EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4):

Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 21 flavouring substances in the Flavouring Group Evaluation 9, Revision 4, using the Procedure in Commission Regulation (EC) No 1565/2000. The present revision of FGE.09 includes the assessment of four additional flavouring substances, *p*-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], *l*-piperitone [FL-no: 07.255] and menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843]. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the 20 substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For the remaining candidate substance [FL-no: 07.207], additional toxicity data are requested (further metabolism and/or toxicity studies). Besides the safety assessment of these flavouring

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[1] On request from the Commission, Question No EFSA-Q-2011-01245, EFSA-Q-2011-01244, EFSA-Q-2011-01243, EFSA-Q-2011-01242, adopted on 5 July 2012.

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substances, the specifications for the materials of commerce have been considered. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all candidate substances.

KEYWORDS

Flavourings, alcohols, ketones, esters, secondary alicyclic, saturated, unsaturated, food safety, FGE.09.

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 asked to evaluate 21 flavouring substance in the Flavouring Group Evaluation 9, Revision 4 (FGE.09Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These flavouring substances belong to chemical group 8, 25 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.09, FGE.09Rev4, includes the assessment of four additional flavouring substances, *p*-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], *l*-piperitone [FL-no: 07.255] and menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843].

The present flavouring group evaluation deals with 21 secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols. The flavouring substances are structurally related to 27 flavouring substances evaluated at the 51st, 59th and 63rd meetings of the Joint FAO/WHO Expert Committee on Food Additives (the JECFA).

Seventeen of the flavouring substances have one or more chiral centres.

Fourteen of the flavouring substances belong to structural class I, six belong to structural class II and one to structural class III, according to the decision tree approach presented by Cramer et al., 1978.

Thirteen of the flavouring substances have been reported to occur naturally in a wide range of food items.

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

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 14 flavouring substances belonging to structural class I have intakes in Europe ranging from 0.0012 to 830 microgram/*capita*/day, for five of the six substances from structural class II the intakes range from 0.0085 to 530 microgram/*capita*/day, and for the substance from structural class III the intake is 1.2 microgram/*capita*/day, which are all below their respective threshold of concern value for structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively. For one substance [FL-no: 09.520] from structural class II the MSDI is 770 microgram/*capita*/day, which is above the threshold of concern of 540 microgram/person/day.

Genotoxicity data are available only for a limited number of the flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

Twenty of the flavouring substances are expected to be metabolised to innocuous products at their estimated level of use as flavouring substances. The remaining substance, cyclotetradecanone [FL-no: 07.207], could not be assumed to be metabolised to innocuous products.

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

It is considered that, on the basis of the default MSDI approach, 20 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. For one flavouring substance, cyclotetradecanone [FL-no: 07.207], the evaluation has been deferred as additional data on toxicokinetics and/or toxicology are required.

When the estimated intakes were based on the mTAMDI approach they ranged from 420 to 63000 microgram/person/day for 13 flavouring substances in structural class I. For seven of these substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold of concern of 1800 microgram/person/day (class I) and for six substances [FL-no: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] above the threshold. The estimated intakes of the six substances assigned to structural class II, range from 320 to 8700 microgram/person/day, which for five substances [FL-no: 07.059, 07.202, 07.203, 07.207 and 09.520] are above the threshold of concern for structural class II substances of 540 microgram/person/day. The mTAMDI estimate for the one substance from structural class III [FL-no: 06.136] is 0.075 microgram/person/day, which is below the threshold of 90 microgram/person/day. For one flavouring substance [FL-no: 09.929] the mTAMDI could not be calculated due to missing information on use levels. Thus, for eleven flavouring substances [FL-no: 07.059, 07.202, 07.203, 07.207, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], and for [FL-no: 09.929] for which use levels are missing, 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 substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.255, 09.355, 09.621 and 09.870], which have mTAMDI estimates below the threshold of concern, are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 21 flavouring substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 21 flavouring substances evaluated through the Procedure.

For cyclotetradecanone [FL-no: 07.207] additional data are requested (further metabolism and/or toxicity studies). For the remaining 20 substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

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

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

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

HISTORY OF THE EVALUATION

The Flavouring Group Evaluation 09, FGE.09 dealt with 10 candidate substances, nine secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols, and one ester of a phenol carboxylic acid and a secondary alicyclic alcohol.

The first Revision of FGE.09, FGE.09Rev1, included the assessment of five additional flavouring substances [FL-no: 06.136, 09.154, 09.520, 09.929 and 09.935]. No new toxicity or metabolism data were provided for four of the five substances. For [FL-no: 09.520] acute and short term toxicity data and *in vitro* and *in vivo* genotoxicity data were provided. Additional data on five substances [FL-no: 02.075, 02.167, 09.355, 09.619 and 09.621] was made available since the FGE.09 was published.

The second Revision of FGE.09, FGE.09Rev2, included the assessment of one additional substance, carvyl-3-methylbutyrate [FL-no: 09.870]. No toxicity and/or metabolism data were provided for this substance. Carvyl-3-methylbutyrate has initially been considered in FGE.212 with respect to genotoxicity, together with other alpha,beta-unsaturated substances from subgroup 2.6 of FGE.19, where the Panel concluded that "*d*-Carvone [FL-no: 07.146] was found genotoxic *in vitro*. However, *d*-carvone was not carcinogenic in mice. Therefore, the Panel concluded that this substance together with the structurally related l-carvone as well as carveol and the carvylderivatives [FL-no: 02.062, 07.147, 09.143, 09.215 and 09.870] could be evaluated through the Procedure".

The third Revision of FGE.09, FGE.09Rev3, included the assessment of one additional substance, *L*-menthyl (S)-3-hydroxybutyrate [FL-no: 09.949]. No toxicity and/or metabolism data were provided for this substance.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.09	9 December 2004	http://www.efsa.eu.int/science/afc/afc_opinions/814_en.html	10
FGE.09Rev1	1 April 2008	http://www.efsa.europa.eu/en/efsajournal/doc/927.pdf	15
FGE.09Rev2	13 May 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1454.htm	16
FGE.09Rev3	28 September 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2396.htm	17
FGE.09Rev4	5 July 2012		21

The present Revision of FGE.09, FGE.09Rev4, includes the assessment of four additional substances [FL-no: 07.059, 07.202, 07.255 and 09.843].

Two of these substances [FL-no: 07.202 and 07.255] are alpha,beta-unsaturated ketones originally allocated to FGE.212Rev1. The two substances have been considered with respect to genotoxicity (EFSA, 2011f) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the two substances can be evaluated through the Procedure.

No toxicity or metabolism data were provided for the new substances. A search in the open literature for these substances did not provide any further data on toxicity or metabolism.

Industry has informed that the substance [FL-no: 07.207] is no longer supported for use as flavouring substance in Europe (EFFA, 2009c).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letter of the 7th May 2010, the Commission requested EFSA, based on additional submitted data on genotoxicity, to carry out re-evaluation of the flavouring substances 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202] and *l*-piperitone [FL-no: 07.255] and depending on the outcome to proceed to the evaluation of this flavouring substance through the Procedure, also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 9, Revision 4

1.1. Description

The present Flavouring Group Evaluation (FGE.09Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Annex I of this FGE), deals with nine secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and 11 esters containing secondary alicyclic alcohols. These 21 flavouring substances (candidate substances) belong to chemical groups 08, 25 and 30 of Annex I of Regulation (EC) No 1565/2000 (EC, 2000a).

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

A summary of the outcome of the safety evaluation of the candidate substances is listed in Table 2a

The hydrolysis products of the candidate substances and their evaluation status as flavouring substances are listed in Table 2b.

The candidate substances are structurally related to 27 flavouring substances (supporting substances) evaluated at the 51st, 59th and 63rd meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the groups "Substances structurally related to menthol", "Carvone and structurally related substances", "Alicyclic ketones, secondary alcohols and related esters" and "Monocyclic and bicyclic secondary alcohols, ketones and related esters" (JECFA, 1999a; JECFA, 2003a; JECFA, 2006a). In addition the racemate of menthyl-3-hydroxybutyrate has been evaluated by the JECFA at the 69th meeting (JECFA, 2009a) in the group of "Substances structurally related to menthol".

The names and structures for the 27 supporting substances are listed in Table 3, together with their evaluation status.

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.).

Two candidate substances possess one chiral centre [FL-no: 07.203 and 07.255] and 15 substances possess two or more chiral centres [FL-no: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949].

1.3. Natural Occurrence in Food

Thirteen candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618 and 09.619] have been reported to occur in fruits, spices, butter, chicken, wine, drinks, tea, juice and essential oils. Quantitative data on the natural occurrence of these substances in food have been reported for three substances (TNO, 2000; TNO, 2011).

These reports include:

- Cyclohexanol [FL-no: 02.070]: up to 0.1 mg/kg in fruits (passionfruit) and 0.006 mg/kg in white wine.
- Cyclopentanol [FL-no: 02.135]: 0.01 - 0.1 mg/kg in passiflora juice, 0.01 - 0.1 mg/kg in passiflora mollisima, 0.01 - 0.02 mg/kg in oysters and 0.01 mg/kg in Chinese quince flesh.
- 2,6,6-Trimethylcyclohex-2-en-1-one [FL-no: 07.202]: 2000mg/kg in maize, 1 mg/kg in tea and up to 0.23 mg/kg in citrus fruits.

According to TNO (TNO, 2000; TNO, 2010; TNO, 2011), the remaining eight candidate substances have not been reported to occur naturally in any food items. These substances are 6-isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [FL-no: 06.136], cyclotetradecanone [FL-no: 07.207], menthyl salicylate [FL-no: 09.621], menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843],

carvyl-3-methylbutyrate [FL-no: 09.870], L-monomenthyl glutarate [FL-no: 09.929], dimenthyl glutarate [FL-no: 09.935] and L-menthyl (S)-3-hydroxybutyrate [FL-no: 09.949].

2. Specifications

Purity criteria for the 21 candidate substances have been provided by the Flavouring Industry (see Table 1 (EFFA, 2003a; EFFA, 2010a; EFFA, 2011e; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f). Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the specifications are adequate for all 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, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995a). The intake approach does not consider the possible natural occurrence in food.

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

In the present FGE.09Rev4, the total annual volume of production of the candidate substances from use as flavouring substances in Europe has been reported to be approximately 19000 kg (EFFA, 2003a; EFFA, 2003b; EFFA, 2011e; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k). For the 26 of 27 supporting substances, for which production figures are available for Europe, the total annual volume of production is approximately 138500 kg (JECFA, 1999a; JECFA, 2003a; JECFA, 2006a).

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

96 % of the total annual volumes of production for the candidate substances is accounted for by four of these flavourings, *p*-menthan-3-one [FL-no: 07.059], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843] and L-monomenthyl glutarate [FL-no: 09.929]. The estimated daily *per capita* intakes from use as flavouring substances are 530, 770, 830 and 110, microgram, respectively. The daily per capita intakes for each of the remaining substances are below 37 microgram (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 present evaluation of the candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k). For 20 candidate substances the use in flavoured food products divided into the food categories as outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), is shown in Table 3.1. For L-monomenthyl glutarate [FL-no: 09.929] the use has not been reported in accordance with the Commission Regulation.

