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EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 12, Revision 2 (FGE.12Rev2): Primary saturated or unsaturated alicyclic alcohol, aldehyde, acid, and esters from chemical group 7

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 12, Revision 2 (FGE.12Rev2):

Primary saturated or unsaturated alicyclic alcohol, aldehyde, acid, and esters from chemical group 7¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

KEYWORDS

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

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 nine flavouring substances in the Flavouring Group Evaluation 12, Revision 2 (FGE.12Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These nine flavouring substances belong to chemical group 7, Annex I of the Commission Regulation (EC) No 1565/2000.

FGE.12Rev2 includes the assessment of two additional flavouring substances compared to FGE.12Rev1. The present FGE.12Rev2 deals in total with nine primary saturated or unsaturated alicyclic alcohol, aldehyde, acid and esters belonging to chemical group 7.

1 On request from the Commission, Question No EFSA-Q-2009-00578, adopted on 30 September 2010.

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Seven of the nine flavouring substances possess one or more chiral centres and additionally, and due to the presence of a double bond, one of these substances can exist as geometric isomer. For two of these substances, the stereoisomeric composition has not been specified.

The nine flavouring substances are classified into structural class I.

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

In its evaluation, the Panel as a default used the “Maximised Survey-derived Daily 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 nine flavouring substances in this group have intakes in Europe from 0.011 to 43 micrograms/*capita*/day, which are below the threshold of concern value for structural class I (1800 micrograms/person/day) substances. The flavouring substances are expected to be metabolised to innocuous products at the estimated levels of intake as flavouring 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 (SCF, 1999a).

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

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 5000 micrograms/person/day for the nine 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 micrograms/person/day. Thus, for these six of the nine 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 three 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 for the materials of commerce have been provided for the nine flavouring substances. Information on the stereoisomeric composition for four of these substances [FL-no: 02.186, 05.157,

05.198 and 09.670] has not been specified sufficiently, as the Flavour Industry has informed that these substances consists of a “mixture of isomers”. However, the isomeric composition of the mixtures have to be provided. Thus, the final evaluation of the materials of commerce cannot be performed for these four substances, pending further information.

The five remaining substances [FL-no: 02.134, 05.183, 08.135, 09.342 and 09.829] would present no safety concern at the estimated levels of intake based on the MSDI approach.

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BACKGROUND

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

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

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

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

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

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.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	29 September 2010		9

The present 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. A search in open literature for these substances did not provide any further data on toxicity or metabolism.

Since the publication of FGE.12Rev1 information on stereoisomeric composition and a melting point has been provided by Industry on the following four substances: [FL-no: 02.186, 05.157, 05.198, 09.670] (EFFA, 2010a).

TERMS OF REFERENCE

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

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 12, Revision 2

1.1. Description

The present Flavouring Group Evaluation 12, Revision 2 (FGE.12Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with nine candidate substances from chemical group 7, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The nine 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 nine 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 three are alicyclic unsaturated aldehydes [FL-no: 05.157, 05.183 and 05.198].

The outcome of the safety evaluation is summarised in Table 2a.

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

Seven of the nine flavouring substances possess one or more chiral centres and additional, due to the presence of a double bond, one of these substances can exist as geometric isomer. For four of these substances [FL-no: 02.186, 05.157, 05.198 and 09.670], the stereoisomeric composition has not been specified sufficiently, as the Flavour Industry has informed that the substances [FL-no: 02.186, 05.198 and 09.670] are a “mixture of diastereoisomers”, however, the ratios of the diastereoisomers are needed (see Table 1) and for [FL-no: 05.157] is stated that it is a “mixture of two positional isomers”, For this substance should the stereoisomeric composition as well as the range of the mixture be given (see Table 1).

1.3. Natural Occurrence in Food

Three of the nine candidate substances 2-cyclohexylethan-1-ol [FL-no: 02.134], myrtnanol [FL-no: 02.186] and myrtanyl acetate [FL-no: 09.670] have been reported to occur in essential oils. 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 nine 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 the nine candidate substances, except that additional information on stereoisomeric mixtures is required for four of the nine 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, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population⁴ (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.12Rev2) the total annual volume of production of the nine candidate substances for use as flavouring substances in Europe has been reported to be approximately 370 kg (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; Flavour Industry, 2009i). 350 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 nine 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 each of the remaining substances the estimated daily *per capita* intake is less than 1 microgram for 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 nine 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; Flavour Industry, 2009i).

