tyburcy KR, 1979. *In vitro* hydrolysis of flavoring esters in a simulated gastric system and a simulated intestinal system. Unpublished report submittd by EFFA to FLAVIS Secretariat.

Moran EJ, Easterday DD and Oser BL, 1980. Acute oral toxicity of selected flavor chemicals. Drug and Chemical Toxicology 3(3), 249-258.

Moreno OM, 1977l. Acute oral toxicity in rats. Dermal toxicity in rabbits. Cetonal. MB Research Laboratories Inc. Project no. MB 77-1941. October 7, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1977u. Acute oral toxicity in rats. Dermal toxicity in rabbits. P-Menth-1,8-dien-7-ol, 10-hydroxymethylene-2-pinene. MB Research Laboratories, Inc. Project no. MB 77-2011. October 20, 1977. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Moreno OM, 1978h. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Cyclohexaneethyl acetate, 2,2,3-trimethylcyclo-pent-3-en-1-yl acetaldehyde. Acute oral toxicity in mice. Acute dermal toxicity in guinea pigs. P-menth-1,8-dien-7-al. MB Research Laboratories, Inc. Project no. MB 78-2932. 9/28/78. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Phillips LR, Malspeis L and Supko JG, 1995. Pharmacokinetics of active drug metabolites after oral administration of perillyl alcohol, an investigational antineoplastic agent, to the dog. Drug Metabolism and Disposition 23(7), 676-680.

Piccirillo VJ, Leatherwood L, Swidersky P and Shopp G, 1979. Acute oral LD50 determination in rats. Campholene Acetate. Borriston Research Laboratories. Study no. 79-022-02. September 28, 1979. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Ripple GH, Gould MN, Stewart JA, Tutsch KD, Arzoomanian RZ, Alberti D, Feierabend C, Pomplun M, Wilding G and Bailey HH, 1998. Phase I clinical trial of perillyl alcohol administered daily. Clinical Cancer Research 4(5), 1159-1164.

Ripple GH, Gould MN, Arzoomanian RZ, Alberti D, Feierabend C, Simon K, Binger K, Tutsch KD, Pomplun M, Wahamaki A, Marnocha R, Wilding G and Bailey HH, 2000. Phase I clinical and pharmacokinetic study of perillyl alcohol administered four times a day. Clinical Cancer Research 6(2), 390-396.

Salzer D, 1998. *In vitro* hydrolysis test. Cis/tr.-1(7),8-p-menthadien-2-yl-acetate. Date 10.02.98. Unpublished report.

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.

TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

TNO, 2009. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

TNO, 2012. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Truhaut R, Dutertre-Catella H and Phu-Lich N, 1970. Biochemical toxicology. First results of a study of metabolism in rabbits, which were administered isophorone, an industrial solvent. Comptes Rendus de l'Académie des Sciences 271, 1333-1336.

Williams RT, 1959a. Detoxication mechanisms. The Metabolism and Detoxification of Drugs, Toxic Substances and Other Organic Compounds. 2nd Ed. Chapman & Hall Ltd, London.

Wohl AJ, 1974c. Acute oral toxicity (rat - 5 gms/kg body weight dose). Dermal roxicity (rabbit - 5 gms/kg body weight dose). Cyclohexyl ethyl acetate. Biological Science Laboratories. April 2, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Wohl AJ, 1974h. Acute oral toxicity (rat - 5 gms/kg body weight dose). Dermal toxicity (rabbit - 5 gms/kg body weight dose). Cyclohexyl ethyl alcohol. Biological Science Laboratories. April 2, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

## ABBREVIATIONS

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AUC | Area Under Curve |
| BW | Body Weight |
| CAS | Chemical Abstract Service |
| CEF | Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service |
| CHO | Chinese hamster ovary (cells) |
| CoE | Council of Europe |
| DNA | Deoxyribonucleic acid |
| EC | European Commission |
| EFFA | European Flavour and Fragrance Association |
| EFSA | The European Food Safety Authority |
| 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) |
| ID | Identity |
| IOFI | International Organization of the Flavour Industry |
| IR | Infrared spectroscopy |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| $LD_{50}$ | Lethal Dose, 50 %; Median lethal dose |
| MS | Mass spectrometry |
| MSDI | Maximised Survey-derived Daily Intake |
| mTAMDI | Modified Theoretical Added Maximum Daily Intake |
| NAD | Nicotinamide Adenine Dinucleotide |
| NADP | Nicotinamide Adenine Dinucleotide Phosphate |
| No | Number |
| NOAEL | No Observed Adverse Effect Level |
| NOEL | No Observed Effect Level |
| NTP | National Toxicology Program |
| PO | Per Oral |
| SCE | Sister Chromatid Exchange |
| SCF | Scientific Committee on Food |
| SMART | Somatic Mutation and Recombination Test |
| TAMDI | Theoretical Added Maximum Daily Intake |

| | |
|---|---|
| UDS | Unscheduled DNA Synthesis |
| WHO | World Health Organisation |