For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

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

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

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

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

Table 3.1 Use of Candidate Substances in Various Food Categories for 20 Candidate Substances for which Data on Use have been provided

Food category	Description	Flavourings used*
01.0	Dairy products, excluding products of category 2	All except [FL-no: 07.059]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 07.059, 07.255, 09.843 and 09.949]
03.0	Edible ices, including sherbet and sorbet	All
04.1	Processed fruits	All except [FL-no: 06.136, 07.059, 07.255 and 09.843]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 09.935]
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 07.059, 07.255 and 09.935]
07.0	Bakery wares	All
08.0	Meat and meat products, including poultry and game	All except [FL-no: 06.136, 07.059, 07.255, 09.843 and 09.949]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 06.136, 07.059, 07.255, 09.843 and 09.949]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no: 06.136 and 07.059]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 07.059, 07.255, 09.843, 09.935 and 09.949]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 09.870]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All
15.0	Ready-to-eat savouries	All except [FL-no: 06.136 and 07.059]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no: 07.059 and 09.843

* No use levels (in accordance with the Commission Regulation) have been submitted for [FL-no: 09.929]

According to the Flavour Industry the candidate substances, for which Industry has provided data on food categories, normal use levels are in the range of 0.0001 - 500 mg/kg food, and the maximum use levels are in the range of 0.0001 - 2000 mg/kg food (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k).

The mTAMDI values for 13 candidate substances from structural class I (see Section 5), for which exposure data have been submitted, range from 420 to 63000 microgram/person/day. For the six candidate substances from structural class II the mTAMDI values range from 320 to 8700 microgram/person/day, respectively. For the remaining substance from structural class III the mTAMDI is 0.075 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

The 11 esters [FL-no: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the data available for the supporting substances (Emberger, 1994a; Emberger, 1994b; White et al., 1990). The resulting carboxylic acids are either metabolised through common physiological pathways like *beta*-oxidation and the citric acid cycle or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994) (see Table 2b and Annex III).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, *p*-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-no: 02.070, 02.075, 02.135, 02.167, 07.059, 07.202, 07.203 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, the hydrolysis products of [FL-no: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are also metabolised via this pathway. Neither menthol nor carveol or dihydrocarveol is anticipated to be oxidised to the corresponding ketone (for detailed discussion, see Annex III).

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion (see Annex III). Thus, it may be anticipated that these 20 substances will be metabolised to innocuous products.

No information is available on toxicokinetics (including metabolism) of cyclotetradecanone [FL-no: 07.207] or on structurally related substances. Cyclotetradecanone [FL-no: 07.207] cannot be assumed to be metabolised to innocuous products.

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 candidate substances the Procedure was applied. The stepwise evaluations are summarised in Table 2a.

Step 1

Fourteen of the candidate substances are classified into structural class I [FL-no: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949], six candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207 07.255 and 09.520] into structural class II and one substance [FL-no: 06.136] is classified into structural class III according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

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

Twenty of the candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and

09.949] are expected to be metabolised to innocuous products and accordingly they proceed via the A-side of the Procedure scheme (Annex I).

It cannot be anticipated that cyclotetradecanone [FL-no: 07.207] is metabolised to innocuous products and accordingly it proceeds via the B-side.

Step A3

The fourteen candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] that have been assigned to structural class I have estimated European daily *per capita* intakes ranging from 0.0012 to 830 microgram (Table 2a). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

The five candidate substance [FL-no: 07.059, 07.202, 07.203, 07.255 and 09.520] assigned to structural class II have European daily *per capita* intakes of 0.0085 and 770 microgram, respectively (Table 2a). For [FL-no: 07.059, 07.202, 07.203 and 07.255] the intakes are below the threshold of concern of 540 microgram/person/day for structural class II. For [FL-no: 09.520] the daily *per capita* intake of 770 microgram is above the threshold of concern for a substance assigned to structural class II. The substance therefore proceeds to step A4.

The candidate substance [FL-no: 06.136] has been assigned to structural class III and has a European daily *per capita* intake of 1.2 microgram (µg). This intake is below the threshold of concern of 90 microgram/person/day for structural class III.

Based on the results of the safety evaluation sequence, 19 candidate substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] proceeding via the A-side of the Procedure do not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substances.

Step A4

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] or its metabolites are not endogenous, the substance therefore proceeds to step A5.

Step A5

A 90 day study in rats has been performed for [FL-no: 09.520] from which a No Observed Adverse Effect Level (NOAEL) of 100 mg/kg body weight (bw)/day could be derived. This NOAEL provides a margin of safety of nearly 10^4 compared to the daily intake of 0.013 mg/kg bw/day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Therefore, [FL-no: 09.520] does not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substance.

Step B3

The estimated intake (MSDI) for cyclotetradecanone [FL-no: 07.207] of 0.061 microgram/capita/day does not exceed the threshold of concern for structural class II (540 microgram/person/day). Accordingly, the evaluation proceeds to step B4.

Step B4

Toxicity data that would permit establishment of a NOAEL are not available for cyclotetradecanone [FL-no: 07.207] or for structurally related substances. Therefore, for the candidate substance cyclotetradecanone [FL-no: 07.207] additional data are required.

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

The estimated intakes, based on the mTAMDI, range from 420 to 63000 microgram/person/day for 13 candidate substances in structural class I. For seven of these substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870] the mTAMDI is below the threshold of concern of 1800 microgram/person/day and for six substances [FL-no: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] the mTAMDI is above the threshold. For L-monomenthyl glutarate [FL-no: 09.929] no mTAMDI could be calculated due to lack of information on use levels in the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For the six substances [FL-no: 07.059, 07.202, 07.203, 07.207, 07.255 and 09.520] assigned to structural class II, the estimated intakes, based on the mTAMDI, range from 320 to 8700 microgram/person/day, which are all above the threshold of concern for structural class II substances of 540 microgram/person/day, except for [FL-no: 07.255].

For [FL-no: 06.136], assigned to structural class III, the estimated intake based on the mTAMDI is 0.075 microgram/person/day, which is below the threshold of concern for a structural class II substance of 90 microgram/person/day.

Thus, for 11 candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949] further information is therefore required. This would include more reliable intake data and then, if required, additional toxicological data. For L-monomenthyl glutarate [FL-no: 09.929] use levels are needed in accordance with the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

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

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

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.070	Cyclohexanol	3.7	1600	Class I	1800
02.075	neo-Dihydrocarveol	2.4	1600	Class I	1800
02.135	Cyclopentanol	0.012	1600	Class I	1800
02.167	Isodihydrocarveol	2.4	1600	Class I	1800
09.154	Menthyl valerate	1.0	3900	Class I	1800
09.355	neo-Dihydrocarvyl acetate	0.012	1600	Class I	1800
09.618	Menthyl formate	0.73	3900	Class I	1800
09.619	Menthyl hexanoate	0.37	3900	Class I	1800
09.621	Menthyl salicylate	0.012	420	Class I	1800
09.843	Menthol 1-and 2-propylene glycol carbonate	830	63000	Class I	1800
09.870	Carvyl-3-methylbutyrate	0.0012	1000	Class I	1800
09.929	L-Monomenthyl glutarate	110		Class I	1800
09.935	Dimenthyl glutarate	30	38000	Class I	1800
09.949	L-Menthyl (S)-3-hydroxybutyrate	37	10600	Class I	1800
07.059	p-Menthan-3-one	530	8700	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	0.12	1600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	0.0085	1600	Class II	540
07.207	Cyclotetradecanone	0.061	3900	Class II	540
07.255	l-Piperitone	12	320	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate	770	3900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one	1.2	0.075	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be

considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

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

On the basis of the reported annual production volumes in Europe (EFFA, 2003a; EFFA, 2003b; EFFA, 2011e; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k), the combined estimated daily *per capita* intake as flavourings of the 14 candidate substances assigned to class I is 1000 microgram, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day.

The candidate substances from structural class I are structurally related to 15 supporting substances for which European intake data are available (European intake data are only available for 15 of the 16 supporting substances from structural class I). The total combined intake of the 14 candidate and 15 supporting substances is approximately 17000 microgram/capita/day, which is above the threshold for structural class I substances of 1800 microgram/person/day. The major contribution (92 %) is provided by one supporting substance, menthol [FL-no: 02.015] (16 mg/*capita*/day). An ADI of 0-4 mg/kg bw was allocated to menthol (JECFA-no: 427) at the 51st meeting (JECFA, 2000a). The ADI is 15 times higher that the MSDI of 16 mg/*capita*/day. The total combined daily *per capita* intake for the remaining substances from structural class I is approximately 1400 microgram, which is below the threshold for structural class I of 1800 microgram/person/day.

On the basis of the reported annual production volumes in Europe, the combined estimated daily *per capita* intake as flavourings of the six candidate substances assigned to structural class II is 1300 microgram, which exceeds the threshold of concern for a compound belonging to structural class II of 540 microgram/person/day. Nearly 60 % of the 1300 microgram derives from one candidate substance, namely methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]. However, for methyl 3-oxo-2-pentyl-1-cyclopentylacetate a NOAEL of 100 mg/kg bw/day has been established, which provides a margin of safety of more than 7000 to the daily *per capita* intake of 770 microgram, corresponding to 13 microgram/kg bw/day. For the remaining five substances the combined daily *per capita* intake is 530 microgram, which is below the threshold of 540 microgram/person/day for a structural class II substance.

The total combined intake from the six candidate and 11 supporting substances from structural class II is approximately 2200 microgram/capita/day, which exceeds the threshold for structural class II substances of 540 microgram/person/day. The major contribution (98 %) is provided by two candidate substances, methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], *p*-menthan-3-one [FL-no: 07.059] and one supporting substance, trans-menthone [FL-no: 07.176]. The estimated intake of [FL-no: 09.520] of 770 microgram/*capita*/day corresponds to 13 microgram/kg bw/day, which is more than 7000 fold lower than the NOAEL of 100 mg/kg bw/day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate.

In human liver microsomes (-)menthone is reported to be biotransformed to (+)neomenthol as the major metabolite and to a minor extent to 7-hydroxymenthone (Miyazawa & Nakanishi, 2006). For *d,l* menthol, which is isomeric to (+)neomenthol, a NOAEL of 375 mg/kg bw is reported after oral dosing of rats for 2 years (National Cancer Institute, 1979). The combined estimated intake of *p*-menthan-3-one [FL-no: 07.059] and trans-menthone [FL-no: 07.176] of 1420 microgram/*capita*/day corresponds to 24 microgram/kg bw/day, which is nearly 16,000 fold lower than the NOAEL of 375 mg/kg bw/day for menthol. The total combined daily *per capita* intake for the remaining 14 candidate and supporting substances from structural class II is approximately 50 microgram, which is below the threshold for structural class II of 540 microgram/person/day.

The only candidate substance from structural class III, 6-isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [FL-no: 06.136] has a daily *per capita* intake of 1.2 microgram, which does not exceed the threshold of 90 microgram/person/day. There are no supporting substances from structural class III.

8. Toxicity

8.1. Acute Toxicity

Data are available for five candidate substances [FL-no: 02.070, 02.135, 06.136, 09.520 and 09.355]. Oral LD_{50} values from studies in the rat range from 625 mg/kg body weight (bw) to > 5000 mg/kg bw.