The nine candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For 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

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All nine
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All nine except [FL-no: 08.135]
03.0	Edible ices, including sherbet and sorbet	All nine except [FL-no: 08.135]
04.1	Processed fruits	All nine 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 nine
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All nine except [FL-no: 08.135]
07.0	Bakery wares	All nine except [FL-no: 08.135]
08.0	Meat and meat products, including poultry and game	All nine except [FL-no: 08.135]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All nine except [FL-no: 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 nine except [FL-no: 08.135]
13.0	Foodstuffs intended for particular nutritional uses	All nine except [FL-no: 08.135]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All nine
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All nine
15.0	Ready-to-eat savouries	All nine except [FL-no: 08.135]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All nine

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; Flavour Industry, 2009i).

The mTAMDI values for the nine candidate substances from structural class I (see Section 5) range from 1600 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 nine candidate substances in this group evaluation contain a monocyclic or bicyclic alicyclic moiety, with substituents containing a primary alcohol, aldehyde 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 substance, after absorption, will be hydrolysed in the liver.

Gastro-intestinal absorption can also be expected for the alcohols and the aldehydes in the present group.

The metabolic fate of the three component alcohols, the two candidate alcohols and the three 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 nine 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 nine 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 as well as the two candidate alcohols and the three aldehydes 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 nine 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 ranges from 0.011 to 43 micrograms/capita/day. These figures are below the threshold of concern value for substances belonging to structural class I (1800 micrograms/person/day).

The estimated intakes for the nine candidate substances in structural class I based on the mTAMDI range from 1600 to 5000 micrograms/person/day. For three of the substances [FL-no: 05.157, 05.183 and 05.198] the mTAMDI is below the threshold of concern of 1800 micrograms/person/day. For the six candidate substances exceeding the threshold of concern [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 ($\mu\text{g}/\text{capita}/\text{day}$)	mTAMDI ($\mu\text{g}/\text{person}/\text{day}$)	Structural class	Threshold of concern ($\mu\text{g}/\text{person}/\text{day}$)
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
05.183	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; Flavour Industry, 2009i), the combined estimated daily *per capita* intake as flavourings of the nine candidate flavouring substances assigned to structural class I is 45 micrograms, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 micrograms/person/day.

The nine candidate substances are structurally related to 15 supporting substances evaluated by JEFCA at its 59th meeting (JECFA, 2003a). The estimated combined intake (in Europe) is approximately 41 micrograms/*capita*/day for 15 the supporting substances assigned to structural class I. The total estimated combined intake of candidate and supporting substances (in Europe) would be approximately 86 micrograms, which does not exceed the threshold of concern for the corresponding structural class I (1800 micrograms/person/day).

8. Toxicity

8.1. Acute Toxicity

Studies were available for three of the nine candidate substances.

Studies were available for nine supporting substances. The oral LD₅₀ in rats ranges 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 nine candidate substances.

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

There are no carcinogenicity studies available neither for the nine 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 nine candidate substances nor for the 15 supporting substances.

8.4. Genotoxicity Studies

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

9. Conclusions

Out of the nine substances one is a alicyclic saturated acid, two are primary alicyclic saturated alcohols, three 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.

Seven of the nine flavouring substances possess one or more chiral centres and additional, and due to the presence of a double bond, one of these substances can exist as geometric isomer. For four of these substances [FL-no: 02.186, 05.157, 05.198 and 09.670], the stereoisomeric composition has not been specified sufficiently, as the Flavour Industry has informed that the substances [FL-no: 02.186, 05.198 and 09.670] are a “mixture of diastereoisomers”, however, the ratios of the diastereoisomers are needed and for [FL-no: 05.157] is stated that it is a “mixture of two positional isomers”, For this substance should the stereoisomeric composition as well as the range of the mixture be given.

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

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

According to the default MSDI approach, the nine 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 nine 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 nine 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 nine 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 six 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 nine candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 5000 micrograms/person/day for the nine flavouring substances from structural class I. The intakes were above the threshold of concern for structural class I of 1800 micrograms/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 three flavouring substances [FL-no: 05.157, 05.183 and 05.198]. These three substances are also expected to be metabolised to innocuous products.

Thus, for six of the nine 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 three 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 nine 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 the nine flavouring substances. Information on the stereoisomeric composition for four of these substances [FL-no: 02.186, 05.157, 05.198 and 09.670] has not been specified sufficiently, as the Flavour Industry has informed that these substances consists of a "mixture of isomers". However, the isomeric composition of the mixtures have to be provided. Thus, the final evaluation of the materials of commerce cannot be performed for these four substances, pending further information.

The five remaining substances [FL-no: 02.134, 05.183, 08.135, 09.342 and 09.829] 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, REVISION2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 12, Revision 2

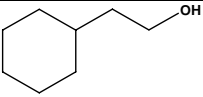
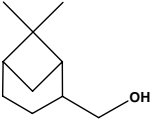
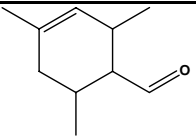
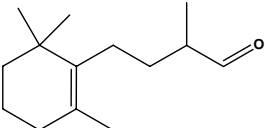
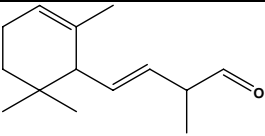
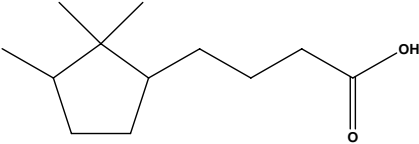
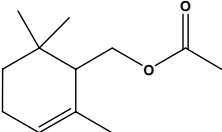
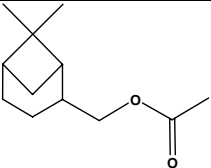
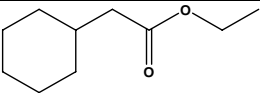
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.134	2-Cyclohexylethan-1-ol		4442-79-9	Liquid C ₈ H ₁₆ O 128.21	Slightly soluble Freely soluble	222 MS 95 %	1.463-1.469 0.918-0.924	
02.186	Myrtanol		514-99-8	Solid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	116 (16 hPa) 77 MS 95 %	n.a. n.a.	Mixture of R,S-enantiomers, i.e. the (+)- and (-)- (cis)- and (trans)-isomers, mixture of all diastereoisomers (EFFA, 2010a). Range should be given. CASrn in Register does not specify stereoisomeric composition.
05.157	Isocyclocitral 6)		1335-66-6	Liquid C ₁₀ H ₁₆ O 152.23	Practically insoluble or insoluble Freely soluble	214 -55 MS 95 %	1.484-1.490 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). The range should be given. CASnr in Register refers to "Incomplet defined structure" (positions of two methyl groups not assigned).
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal		65405-84-7	Liquid C ₁₄ H ₂₄ O 210.36	Practically insoluble or insoluble Freely soluble	305 MS 95 %	1.468-1.474 0.924-0.930	Racemate. CASrn in Register to be changed to 73398-85-3. New CASrn refers to the racemate.
05.198	alpha-Methyl ional		58102-02-6	Liquid C ₁₄ H ₂₂ O 206.33	Practically insoluble or insoluble Freely soluble	90 (0.1 hPa) MS 95 %	1.485-1.491 0.911-0.917	Mixture of (Z)- & (E)-isomers (EFFA, 2010a). Range should be given. Name to be changed to 3-Butenal, 2-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl) (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 12, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid		4529 957136-80-0	Liquid C ₁₂ H ₂₂ O ₂ 198	Slightly soluble Partially soluble	140 - 143 NMR MS 99 %	1.461-1.467 0.955-0.961	CASrn refers to the racemate. Composition of mixture: 50 - 60 % 4-((1S,3R)-2,2,3-trimethylcyclopentyl)butanoic acid; 32 - 40 % 4-((1R,3S)- ; 0 - 6 % 4-((1S,3S)- ; 0 - 4 % 4-((1R,3R)-.
09.342	Cyclogeranyl acetate		54993-30-5	Liquid C ₁₂ H ₂₀ O ₂ 196.29	Practically insoluble or insoluble Freely soluble	98 (13 hPa) MS 95 %	1.464-1.470 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 C ₁₂ H ₂₀ O ₂ 196.29	Practically insoluble or insoluble Freely soluble	106 (9 hPa) MS 95 %	1.470-1.476 0.969-0.975	Mixture of R,S-enantiomers, i.e. the (+)- and (-)- (cis)- and (trans)-isomers, mixture of all diastereoisomers (EFFA, 2010a). Range should be given. CASrn in Register does not specify stereoisomeric composition.
09.829	Ethyl cyclohexyl acetate		2348 218 5452-75-5	Liquid C ₁₀ H ₁₈ O ₂ 170.25	Practically insoluble or insoluble Freely soluble	211 NMR MS 95 %	1.442-1.450 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)