Ten supporting substances [FL-no: 02.015, 02.061, 02.062, 02.209, 07.111, 07.148, 07.176, 09.016, 09.215 and 09.216] were tested for acute toxicity in the mouse, rat, rabbit, dog and guinea pig. The LD_{50} values ranged from 930 mg/kg bw to > 5000 mg/kg bw.

The magnitudes of the LD_{50} values indicate that the oral acute toxicity is rather low for the candidate substances and supporting substances.

All acute toxicity studies are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Only one conventional subchronic oral study has been conducted on the candidate substances (for [FL-no: 09.520]). For cyclohexanol [FL-no: 02.070] data were available from a study designed to investigate only peripheral neuropathy in which rats were given intraperitoneal doses of 200 mg cyclohexanol once or twice daily for up to six weeks. No effects on the peripheral nervous system were observed but the experiment was terminated early because the animals were in poor condition, there was a decrement of weight gain and two animals died prematurely. No general gross or histopathological examinations were reported and no No Observed Adverse Effect Level (NOAEL) was established. This study was not considered applicable to the evaluation of the oral toxicity of cyclohexanol (Perbellini et al., 1981).

For methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] a study, following the current OECD guidelines, was performed in rats. Male and female Sprague-Dawley CD rats were given in the diet daily doses of 0 (basal diet), 10, 50 or 100 mg/kg bw over a 90-day period (10 male and 10 female rats per dose group). No treatment related changes were observed in mortality, expanded clinical observations, ophthalmic examination, body weight gain, body weight change, food consumption, haematology, clinical chemistry, urinalysis, organ weights and macroscopic examination. There were no treatment related histopathological changes in the tissues from rats of any of the treatment groups. The NOAEL was therefore considered to be 100 mg/kg bw/day (the highest dose tested) (Kelly and Bolte, 2000).

Carcinogenicity studies are available for the two supporting substances, cyclohexanone and menthol [FL-no: 07.148 and 02.015].

Cyclohexanone [FL-no: 07.148] was given to male and female rats in the drinking water (3300 and 6500 ppm) and male and female mice (6500, 13000 and 25000 ppm (only female)) for two years. A reduction in weight gain (15 - 20 %) was observed in all groups at the highest doses. An increase in the incidence of lymphomas was observed at a lower dose level, but it was not dose related (Lijinsky and Kovatch, 1986).

In two other studies, two doses of dl-menthol were given to rats in the diet (3750 and 7500 ppm) and mice (2000 and 4000 ppm) for 103 weeks. A small reduction in survival was seen in the treated female

mice. An increase of incidence of mammary gland fibroadenomas or mammary adenocarcinomas was observed in female rats at the lower dose level, but this was not dose related. In male rats, dl-menthol was not considered toxic or carcinogenic. In mice, a small increase in the incidence of hepatocellular carcinomas was observed. However, this increase was within the normal range of tumour incidence in the historical control groups, and the authors concluded that dl-menthol was not carcinogenic in rats and mice in the performed studies (National Cancer Institute, 1979).

For five supporting substances [FL-no: 02.015, 07.095, 07.111, 07.176 and 07.148] there are further oral subchronic toxicity data.

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

8.3. Developmental / Reproductive Toxicity Studies

There is a study available for one candidate substance [FL-no: 02.070], with a NOAEL of < 1500 mg/kg bw/day. For one supporting substance [FL-no: 02.015] there are several studies (see Annex IV, Table IV.3).

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

8.4. Genotoxicity Studies

Due to the presence of a structural alert for genotoxicity ("alpha,beta-unsaturated carbonyl moiety") for three candidate substances [FL-no: 07.202, 07.255 and 09.870] in the current revision of FGE.09, the genotoxicity of these substances was further assessed in FGE.212 and FGE.212Rev1.

In FGE.212 the concern for carvyl-3-methylbutyrate [FL-no: 09.870] was alleviated and the Panel concluded that this substance could be evaluated through the Procedure.

Since it was concluded in FGE.212Rev1, that based on additional information, the concern for genotoxic potential for isophorone [FL-no: 07.126] has been alleviated, a genotoxic potential can also be ruled out for substances structurally related to isophorone, including [FL-no: 07.202 and 07.255]. Therefore, these two substances [FL-no: 07.202 and 07.255] can be evaluated using the Procedure.

Genoxicity data are available for only three of the remaining candidate substances: cyclohexanol [FL-no: 02.070], cyclopentanol [FL-no: 02.135], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] and for nine supporting substances and one structurally related substance.

Cyclohexanol [FL-no: 02.070] was not genotoxic in two Ames tests (Barsky, 1976; Haworth et al., 1983) and in an *in vivo* micronucleus assay (Gelbke, 1991), which are all considered as valid studies. However, the results of the *in vivo* study are of limited relevance, due to the lack of evidence that the substance did reach the bone marrow. Inconclusive results were reported in an *in vitro* chromosomal aberration assay with human leukocytes (Collin, 1971) and negative results were reported in a dominant lethal mutations assay with *Drosophila melanogaster* (Goncharova, 1970)*;* both studies were considered inadequate.

Cyclopentanol [FL-no: 02.135] was studied in a valid Ames test (McMahon et al., 1979). No mutagenicity was found.

A battery of *in vitro* and *in vivo* genotoxicity studies were conducted on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] including valid negative reverse mutation tests in *Escherichia coli* (Wagner and Klug, 2000) and *Salmonella typhimurium* (Thompson, 2000).

In a mouse lymphoma test on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], pre-dating GLP, a more than 2-fold increase of the mutant frequency over the solvent treated control values was

found at the highest tested cytotoxic concentration of 300 µg/ml in the presence of metabolic activation, and at the two highest tested cytotoxic concentrations of 200 and 300 µg/ml in the absence of metabolic activation. Only limited documentation is provided in the study report; together with the fact that several cultures were infected and a lack of a confirmatory test, it is impossible to assess the reliability of these results (Ross and Harris, 1979b).

No induction of forward mutations at the TK locus in L5178Y mouse lymphoma cells were found in a study performed in compliance with the current OECD test guidelines, both in the absence and in the presence of metabolic activation, up to and including cytotoxic concentrations (Cifone, 2001).

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was tested in a bone marrow micronucleus test in mice following a single intraperitoneal administration of 0, 280, 560 or 1120 mg/kg bw in corn oil. The study was performed in compliance with the current OECD test guidelines. The two highest doses chosen induced clear signs of toxicity; slight reductions (up to 12 %) in the ratio of polychromatic erythrocytes to total erythrocytes were found, indicating that the test material had reached the target cells. No increase in micronucleated cells was found in the groups treated with the test material. The positive control induced the expected increases (Gudi and Krsmanovic, 1998).

In an Unscheduled DNA Synthesis (UDS) study, the ability of methyl 3-oxo-2-pentyl-1-cyclopentylacetate to induce DNA repair was studied in isolated rat hepatocytes after administration *in vivo*. The study was performed in compliance with the current OECD Guideline 486 (OECD, 1997a). Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was administered to male Sprague-Dawley CD rats by intraperitoneal injection in doses of 333.3 and 1000 mg/kg bw (the latter dose was the maximum tolerated dose) followed by liver perfusion at 2 or 16 hours after dosing. No marked increase in the incidence of UDS was observed at either dose level or perfusion time. Statistically significant differences were revealed in the positive control groups when compared to the negative control group and the test article (Durward, 2001).

Genotoxicity data are available for nine supporting substances [FL-no: 02.015, 02.062, 07.148, 07.176, 09.027, 09.215, 09.230, 07.149 and 07.045].

Cyclohexanone [FL-no: 07.148], structurally related to the alicyclic ketones and secondary alcohols in this FGE, was not mutagenic in an Ames test, considered to be valid (Haworth et al., 1983). Negative and positive results were reported in several other *in vitro* studies at gene and chromosomal level, as well as a negative result in a sex-linked recessive lethal mutations in *D. melanogaster*. However, these studies were considered inadequate.

Menthol [FL-no: 02.015] gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes (Storer et al., 1996). With the same substance equivocal results in an *in vivo* host mediated mutation assay were observed at high dose levels (Food and Drug Res. Lab., Inc., 1975a) and negative results in several Ames tests, a TK+/- mouse lymphoma assay (Myhr and Caspary, 1991), sister chromatid exchange (SCE) tests in Chinese hamster ovary (CHO) cells (Ivett et al., 1989) and human lymphocytes (Murthy et al., 1991), and chromosomal aberration assays with human embryonic lung cells (Food and Drug Res. Lab., Inc., 1975a), human lymphocytes (Murthy et al., 1991) and CHO cells (Ivett et al.,m 1989). Negative results were also reported in two *in vivo* micronucleus (Shelby et al., 1993) and chromosomal aberration assays (Food and Drug Res. Lab., Inc., 1975a). However, the results of these studies have a limited relevance, due to the lack of bone marrow toxicity. In addition, an *in vivo* dominant lethal assay was available, from which also negative results were obtained.

trans-Menthone [FL-no: 07.176] was genotoxic in an Ames test (Andersen and Jensen, 1984b) and in a somatic mutation and recombination test (SMART) with *Drosophila* (Franzios et al., 1997). The observed effects were not very pronounced. Further, *trans*-menthone is easily converted to menthol, which is estimated to be overall negative in genotoxicity tests.

Carveol and carvyl acetate [FL-no: 02.062 and 09.215] were tested in Ames test at various doses from 10 - 560 µg/plate in the *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without S9 mix in dimethyl sulphoxide. Positive and negative controls were used. No mutagenicity was observed (Mortelmans et al., 1986).

Conclusion on genotoxicity

For three of the candidate substances [FL-no: 07.202, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances.

Only for three of the remaining candidate substances some genotoxicity data are available, and for these three mainly negative results were obtained. For the supporting substances mainly negative, but also some positive results were obtained. The positive results were obtained in poorly reported tests, or in tests which are difficult to interpret with respect to their relevance for genotoxicity.

Overall, the genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

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

9. Conclusions

The present Revision of FGE.09, FGE.09Rev4, includes the assessment of four additional candidate substances compared to FGE.09Rev3, *p*-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], *l*-piperitone [FL-no: 07.255] and menthol 1-and 2-propylene glycol carbonate [FL-no: 09.843].

So, the present FGE.09Rev4 deals with 21 secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols. These flavouring substances belong to chemical groups 8, 25 and 30 of Annex I of Regulation (EC) No 1565/2000.

Two candidate substances [FL-no: 07.203 and 07.255] possess one chiral centre and 15 substances [FL-no: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] possess two or more chiral centres.

Fourteen of the candidate substances belong to structural class I, six substances belong to structural class II and one to structural class III according to the decision tree approach presented by Cramer et al., 1978.

Thirteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach the 14 flavouring substances belonging to structural class I have intakes in Europe ranging from 0.0012 to 830 microgram/*capita*/day, for five of the six substances from structural class II the intakes range from 0.0085 to 530 microgram/*capita*/day, and for the substance from structural class III the intake is 1.2 microgram/*capita*/day, which are all below their respective threshold of concern value for structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively. For one substance [FL-no: 09.520] from structural class II the MSDI is 770 microgram/*capita*/day, which is above the threshold of concern of 540 microgram/person/day.

The total combined intakes of candidate and supporting substances from structural class I and II do not give rise to a safety concern.

For three of the candidate substances [FL-no: 07.202, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances. Genotoxicity data are available only for a limited number of the remaining flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

Twenty of the candidate substances are expected to be metabolised to innocuous products at the estimated levels of use as flavouring substances. The remaining candidate substance, cyclotetradecanone [FL-no: 07.207], could not be assumed to be metabolised to innocuous products due to lack of data on cyclotetradecanone or on closely related substances.

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

It is considered that 20 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances based on the default MSDI approach. For one candidate substance, cyclotetradecanone [FL-no: 07.207], the evaluation has been deferred as additional data on toxicokinetics and/or toxicology are required.

When the estimated intakes were based on the mTAMDI approach they ranged from 420 to 63000 microgram/person/day for 13 candidate substances in structural class I. For seven substances [FL-no: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold of concern of 1800 microgram/person/day and for six [FL-no: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] the mTAMDI is above the threshold. For one flavouring substance [FL-no: 09.929] from structural class I the use levels are missing. The estimated intakes of the six substances assigned to structural class II, range from 320 to 8700 microgram/person/day, which for five substances [FL-no: 07.059, 07.202, 07.203, 07.207 and 09.520] are above the threshold of concern for structural class II substances of 540 microgram/person/day. The mTAMDI estimate for the one substance from structural class III [FL-no: 06.136] is 0.075 microgram/person/day, which is below the threshold of 90 microgram/person/day. Thus, for eleven candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], and for [FL-no: 09.929] for which use levels are missing, 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 nine substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.255, 09.355, 09.621 and 09.870] which have mTAMDI estimates below the threshold of concern are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 21 candidate substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all flavouring substances.

For cyclotetradecanone [FL-no: 07.207] additional toxicity data are requested (further metabolism and/or toxicity studies).

For the remaining 20 substances [FL-no: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.202, 07.203, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 9, REVISION 4

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

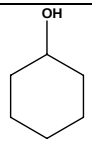
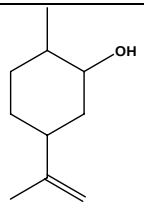
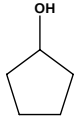
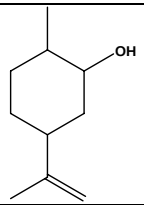
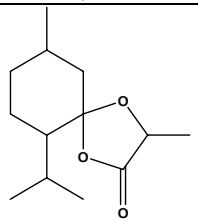
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.070	Cyclohexanol		2138 108-93-0	Solid $C_6H_{12}O$ 100.16	Slightly soluble Freely soluble	158 25 MS 95 %	1.462-1.468 0.942-0.948	
02.075	neo-Dihydrocarveol		2296 18675-34-8	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	107 (33 hPa) MS 95 %	1.476-1.482 0.920-0.926	Register name and CASrn to be changed to (1R,2S,5S)-neo-Dihydrocarveol, 18675-33-7 (EFFA, 2005l).
02.135	Cyclopentanol		10193 96-41-3	Liquid $C_5H_{10}O$ 83.13	Slightly soluble Freely soluble	140 MS 95 %	1.449-1.455 0.945-0.951	
02.167	Isodihydrocarveol		18675-35-9	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	90 (6.7 hPa) MS 95 %	1.475-1.481 0.918-0.924	Register name to be changed to (1R,2R,5S)-Isodihydrocarveol.
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		4285 831213-72-0	Liquid $C_{13}H_{22}O_3$ 226.32	Slightly soluble Soluble	259 IR NMR MS 98.9 %	1.4606-1.4609 1.017-1.021	Mixture of isomers: (3S, 5R, 6S,9R)-isomer: 65.6 % and (3S, 5S, 6S,9R)-isomer: 27.4 %, mixture of other diastereomers: 5.86 % (Flavour Industry, 2006o).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

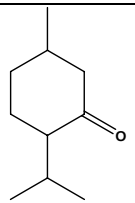
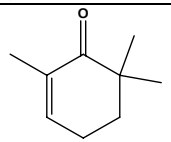
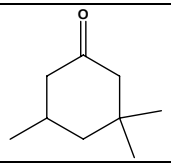
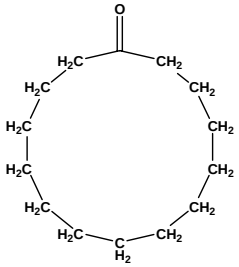
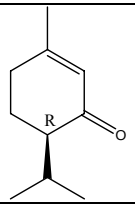
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
07.059	p-Menthan-3-one		2667 2035 10458-14-7	Liquid $C_{10}H_{18}O$ 154.25	Soluble Soluble	207 MS 96 %	1.448-1.453 0.888-0.895	Mixture of diastereoisomers, approximately 25 % of each (EFFA, 2012b).
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		20013-73-4	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Freely soluble	63 (16 hPa) MS 95 %	1.470-1.476 0.924-0.930	
07.203	3,3,5-Trimethylcyclohexan-1-one		873-94-9	Liquid $C_9H_{16}O$ 140.22	Practically insoluble or insoluble Freely soluble	189 MS 95 %	1.442-1.448 0.888-0.894	Racemate (EFFA, 2010a).
07.207	Cyclotetradecanone		3603-99-4	Solid $C_{14}H_{26}O$ 210.36	Practically insoluble or insoluble Freely soluble	159 (16 hPa) 53 NMR 95 %	n.a. n.a.	Substance no longer supported by Industry (EFFA, 2009c).
07.255 1856	l-Piperitone		4200 4573-50-6	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Freely soluble	246 MS 99 %	1.482-1.488 0.929-0.935	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

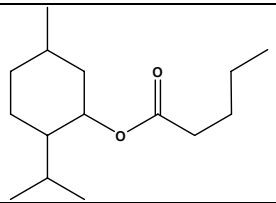
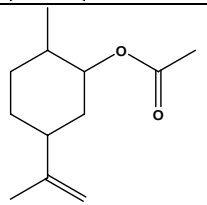
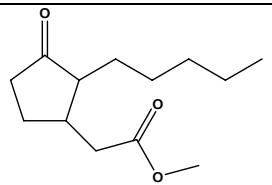
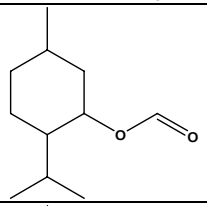
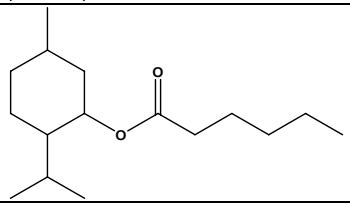
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.154 1852	Menthyl valerate		4156 472 89-47-4	Liquid $C_{15}H_{28}O_2$ 240.39	Practically insoluble or insoluble Freely soluble	261 NMR 95 %	1.445-1.451 0.903-0.909	Register name to be changed to (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl valerate (EFFA, 2010a).
09.355	neo-Dihydrocarvyl acetate		10859 56422-50-5	Liquid $C_{12}H_{20}O_2$ 196.29	Practically insoluble or insoluble Freely soluble	266 MS 95 %	1.453-1.459 0.925-0.931	According to EFFA: Mixture of the two racematic forms (1S,2R,5R) and (1R,2S,5S), which is specified by the name (EFFA, 2005l).
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		3408 10785 24851-98-7	Liquid $C_{13}H_{22}O_3$ 226.32	Slightly soluble Freely soluble	111 (0.1 hPa) NMR 98 %	1.458-1.462 0.997-1.006	According to EFFA: Mixture of the four stereoisomeric forms (RR, RS, SR and SS) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a).
09.618	Menthyl formate		10751 2230-90-2	Liquid $C_{11}H_{20}O_2$ 184.28	Practically insoluble or insoluble Freely soluble	95 (13 hPa) 9 MS 95 %	1.446-1.452 0.933-0.939	According to EFFA: Mixture of the two racematic forms (1S,2R,5S) and (1R,2S,5R), which is specified by the name.
09.619	Menthyl hexanoate		6070-16-2	Liquid $C_{16}H_{30}O_2$ 254.14	Practically insoluble or insoluble Freely soluble	153 (20 hPa) MS 95 %	1.445-1.451 0.898-0.906	Register name to be changed to (1R,2S,5R)-Menthyl hexanoate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

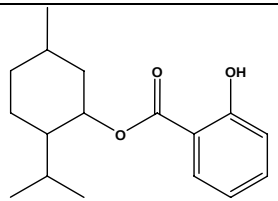
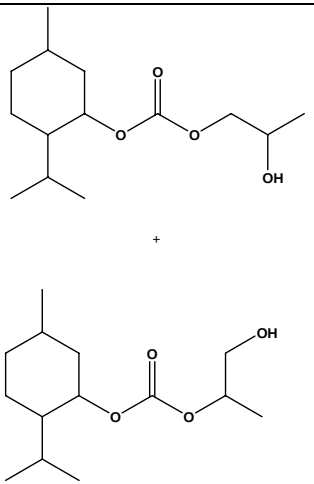
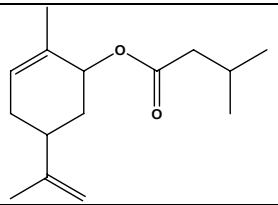
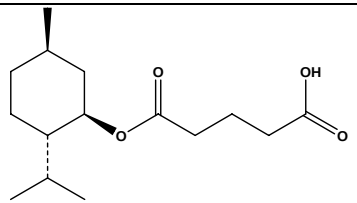
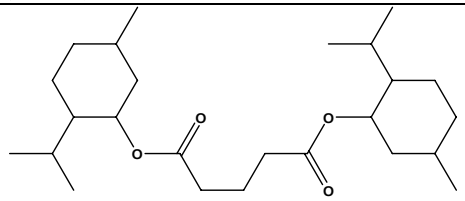
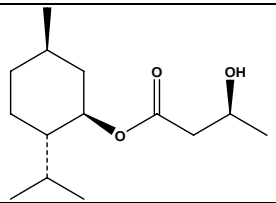
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.621	Menthyl salicylate		 89-46-3	Liquid $C_{17}H_{24}O_3$ 276.37	Practically insoluble or insoluble Freely soluble	175 (13 hPa) MS 95 %	1.509-1.515 1.047-1.053	Register name to be changed to (1R,2S,5R)-Menthyl salicylate.
09.843	Menthol 1-and 2-propylene glycol carbonate		3806 30304-82-6	Liquid $C_{14}H_{26}O_4$ 258.36	Insoluble Soluble	143 IR MS 98 %	1.458-1.458 1.013-1.014	According to EFFA: [FL-no: 09.843] is a mixture of 60 % Menthol 1-propylene glycol carbonate (which is a mixture of four stereoisomers, 15 % of each) and 40 % Menthol 2-propylene glycol carbonate (which is a mixture of four stereoisomers 10 % of each) (EFFA, 2012b)
09.870	Carvyl-3-methylbutyrate		 94386-39-7	Liquid $C_{15}H_{24}O_2$ 236.37	Practically insoluble or insoluble Freely soluble	343 MS 95 %	1.462-1.468 0.932-0.938	According to EFFA: Mixture of the four stereoisomeric forms (RR, RS, SR and SS) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 9, Revision 4

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
09.929	L-Monomenthyl glutarate		4006 220621-22-7	Liquid $C_{15}H_{26}O_4$ 270	Sparingly soluble Soluble	390 (decomp) n.a. IR NMR 95 %	1.462-1.470 1.026-1.036	
09.935	Dimenthyl glutarate		 406179-71-3	Solid $C_{25}H_{44}O_4$ 408	Insoluble Soluble	 48-50 NMR MS 98 %	n.a. n.a.	According to EFFA: Menthyl moiety mixture of the two racematic forms (1S,2R,5S) and (1R,2S,5R), which is specified by the name. Since there are two menthyl moieties, three combinations exist, approximately 25 % +/+ 25 % - /- and 50 % +/- (EFFA, 2010a).
09.949	L-Menthyl (S)-3-hydroxybutyrate		4308 115869-76-6	Liquid $C_{14}H_{26}O_3$ 242.35	Slightly soluble Soluble	95-97 (0.7hPa) IR MS 98 %	1.454 - 1.464 0.969 - 0.979	Stereoisomeric composition of (S)-form : > 80 % ee and (R)-form < 20 %.ee (ee=enatiomeric excess).