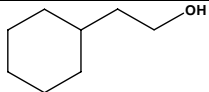
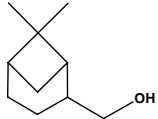
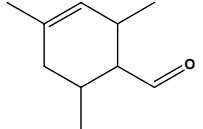
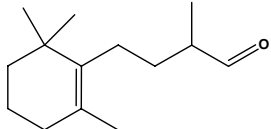
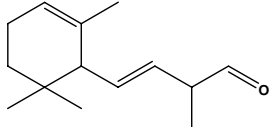
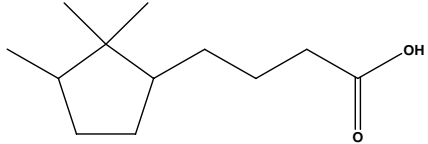
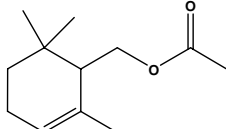
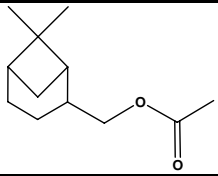
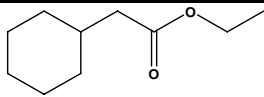
FL-no	EU Register name	Structural formula	MSDI 1) ($\mu\text{g}/\text{capita}/\text{day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.134	2-Cyclohexylethan-1-ol		0.011	Class I A3: Intake below threshold	4)	6)	
02.186	Myrtanol		0.37	Class I A3: Intake below threshold	4)	7)	
05.157	Isocyclocitral		0.011	Class I A3: Intake below threshold	4)	7)	
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal		0.012	Class I A3: Intake below threshold	4)	6)	
05.198	alpha-Methyl ional		0.011	Class I A3: Intake below threshold	4)	7)	
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid		43	Class I A3: Intake below threshold	4)	6)	
09.342	Cyclogeranyl acetate		0.24	Class I 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 ($\mu\text{g}/\text{capita}/\text{day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.670	Myrtanyl acetate		0.58	Class I A3: Intake below threshold	4)	7)	
09.829	Ethyl cyclohexyl acetate		0.61	Class I 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) = $\mu\text{g}/\text{capita}/\text{day}$.*

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

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

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

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

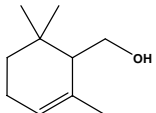
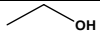
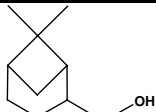
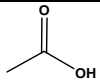
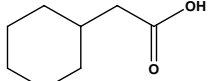
6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).*

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

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

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Cyclogeraniol		Not evaluated as flavouring substance		Not in EU-Register
02.078	Ethanol 41		Category 1 a) 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 A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 a) No safety concern c) Category A d)	Class I A3: Intake above threshold, A4: Endogenous	
08.034	Cyclohexylacetic acid 965		No safety concern e) Category B d)	Class I 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).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

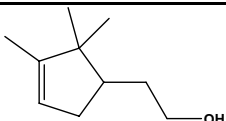
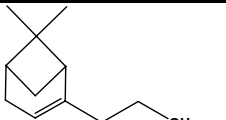
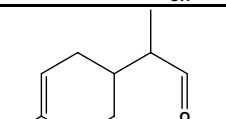
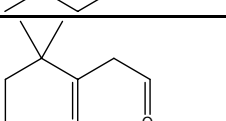
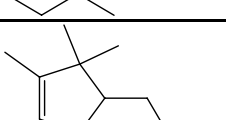
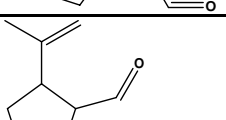
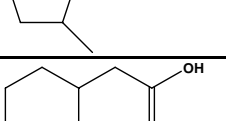
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.114	2-(2,2,3-Trimethylcyclopent-3-enyl)ethan-1-ol		3741 1901-38-8	970 JECFA specification (JECFA, 2002d)	0.012	No safety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

Table 3: Supporting Substances Summary

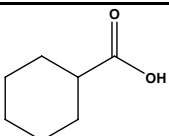
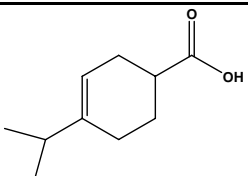
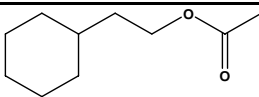
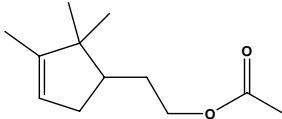
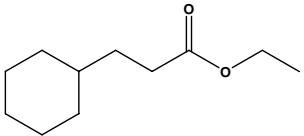
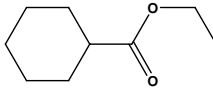
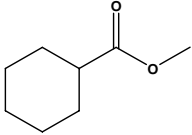
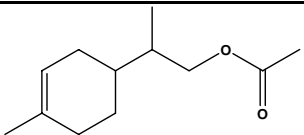
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.060	Cyclohexanecarboxylic acid		3531 11911 98-89-5	961 JECFA specification (JECFA, 2002d)	0.061	No safety concern a)	
08.067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

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.615	p-Menth-1-en-9-yl acetate		3566 10748 28839-13-6	972 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).