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

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

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

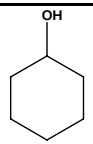
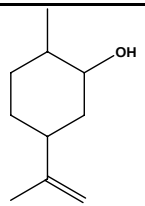
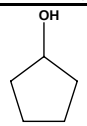
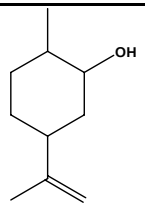
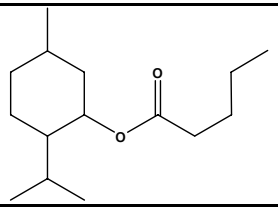
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.070	Cyclohexanol		3.7	Class I A3: Intake below threshold	4)	6)	
02.075	neo-Dihydrocarveol		2.4	Class I A3: Intake below threshold	4)	6)	
02.135	Cyclopentanol		0.012	Class I A3: Intake below threshold	4)	6)	
02.167	Isodihydrocarveol		2.4	Class I A3: Intake below threshold	4)	6)	
09.154 1852	Menthyl valerate		1.0	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)

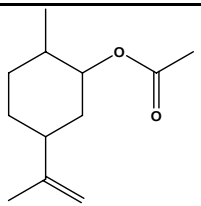
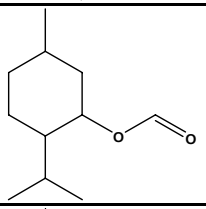
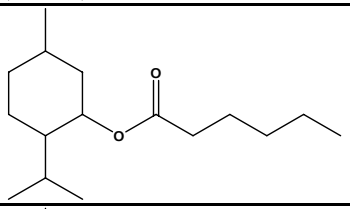
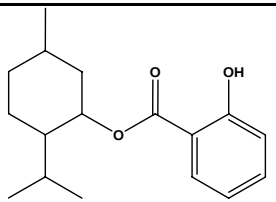
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.355	neo-Dihydrocarvyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.618	Menthyl formate		0.73	Class I A3: Intake below threshold	4)	6)	
09.619	Menthyl hexanoate		0.37	Class I A3: Intake below threshold	4)	6)	
09.621	Menthyl salicylate		0.012	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)

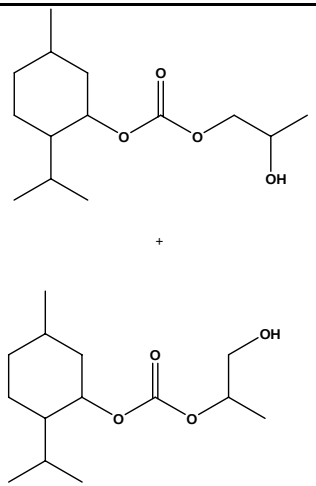
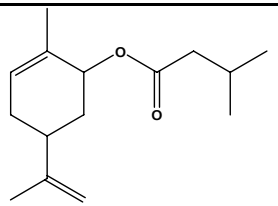
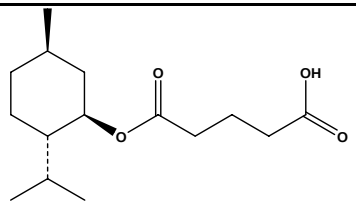
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.843	Menthol 1-and 2-propylene glycol carbonate		830 380	Class I A3: Intake below threshold	4)	6)	
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.929	L-Monomenthyl glutarate		110	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)

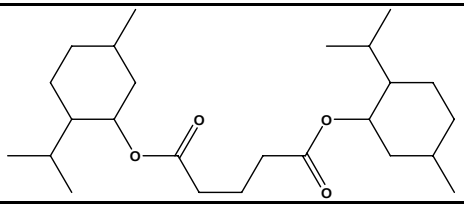
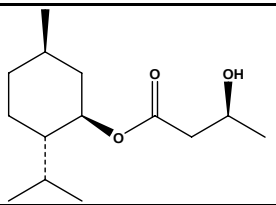
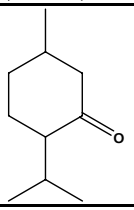
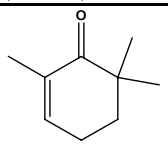
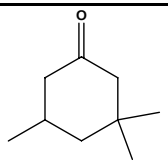
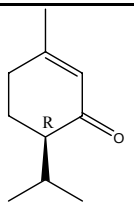
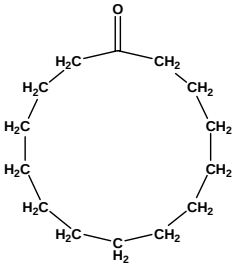
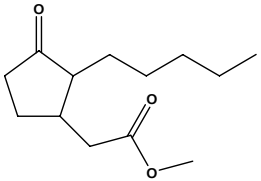
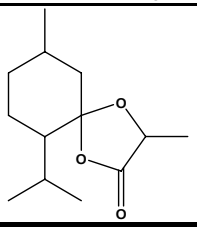
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.935	Dimenthyl glutarate		30	Class I A3: Intake below threshold	4)	6)	
09.949	L-Menthyl (S)-3-hydroxybutyrate		37	Class I A3: Intake below threshold	4)	6)	
07.059	p-Menthan-3-one		530 2500	Class II A3: Intake below threshold	4)	6)	
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		0.12	Class II A3: Intake below threshold	4)	6)	b)
07.203	3,3,5-Trimethylcyclohexan-1-one		0.0085	Class II A3: Intake below threshold	4)	6)	

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
07.255 1856	l-Piperitone		12	Class II A3: Intake below threshold	4)	6)	b)
07.207	Cyclotetradecanone		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		c)
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		770	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6)	
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		1.2	Class III A3: Intake below threshold	4)	6)	

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

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

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

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

5) Data must be available on the substance or closely related substances to perform a safety evaluation.
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).
7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.
a) Evaluated in FGE.212, genotoxic concern could be ruled out.
b) Evaluated in FGE.212Rev1, genotoxic concern could be ruled out.
c) Substance not supported by Industry (EFFA, 2009c).

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

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

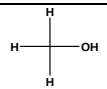
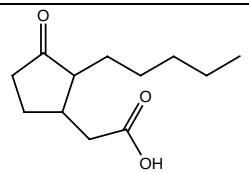
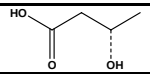
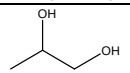
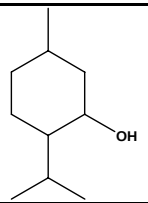
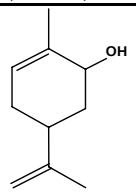
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
Not in Register	Methanol		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	3-Oxo-2-pentyl-1-cyclopentyl acetic acid		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	(S)-3-Hydroxybutyric acid		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	1,2-Propandiol		Not evaluated as flavouring substance		Not a Register substance.
02.015	Menthol 427		No safety concern a) Category A b)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	NOAEL: 380 mg/kg bw/day
02.062	Carveol 381		No safety concern a) Category B b)	Class I A3: Intake below threshold	

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

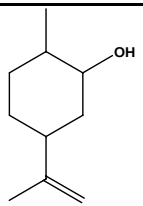
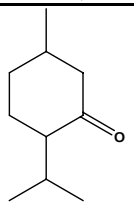
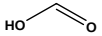
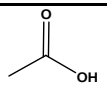
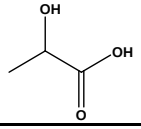
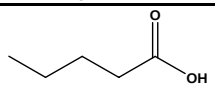
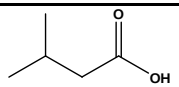
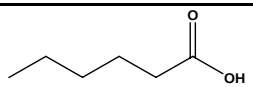
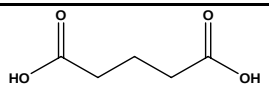
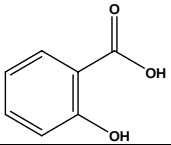
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.075	neo-Dihydrocarveol		Category B b) FGE.09	Class I A3: Intake below threshold	
07.059	p-Menthan-3-one		Category B b) FGE.37	Class II A3: Intake below threshold	
08.001	Formic acid 79		Category 1 c) No safety concern d) Deleted b)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern e) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	

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
08.082	Glutaric acid		FGE.10	Class I A3: Intake below threshold	
08.112	Salicylic acid 958		No safety concern e)	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) (JECFA, 2000a).

b) (CoE, 1992).

c) (SCF, 1995).

d) (JECFA, 1999b).

e) (JECFA, 2002b).

ND: Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

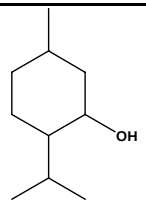
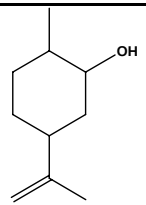
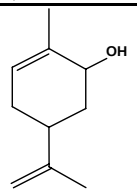
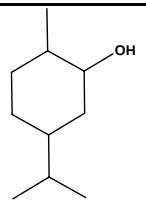
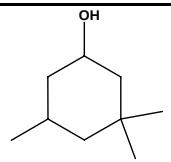
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998b)	16000	No safety concern a) Category A b)	ADI: 0-4 (JECFA, 2000a).
02.061	Dihydrocarveol		2379 2025 619-01-2	378 JECFA specification (JECFA, 1998b)	0.37	No safety concern a) Category B b)	
02.062	Carveol		2247 2027 99-48-9	381 JECFA specification (JECFA, 1998b)	9.5	No safety concern a) Category B b)	
02.071	p-Menthan-2-ol		3562 2228 499-69-4	376 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category B b)	
02.209	3,3,5-Trimethylcyclohexan-1-ol		3962 116-02-9	1099 JECFA specification (JECFA, 2002d)	0.12	No safety concern c)	JECFA name: 3,3,5-Trimethyl cyclohexanol.

Table 3: Supporting Substances Summary

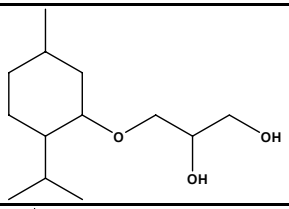
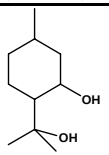
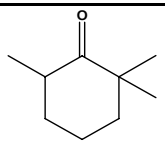
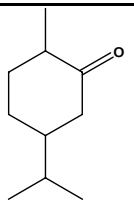
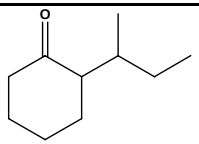
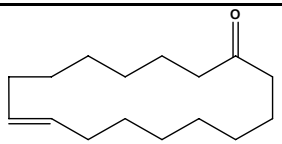
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.224	3-(1-Menthoxy)propane-1,2-diol		3784 87061-04-9	1408 JECFA specification (JECFA, 2005b).	4.1	No safety concern d)	JECFA name: 3-L-Menthoxypropane-1,2-diol.
02.246	p-Menthane-3,8-diol		4053 42822-86-6	1416 JECFA specification (JECFA, 2005b).	39	No safety concern d)	
07.045	2,2,6-Trimethylcyclohexanone		3473 686 2408-37-9	1108 JECFA specification (JECFA, 2002d)	2.1	No safety concern c) Category B b)	Stereoisomeric composition to be specified.
07.092	p-Menthan-2-one		3176 11128 499-70-7	375 JECFA specification (JECFA, 1998b)	0.012	No safety concern a)	
07.095	2-(sec-Butyl)cyclohexanone		3261 11044 14765-30-1	1109 JECFA specification (JECFA, 2002d)	5.1	No safety concern c)	
07.110	Cycloheptadec-9-en-1-one		3425 11744 542-46-1	1401 JECFA specification (JECFA, 2005b).	0.24	No safety concern d)	

Table 3: Supporting Substances Summary

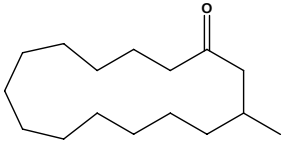
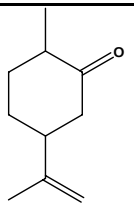
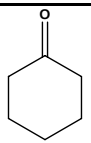
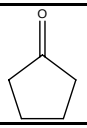
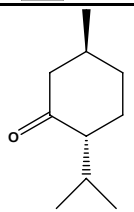
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.111	3-Methylcyclopentadecan-1-one		3434 11135 541-91-3	1402 JECFA specification (JECFA, 2005b).	0.37	No safety concern d)	JECFA name: 3-Methyl-1-cyclopentadecanone.
07.128	Dihydrocarvone		3565 11703 7764-50-3	377 JECFA specification (JECFA, 2000d)	0.012	No safety concern a)	
07.148	Cyclohexanone		3909 11047 108-94-1	1100 JECFA specification (JECFA, 2002d)	0.12	No safety concern c)	
07.149	Cyclopentanone		3910 11050 120-92-3	1101 JECFA specification (JECFA, 2002d)	0.018	No safety concern c)	
07.176	trans-Menthone	 (-)menthone shown	2667 2035 89-80-5	429 JECFA specification (JECFA, 1998b)	890	No safety concern a)	JECFA name: Menthone. CASrn in Register refers to cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R,5S)-rel-.

Table 3: Supporting Substances Summary

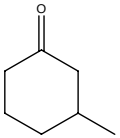
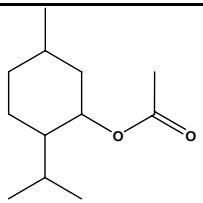
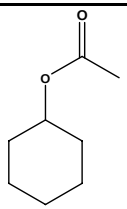
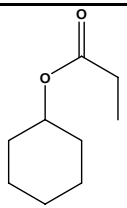
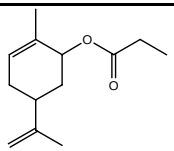
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.180	3-Methylcyclohexanone		3947 591-24-2	1103 JECFA specification (JECFA, 2002d)	0.12	No safety concern c)	
09.016	Menthyl acetate		2668 206 29066-34-0	431 JECFA specification (JECFA, 1998b)	270	No safety concern a) Category B b)	JECFA evaluated menthyl acetate (CASrn 16409-45-3 which does not specify isomer). CASrn in Register replaced by 89-48-5 which refers to Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate, (1R,2S,5R) (SciFinder).
09.027	Cyclohexyl acetate		2349 217 622-45-7	1093 JECFA specification (JECFA, 2002d)	12	No safety concern c) Category B b)	
09.140	Cyclohexyl propionate		2354 421 6222-35-1	1097 JECFA specification (JECFA, 2002d)	0.012	No safety concern c) Category B b)	
09.143	Carvyl propionate		2251 424 97-45-0	383 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

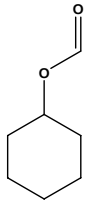
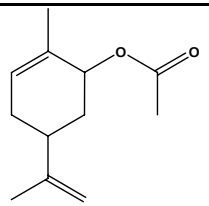
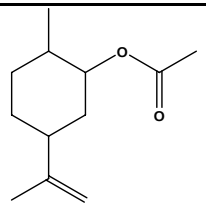
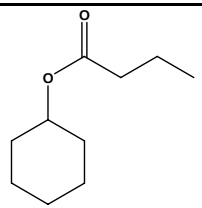
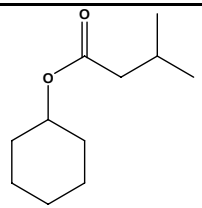
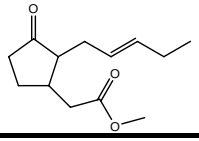
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.160	Cyclohexyl formate		2353 498 4351-54-6	1095 JECFA specification (JECFA, 2002d)	0.012	No safety concern c) Category B b)	
09.215	Carvyl acetate		2250 2063 97-42-7	382 JECFA specification (JECFA, 1998b)	4.0	No safety concern a) Category B b)	
09.216	Dihydrocarvyl acetate		2380 2064 20777-49-5	379 JECFA specification (JECFA, 1998b)	9.7	No safety concern a) Category B b)	
09.230	Cyclohexyl butyrate		2351 2082 1551-44-6	1094 JECFA specification (JECFA, 2002d)	0.89	No safety concern c) Category B b)	
09.464	Cyclohexyl isovalerate		2355 459 7774-44-9	1096 JECFA specification (JECFA, 2002d)	0.28	No safety concern c) 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
09.521	Methyl 3-oxo-2-pent-2-enyl-1-cyclopentylacetate		3410 10821 39924-52-2	1400 JECFA specification (JECFA, 2005b).	26	No safety concern d)	JECFA evaluated methyl jasmonate (CASrn 1211-29-6). (R)- or (S)- nor (E)- or (Z)- not specified by Register CASrn.

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, 2000a).

b) (CoE, 1992).

c) (JECFA, 2002c).

d) (JECFA, 2005c).

ND) No intake data reported

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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

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

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

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

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

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

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

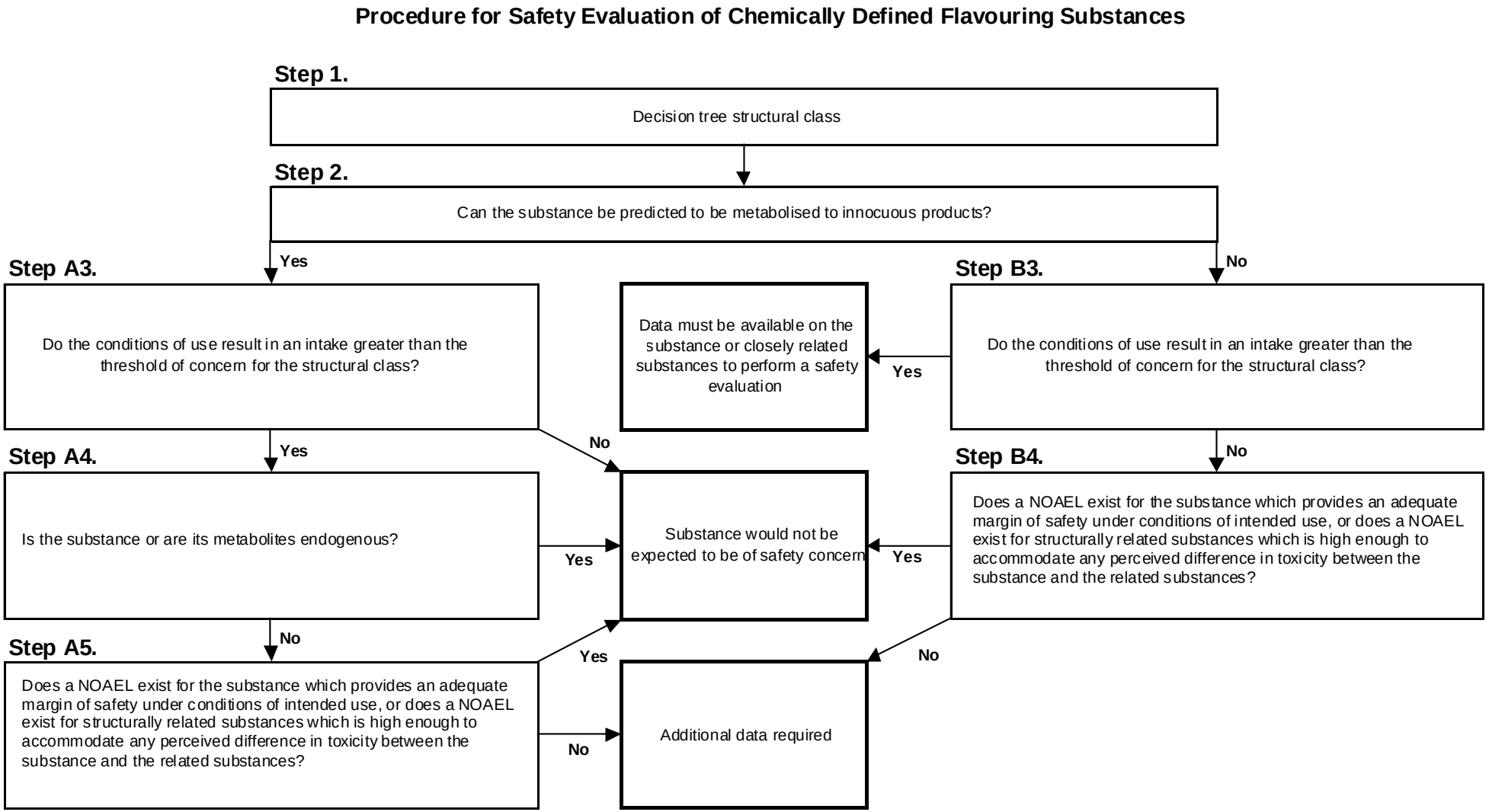


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 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 (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k) for 20 of the 21 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.09Rev4 (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k).

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
02.070	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
02.075	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
02.135	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
02.167	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
06.136	0,0001 0,0005	0,0001 0,0001	0,0001 0,0008	- -	- -	0,001 0,005	0,0001 0,0005	0,0001 0,0005	-	-	-	-	-	0,0001 0,0005	0,0001 0,0005	0,0001 0,0008	- -	0,0001 0,0005
07.059	- -	- -	15,32 22,99	- -	- -	33,27 52,97	- -	47,89 68,1	- -	- -	- -	- -	- -	- -	4,22 5,86	0,87 2,59	- -	- -

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.09Rev4 (Burdock, 1995; EFFA, 2003a; EFFA, 2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k).