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 products⁸ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous⁹ (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).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

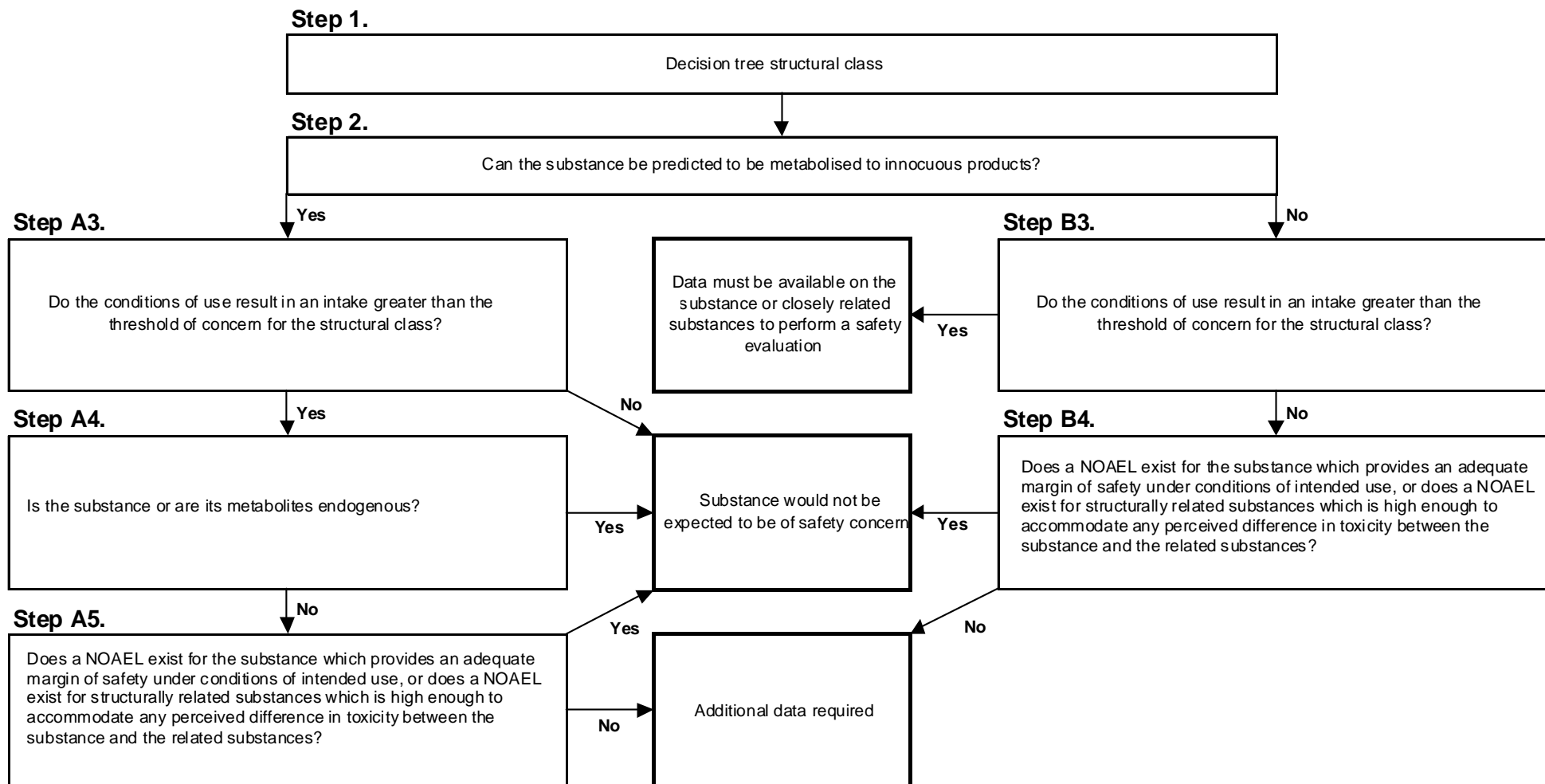


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 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 nine 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; Flavour Industry, 2009i).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
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
05.183	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
	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		

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	
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 each of the nine flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003i; EFFA, 2004z; EFFA, 2007a; Flavour Industry, 2009i). The mTAMDI values are only given for the highest reported normal use levels.

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.134	2-Cyclohexylethan-1-ol	3900	Class I	1800
02.186	Myrtanol	3900	Class I	1800
05.157	Isocyclocitral	1600	Class I	1800
05.183	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 nine candidate flavouring substances in this group evaluation are 2-cyclohexylethan-1-ol [FL-no: 02.134], myrtaanol and its acetate [FL-no: 02.186 and 09.670, respectively], three aldehydes: isocyclocitral [FL-no: 05.157], 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 59th 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 & Heymann, 1979; Leegwater & Straten, 1974a; Leegwater & Straten, 1974b; Longland et al., 1977; Grundschober, 1977; Graffner-Nordberg et al., 1998). Results of *in vitro* studies indicate that the affinity of the esterases for their substrates increases as the length of the ester increases and that the rate of hydrolyses of the straight-chain esters is approximately 100 times faster than the rate of hydrolysis of the branched-chain esters (Arndt & Krisch, 1973; Butterworth et al., 1975a; Junge & Heymann, 1979).

Cyclohexanecarboxylate methyl ester and cyclohexanecarboxylate ethyl ester were incubated separately with 50 ml simulated gastric fluid at 37° C, for six hours. Results showed approximately 20 % hydrolysis of each ester in the gastric fluid system. After a five-hour incubation in simulated intestinal fluid, approximately 40 and 50 % of cyclohexanecarboxylate methyl- and ethyl esters were hydrolysed, respectively (Moran & Tyburcy, 1979). In an *in vitro* hydrolysis study, 100 % of cyclohexanepropionate ethyl ester was hydrolysed after two-hours of incubation in 5 % pancreatin (Grundschober, 1977; Leegwater & Straten, 1974a).

The *in vitro* hydrolysis of the structurally related ester, *p*-1-(7)8-menthadien-2-yl acetate¹⁰, 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 gastrointestinal 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 alcohol¹⁰.

Cyclohexanecarboxylic acid

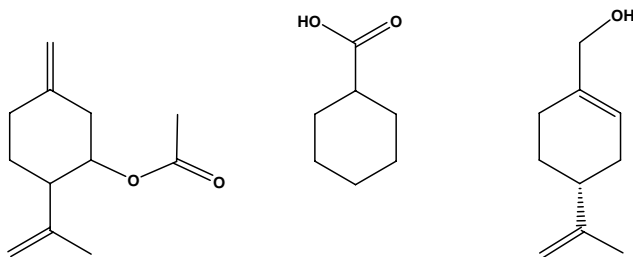
Cyclohexanecarboxylate sodium salt, with a ¹⁴C-labelled ring was orally administered to male Wistar albino rats at a dose of 100 mg/kg bw. Results showed that > 98 % of the original dose was excreted as urinary metabolites within seven hours. Less than 1 % was excreted *via* the faeces or expired air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

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

Perillyl alcohol

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

Within four hours after a single dose of 1000 mg perillyl alcohol/kg bw, administered to female Wistar-Furth rats *via* gavage, major plasma metabolites were identified as oxidised metabolites of perillyl alcohol (perillic



¹⁰ *p*-1-(7)8-menthadien-2-yl acetate cyclohexanecarboxylic acid perillyl alcohol

acid and dihydroperillic acid). No trace of perillyl alcohol was detected in the plasma at any time point, including 15-minutes post-gavage (Haag & Gould, 1994).

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

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

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

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

III.2.3 Biotransformation

Cyclohexyl Derivatives

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

The metabolic options available to alicyclic substances increase as the number and types of functional groups and ring substituents in the molecule increase. If a primary alcohol, aldehyde or carboxylic acid function is present on an alkyl side-chain of the ring, the substance may undergo beta-oxidation at the side chain. For the present group of flavouring substances, this seems in particular important for [FL-no: 05.183 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 & 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 substance 4-(2,6,6-trimethyl-cyclohexenyl)-2-methylbutanal, [FL-no: 05.183], and candidate

substance myrtenol [FL-no: 02.186] and supporting substances) can be obtained from the biotransformations of representative supporting substance aldehydes *p*-mentha-1,8-dien-7-al (i.e. perillaldehyde) and 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (i.e. myrtenal), which have been described below. In addition, for the metabolism of the flavouring substances cyclogeranyl acetate [FL-no: 09.342] and 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutenal [FL-no: 05.183], in which multiple and cycloalkene methyl side-chains occur, the metabolism of isophorone (3,5,5-trimethylcyclohex-2-ene-1-one [FL-no: 07.126])¹¹ might be used as an example.