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
07.202	3 15	2 10	3 15	2 10	-	4 20	2 10	5 25	1 5	1 5	-	-	2 10	3 15	2 10	4 20	5 25	2 10
07.203	3 15	2 10	3 15	2 10	-	4 20	2 10	5 25	1 5	1 5	-	-	2 10	3 15	2 10	4 20	5 25	2 10
07.207	7 35	5 25	10 50	7 35	-	10 50	5 25	10 50	2 10	2 10	-	-	5 25	10 50	5 25	10 50	20 100	5 25
07.255	0,5 5	- -	0,2 2	- -	-	0,5 5	- -	1 10	- -	- -	-	-	0,2 2	- -	0,2 1	0,2 2	5 50	0,2 2
09.154	7 35	5 25	10 50	7 35	-	10 50	5 25	10 50	2 10	2 10	-	-	5 25	10 50	5 25	10 50	20 100	5 25
09.355	3 15	2 10	3 15	2 10	-	4 20	2 10	5 25	1 5	1 5	-	-	2 10	3 15	2 10	4 20	5 25	2 10
09.520	7 35	5 25	10 50	7 35	-	10 50	5 25	10 50	2 10	2 10	-	-	5 25	10 50	5 25	10 50	20 100	5 25
09.618	7 35	5 25	10 50	7 35	-	10 50	5 25	10 50	2 10	2 10	-	-	5 25	10 50	5 25	10 50	20 100	5 25
09.619	7 35	5 25	1 50	7 35	-	10 50	5 25	10 50	2 10	2 10	-	-	5 25	10 50	5 25	10 50	20 100	5 25
09.621	0,5 2,5	0,2 1	0,5 2,5	0,4 2	-	1 5	0,2 1	2 10	0,2 1	0,2 1	-	-	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
09.843	200 800	- -	100 400	- -	-	500 2000	15 60	60 250	- -	- -	-	-	25 100	- -	30 120	100 400	25 100	- -
09.870	3 15	2 10	3 15	2 10	-	4 20	2 10	5 25	1 5	1 5	-	-	2 10	3 15	- -	4 20	5 25	2 10
09.935	1 15	1 15	10 150	1 15	1 15	100 1500	- -	10 150	1 15	1 15	-	-	1 15	- -	100 1500	100 1500	1 15	1 15
09.949	30 150	- -	10 50	20 100	-	50 200	5 20	20 100	- -	- -	-	-	10 30	- -	10 50	10 50	30 150	10 30

The candidate substances [FL-no. 07.059, 09.843 and 09.949] are also used in chewing gum, which is not covered by any of the above food categories. The normal/maximum use levels for chewing gum are reported to be 14.34/14.34 mg/kg for [FL-no: 07.059], 5000/20000 mg/kg for [FL-no: 09.0843] and 500/1000 mg/kg for [FL-no: 09.949]. Under the assumptions that all of the flavouring substances are released from the chewing gum and that the intake estimate is 2 g chewing gum/day, the calculation of the mTAMDI of the candidate substance based on the 16 food categories and the use of chewing gum sum up to 8700, 63000 and 10600 µg/person/day, respectively. These figures are presented in tables II.2.3 and 6.1.

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

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)
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

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

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

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

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

The mTAMDI values (see Table II.2.3) are presented for each of the flavouring substances in the present flavouring group, for which Industry has provided use and use levels (Burdock, 1995; EFFA, 2003a; EFFA,

2007a; Flavour Industry, 2004h; Flavour Industry, 2006c; Flavour Industry, 2006o; Flavouring Industry, 2007n; Flavour Industry, 2010f; Flavour Industry, 2010k). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.070	Cyclohexanol	1600	Class I	1800
02.075	neo-Dihydrocarveol	1600	Class I	1800
02.135	Cyclopentanol	1600	Class I	1800
02.167	Isodihydrocarveol	1600	Class I	1800
09.154	Menthyl valerate	3900	Class I	1800
09.355	neo-Dihydrocarvyl acetate	1600	Class I	1800
09.618	Menthyl formate	3900	Class I	1800
09.619	Menthyl hexanoate	3900	Class I	1800
09.621	Menthyl salicylate	420	Class I	1800
09.843	Menthol 1-and 2-propylene glycol carbonate	63000	Class I	1800
09.870	Carvyl-3-methylbutyrate	1000	Class I	1800
09.929	L-Monomenthyl glutarate		Class I	1800
09.935	Dimenthyl glutarate	38000	Class I	1800
09.949	L-Menthyl (S)-3-hydroxybutyrate	10600	Class I	1800
07.059	p-Menthan-3-one	8700	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	1600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	1600	Class II	540
07.255	l-Piperitone	320	Class II	540
07.207	Cyclotetradecanone	3900	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate	3900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one	0.075	Class III	90

ANNEX III: METABOLISM

III.1. Absorption, Distribution and Elimination

The candidate substances of secondary alicyclic saturated and unsaturated alcohols, ketones and esters of the present flavouring group evaluation are expected to be rapidly absorbed from the gastrointestinal tract. Supporting substances evaluated by the JECFA sustain this view (JECFA, 1999a; JECFA, 2003a).

III.2. Biotransformation

The candidate substances are expected to be metabolised through several alternative metabolic pathways. Depending on their chemical structure, the possible metabolic reactions are the following:

III.2.1 Ester hydrolysis

III.2.2 Reduction of ketone groups and oxidation of alcohol groups

III.2.3 Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

III.2.4 Metabolism to glucuronides

III.2.5 Metabolism to sulphates

No information is available on the toxicokinetics of the candidate substance cyclotetradecanone [FL-no: 07.207] or any related supporting substances in the present FGE.

III.2.1. Ester hydrolysis

The esters included in this FGE are expected to be hydrolysed enzymatically to carboxylic acids and alcohols via carboxylesterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980). For the one hemiketal ester [FL-no: 06.136] hydrolysis to the corresponding cyclic ketone, p-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004] is expected.

The supporting substances menthyl acetate [FL-no: 09.016] and dihydrocarvyl acetate [FL-no: 09.216] were previously evaluated by the JECFA (JECFA, 1999a), but no metabolism studies were available for these supporting substances, structurally related to the candidate substances menthyl valerate, neo-dihydrocarvyl acetate, menthyl formate and menthyl hexanoate [FL-no: 09.154, 09.355, 09.618 and 09.619]. The JECFA evaluation was based on a study demonstrating about 75 % and 85 % hydrolysis of 1-menthol propylene glycol carbonate and 1-menthol ethylene glycol carbonate, respectively, after four hours in liver homogenate. Less than 20 % of these two substances were hydrolysed in gastric juice and intestinal fluid (Emberger, 1994a; Emberger, 1994b). More than 80 % of a radioactively labelled mandelic acid of 3,3,5-trimethylcyclohexanol, a cyclohexyl ester structurally related to the candidate substance menthyl salicylate [FL-no: 09.621], was hydrolysed after 15 minutes of incubation with rat hepatic microsomes (White et al., 1990).

Based on these data, it is anticipated that candidate esters and the one hemiketal ester [FL-no: 06.136, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949], after intestinal absorption are hydrolysed to the corresponding alcohols/ketone and their corresponding carboxylic acids (see Table 2b). The simple mono- and di-carboxylic acids [FL-no: 08.001, 08.002, 08.004, 08.007, 08.008,

08.009 and 08.082] and 3-hydroxybutyric acid are expected to be completely metabolised through common routes of biotransformations. The acids salicylic acid [FL-no: 08.112] and 3-oxo-2-pentyl-1-cyclopentyl acetic acid (formed from [FL-no: 09.520]) are anticipated to be conjugated and excreted with the urine.

III.2.2. Reduction of ketone groups and oxidation of alcohol groups

Six of the candidate substances [FL-no: 07.059, 07.202, 07.203, 07.207, 07.255 and 09.520] contain a ketone group, which may be metabolically reduced to a hydroxyl group. This may also be expected for the hemiketal ester [FL-no: 06.136] after hydrolysis to ketone.

Incubation of human liver microsomes with the supporting substance trans-menthone resulted in formation of two metabolites. The major metabolite was a reduction product, (+)neomenthol and a hydroxylation product, 7-hydroxymenthone, was a minor metabolite (Miyazawa & Nakanishi, 2006)

Metabolism of the supporting substance carveol [FL-no: 02.062], the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870], was studied *in vitro*. (+)-Carveol and (+)-carvone were incubated with liver microsomes from dogs, rabbits, guinea pigs, mice, rats, monkeys and humans. (+)-Carveol was oxidized to (+)-carvone by liver microsomes of dogs, rabbits and guinea pigs, but not by those of humans, monkeys, rats and mice. On the other hand the (+)-carvone was reduced to (+)-carveol by liver microsomes of all animals examined. These results suggest a species specific metabolism of (+)-carveol, and shows that carveol is not converted to carvone in the liver of humans (Shimada et al., 2002).

In vivo metabolism of *l*-menthol was studied in adult male rats by giving the rats 800 mg/kg bw *l*-menthol solved in 1 % methyl cellulose solution by gavage every day for 20 days. Control rats were given vehicle only. The following metabolites of *l*-menthol were found in the urine; *p*-menthane-3,8-diol, *p*-menthane-3,9-diol, 3,8-oxy-*p*-menthane-7-carboxylic acid and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. The main urinary metabolites were *p*-menthane-3,9-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. Menthone was not detected (Madyastha and Srivatsan, 1988a).

III.2.3. Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

Oxidation of alkyl groups have been observed for menthol and for the hydrolysis product of the candidate esters neo-dihydrocarvyl acetate [FL-no: 09.355] and menthyl formate [FL-no: 09.618], and for the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] (Truhaut et al., 1970; Yamaguchi et al., 1994).

III.2.4. Metabolism to glucuronides

The hydrolysis product menthol as such or after the oxidation of the alkyl ring substituents is mainly conjugated with glucuronic acid and excreted *via* the bile in rats. Low levels of oxidation products were found in the urine, but no unchanged menthol was detected in the urine, faeces or bile after oral administration of radioactive labelled menthol (Yamaguchi et al., 1994).

The candidate substances isodihydrocarveol and neo-dihydrocarveol [FL-no: 02.167 and 02.075] are also anticipated to be conjugated with glucuronic acid, since dihydrocarveol after application by gavage to rabbits was found in the urine as the glucuronide (Hämäläinen, 1912; JECFA, 1999a). However, dihydrocarveol was also found to be excreted unchanged (Fischer and Bielig, 1940; JECFA, 1999a). In rabbits, carvone is reduced to yield carveol, which then is converted to the glucuronic acid conjugate and excreted in the urine (Fischer and Bielig, 1940). Carveol, the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870] is therefore anticipated to be conjugated with glucuronic acid and excreted in the urine.

Salicylic acid (resulting from hydrolysis of menthyl salicylate [FL-no: 09.621]) is either excreted unchanged, or as salicyluric acid and salicylic glucuronide (Vree et al., 1994).

The candidate alicyclic ketones (p-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203], l-piperitone [FL-no: 07.255] and methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]) are anticipated to be reduced to the corresponding secondary alcohols. These secondary alcohols and the candidate secondary alcohols cyclohexanol [FL-no: 02.070], neo-dihydrocarveol [FL-no: 02.075], isodihydrocarveol [FL-no: 02.167] and cyclopentanol [FL-no: 02.135] are mainly excreted as conjugates with glucuronic acid. Studies in rabbits with the supporting substance cyclohexanone [FL-no: 07.148] and with cyclopentanone and cycloheptanone show that 50 - 70 % of these substances are reduced to the corresponding alcohols (the candidate substances cyclohexanol [FL-no: 02.070] and cyclopentanol [FL-no: 02.135] and cycloheptanol), which are conjugated with glucuronic acid and excreted (Elliott et al., 1959; James and Waring, 1971). Workers employed in a shoe factory were exposed to small amounts of cyclohexane in the air. Cyclohexanol and cyclohexanone were found in the urine of these workers, indicating that the same metabolic pathways are also found in humans (Governa et al., 1987). A recent study in humans shows that the main metabolite in urine after cyclohexanone or cyclohexanol exposure is not cyclohexanol-glucuronide as in rabbit and rats, but 1,2-cyclohexanediol-glucuronide (Mráz et al., 1994; Mráz et al., 1998).