Isophorone

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

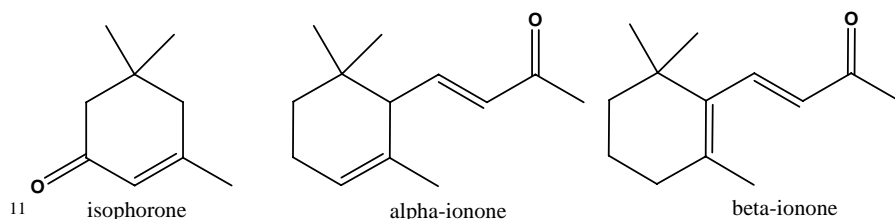
Alpha- and Beta-ionone

The two candidate substances 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183] and cyclogeranyl acetate [FL-no: 09.342] are structurally related to beta-ionone [FL-no: 07.008] and alpha-ionone [FL-no: 07.007]¹¹, respectively. Available metabolic data on these two ionones indicate that they may undergo allylic ring hydroxylation and possible further oxidation to keto groups. These reactions result in the formation of polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acid (JECFA, 1999a). It is anticipated the the two candidate substances [FL-no: 05.183 and 09.342], at least partially, may form similar polar metabolites and be excreted with the urine.

Perillyl Alcohol and Perillaldehyde

The metabolism of perillyl alcohol, perillaldehyde and perillic acid was determined after intravenous injection in male Wistar rats and in exposed isolated rat hepatocytes. Although perillaldehyde can react spontaneously with glutathione, no indication of the formation of GSH conjugates was found either *in vivo* or in hepatocytes. After dosing with perillaldehyde about 50 % of the 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*-isopropenylbenzoic 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.

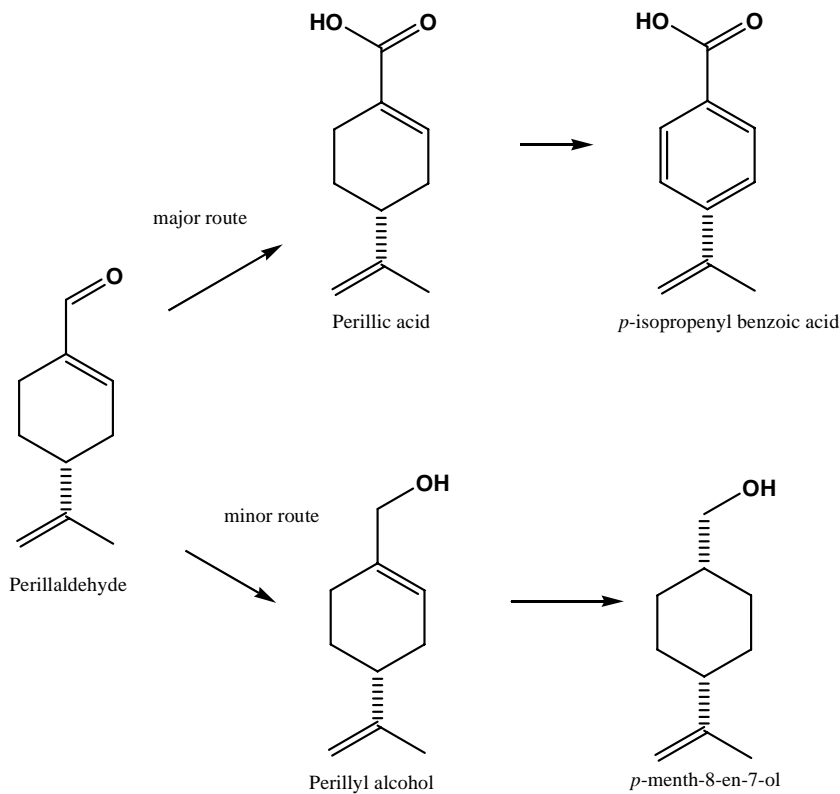


Figure III.1 Metabolism of perillaldehyde in rabbits

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, neither after the gavage dose, not even at 15 minutes post gavage, and also not after sub-chronic feeding. These results indicate that perillyl alcohol is rapidly absorbed from the gastrointestinal tract and metabolised. The presence of dihydroperillic acid indicates that the endocyclic double bond was hydrogenated. After acute exposure the ratio perillic acid / dihydroperillic acid amounted to > 10, while after 3 – 10 weeks of exposure via the diet this ratio had dropped to 2 – 3 (Haag & Gould, 1994). An *in vivo* study conducted in male Wistar rats confirmed that the oxidation of perillyl alcohol to perillic acid involved perillaldehyde as an intermediate. Rats were administered intravenously perillyl alcohol, perillaldehyde or perillic acid at a dose of 80 micromol/kg bw (approximately 12.2, 12.0 or 13.3 mg/kg bw, respectively). Urine and bile were collected for two consecutive hours post administration. In all cases, the glucuronic acid conjugate of perillic acid was the predominant metabolite detected in the urine (10 % of the dose) and bile (46 % of the dose). The glucuronic acid conjugate of perillyl alcohol was also a major biliary metabolite following the intravenous administration of perillyl alcohol (5 %), while urinary excretion of this conjugate amounted to 1 % of the dose. Based on the results, the authors concluded that within two hours, approximately 56 % of the original dose had been oxidised to perillic acid through perillaldehyde, and eventually excretion as a glucuronic acid conjugate (Boon et al., 2000).

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

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

Myrtenal

Six male rabbits received an oral dose of 2000 mg of 2-formyl-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene (= (-)-myrtenal) per animal. In the urine of these animals myrtenol, dihydromyrtanol, myrtenic acid and perillic acid could be detected. Myrtenol and dihydromyrtanol 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 dihydromyrtanol (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 the glucuronic acid conjugate of myrtenol (2-hydroxymethyl-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene; [FL-no: 02.091]; the component alcohol in candidate flavouring substances [FL-no: 09.899 and 09.900]) in the urine (Eriksson & Levin, 1990). The myrtenol was not detected in the sawdust (Eriksson & Levin, 1990), but could have originated from side-chain oxidation of alpha-pinene (= 2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene; [FL-no: 01.004]; (Ishida et al., 1981).

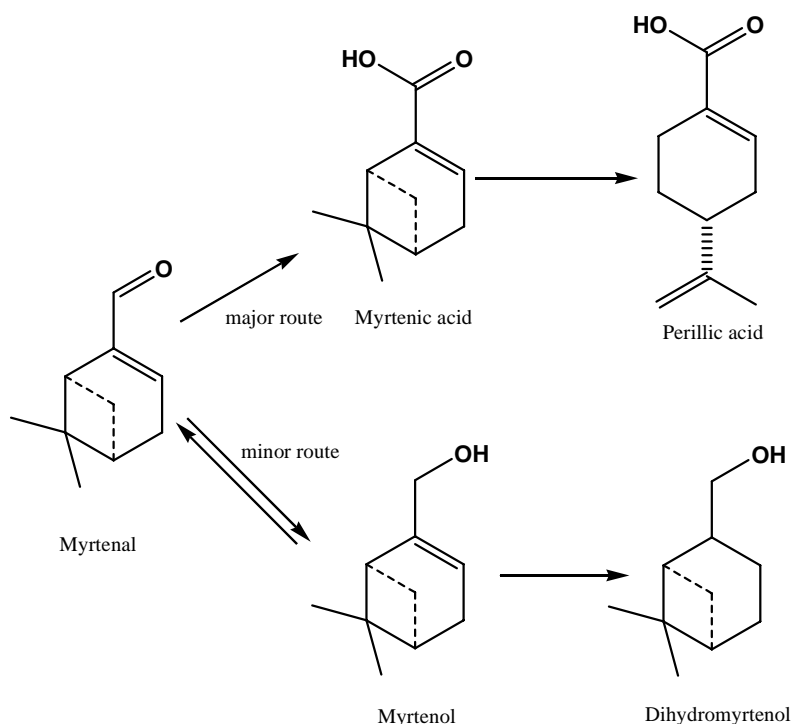


Figure III.2 Metabolism of myrtenal in rabbits

In summary, in mammals, monocyclic or bicyclic terpenoid primary alcohols (e.g. candidate substances cyclogeraniol [from FL-no: 09.342] and myrtanol [FL-no: 02.186], and the structurally related substance perillyl alcohol) are generally oxidised to the corresponding carboxylic acid, conjugated with glucuronic acid, and are excreted as urinary metabolites. The same is true for the monocyclic aldehyde (candidate substance 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183]) and structurally related substances 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 & Gould, 1994). If an endocyclic double bond is present, reduction of this double bond may occur.

III.3. Summary and Conclusions

The nine 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 three 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. 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 LD50 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 F: 3000	(Piccirillo et al., 1979)	The study is considered valid.
(<i>alpha</i> -Campholenic alcohol [02.114])	Rat	NR	Gavage	1000 – 2000	(Levenstein, 1982a)	Study is inadequate for determination of LD ₅₀ . 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 . 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 . Also substance name given as 'catonal'. 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)	
Isoocyclocitral [05.157]	Rat	NR	Oral	4.5 ml/kg bw	(Levenstein, 1973f)	

NR = Not reported.

M = Male; F = Female.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for none of the candidate substances of the present Flavouring Group Evaluation but for one supporting substance evaluated by the JECFA at the 59th meeting. The supporting substance is listed in brackets.

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

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(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 59th meeting.

TABLE IV.4: GENOTOXICITY (IN VITRO)

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

TABLE IV.5: GENOTOXICITY (IN VIVO)

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

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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