When the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] was given to rats and rabbits glucuronides of 3,5,5-trimethylcyclohexanol were detected in the urine (Truhaut et al., 1979).

III.2.5. Metabolism to sulphates

A small fraction of the two candidate substances, cyclopentanol and cyclohexanol [FL-no: 02.135 and 02.070], is anticipated to be conjugated with sulphate and excreted in the urine. This is based on studies on the structurally related substances cyclopentanone, cyclohexanone and cycloheptanone, which were given by gavage to rabbits (1.7 - 2.3 mmol/kg) and rats (1.8 - 2.5 mmol/kg), and 1 - 3 % of the dose was found in the urine as sulphate conjugates (James and Waring, 1971).

III.3. Summary and Conclusions

The 11 esters [FL-no: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the evaluation of supporting substances (Heymann, 1980; Anders, 1989; Emberger, 1994a; Emberger, 1994b; White et al., 1990). The resulting carboxylic acids are either metabolised through common physiological pathways like *beta*-oxidation and the citric acid cycle or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, *p*-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-no: 02.070, 02.075, 02.135, 02.167, 07.059, 07.202, 07.203 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, hydrolysis products of [FL-no: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are also metabolised via this pathway. Neither menthol nor carveol or dihydrocarveol is anticipated to be oxidised to the corresponding ketone.

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion.

Thus, it may be anticipated that these 20 substances will be metabolised to innocuous products.

No information is available on toxicokinetics (including metabolism) of cyclotetradecanone [FL-no: 07.207] or on structurally related substances. Cyclotetradecanone [FL-no: 07.207] cannot be assumed to be metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for five candidate substances of the present FGE and for ten supporting substances evaluated by the JECFA at the 51st, 59th and 63rd meetings. The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Menthol [02.015])	Mouse	M	Gavage	2652	(Food and Drug Research Laboratories, Inc., 1975a)	
	Mouse	M	Gavage	4384	(Food and Drug Research Laboratories, Inc., 1975a)	
	Mouse	NR	Gavage	3100	(Wokes, 1932)	
	Rat	M, F	Gavage	3180	(Jenner et al., 1964)	
	Rat	M	Gavage	940	(Food and Drug Research Laboratories, Inc., 1975a)	
(trans-Menthone [07.176])	Rat	M, F	Oral	1600 - 1950	(Igimi and Ide, 1974; Levenstein, 1973c)	Testmaterial = racemic menthone
(Menthyl acetate [09.016])	Rat	M, F	Gavage	> 7000	(Levenstein, 1973d)	Testmaterial = racemic menthyl acetate
	Rat	M, F	Oral	> 5000	(Shelanski, 1972a)	Testmaterial = *l*-menthyl acetate
(Dihydrocarveol [02.061])	Rat	NR	Oral	> 5000	(Moreno, 1977k)	
(Dihydrocarvyl acetate [09.216])	Rat	NR	Oral	> 5000	(Moreno, 1980f)	
neo-Dihydrocarvyl acetate [09.355]	Rat	NR	Oral	> 5000	(Moreno, 1980f)	
Cyclopentanol [02.135]	Rat	NR	Gavage	< 625	(Myers et al., 1980)	
(3,5,5-Trimethylcyclohexanol [02.209])	Rat	M, F	Oral	3250	(Smyth and Carpenter, 1948)	
(Cyclohexanone [07.148])	Rat	M, F	Oral	1705	(Kohli et al., 1967)	
	Rat	M, F	Oral	1840	(Deichmann and LeBlanc, 1943)	
	Rat	M, F	Oral	1620	(Smyth et al., 1969a)	
	Rat	M, F	Oral	1800	(Gupta et al., 1979)	
	Mouse	M, F	Oral	2070	(Gupta et al., 1979)	
	Rabbit	M	Gavage	1600	(Treon et al., 1943)	
	Rabbit	M	IP	1540	(Gupta et al., 1979)	
	Guinea pig	M	IP	930	(Price, 1951)	
Cyclohexanol [02.070]	Rat	M	Gavage	1750	(Miller and Sherman, 1965)	
	Rat	NR	Oral	1550	(Birch, 1978a)	
	Rat	NR	Oral	2060[1]	(Smyth et al., 1946c)	
	Rat	NR	Oral	2060	(Bär and Griepentrog, 1967)	
	Rat	M, F	Oral	1120	(Birch et al., 1981)	
	Rabbit	NR	Gavage	2200 - 2600[2]	(Treon et al., 1943)	
(3-Methylcyclopentadecan-1-one [07.111])	Dog	M, F		> 2000	(You et al., 1997)	
	Rat	M, F		> 5000	(Oh et al., 1997)	
6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [06.136]	Rat	NR	Oral	>2000	(Flavour Industry, 2006c)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Rat	NR	Oral	> 2000	(Flavour Industry, 2006c)	
(Carveol [02.062])	Rat	NR	Oral	3000	(Keating, 1972a)	
(Carvyl acetate [09.215])	Rat	NR	Oral	> 5000	(Levenstein, 1976c)	

NR: Not reported, M: Male, F: Female.

[1] Administered as 10 % solution in Tergitol 7.

[2] Minimum lethal dose.

Subacute / subchronic / chronic toxicity data are available two candidate substance of the present flavouring group and for five supporting substances evaluated at the 51st, 59th and 63rd JECFA meetings. The supporting substances are listed in brackets.

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
(Menthol [02.015])	Mouse; M, F 2/50	Diet	2000, 4000 ppm	103 weeks	600[1]	(National Cancer Institute, 1979)	Good quality
	Mouse; F 2/30	Intraperitoneal injection (IP)	500 and 2000 mg/kg 3 times week	24 weeks	A NOAEL was not determined	(Stoner et al., 1973)	Good quality
	Rat; M, F 3/20	Gavage	0, 200, 400 and 800 mg/kg bw day	28 days	< 200[2]	(Thorup et al., 1983a)	Relative good quality
	Rat; M, F 2/80	Diet	100 and 200 mg/kg bw	5.5 weeks	200[1]	(Herken, 1961)	Limited information
	Rat; M, F 2/50	Diet	3750 and 7500 ppm	103 weeks	375[1]	(National Cancer Institute, 1979)	Good quality
(trans-Menthone [07.176])	Rat; M, F 3/20	Gavage	200, 400 and 800 mg/kg bw day	28 days	400	(Madsen et al., 1986)	Good quality
	Mouse; F 2/30	IP	1900 and 4750 mg/kg 3 times week	24 weeks	A NOAEL was not determined[2]	(Stoner et al., 1973)	Good quality
(Cyclohexanone [07.148])	Mouse; M, F 7/20	Drinking Water	400 - 47000 ppm	13 weeks	M: approx. 3300, F: approx. 6500	(Lijinsky and Kovatch, 1986)	Good quality
	Rat; M, F 7/10	Drinking water	190 – 6500 ppm	25 weeks	Approx. 330	(Lijinsky and Kovatch, 1986)	Good quality
	Mouse; M, F 3/84-104	Drinking water	6500, 13000 and 25000 (F) ppm	2 years	Approx. 1600	(Lijinsky and Kovatch, 1986)	Good quality
	Rat; M, F 2/104	Drinking water	3300 and 6500 ppm	2 years	Approx. 330	(Lijinsky and Kovatch, 1986)	Good quality
	Rat 1/7	IP	200 mg/kg bw (twice a day) 5 days/week	13 weeks	A NOAEL was not determied[1]	(Perbellini et al., 1981)	Only neurotoxicity was checked. Limited experimental design
Cyclohexanol [02.070]	Rats 1/7	IP	200 mg/kg bw (twice a day) 5 days/week	3 weeks (twice a day) plus 3 weeks (once a day)	A NOAEL was not determied[1]	(Perbellini et al., 1981)	Limited experimental design
	Rat; M 1/6	Gavage	455 mg/kg day	7 days	455[1]	(Lake et al., 1982)	Limited quality
	Rat; M 1/NR	Drinking water	10 ppm	30 days	1[1]	(Messiha and Lox, 1985)	Limited quality
(2-sec-Butylcyclohexanone [07.095])	Rat 3/NR	Diet		91	370	(Hummler, 1969)	Study not available
(3-Methylcyclopentadecan-1-one [07.111])	Rat, M, F 3/20	Gavage		30	1000[3]	(Oh et al., 1997)	
	Dog, M, F 3/6	Gavaga		28	20[3]	(You et al., 1997)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Rat M, F 10/10	Diet	0, 10, 50 or 100 mg/kg bw day	90	100	(Kelly and Bolte, 2000)	

NR: Not reported.

M: Male, F: Female.

[1] The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

[2] The test substance was administered 3 times per week for 8 weeks; animals were observed for an additional 16 weeks.

[3] Study was performed with either a single dose or multiple doses that produced no advere effect. The value is therefore not a true NOEL, but is the highest dose tested that produced no adverse effects. The actual NOEL may be higher.

Developmental and reproductive toxicity data are only available for one candidate substance of the present FGE and for one supporting substance evaluated at the 51st JECFA meeting. Supporting substance is listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name	Study type Duration	Species/Sex No/group	Route	Dose levels (mg/kg/day)	NOAEL (mg/kg/day) Including information on possible maternal toxicity	Reference
(Menthol [02.015])	Teratology Gestation days 6-15	Mouse; F 22	Gavage	0, 1.85, 8.59, 39.9, 185	185[1]	(Food and Drug Research Laboratories, Inc., 1973)
	Teratology Gestation days 6-15	Rat; F 22-23	Gavage	0, 2.18, 10.15, 47.05, 218	218[1]	(Food and Drug Research Laboratories, Inc., 1973)
	Teratology Gestation days 6-15	Hamster; F 20-22	Gavage	0, 4.05, 21.15, 98.2, 405	405[1]	(Food and Drug Research Laboratories, Inc., 1973)
	Teratology Gestation days 6-18	Rabbit; F 9-11	Gavage	0, 4.25, 19.75, 91.7, 425	425[1]	(Food and Drug Research Laboratories, Inc., 1973)
Cyclohexanol [02.070]	Reproductive NR[2]	Mouse; M, F NR	Diet	ca. 1500 (1 %)	< 1500 (< 1 %)	(Gondry, 1972)

NR: Not reported.

M: Male, F: Female.

[1] The study was performed at a single dose level or multiple dose levels that produced no adverse effects.
[2] Animals were exposed during gestation, lactation and weaning over multiple generations. Total length of exposure not reported.

In vitro mutagenicity/genotoxicity data are available for three candidate substances of the present flavouring group evaluation and for nine supporting substances evaluated at the 51st, 59th and 63rd JECFA meetings and one structurally related substance evaluated at the 69th meeting. Supporting substances are listed in brackets.

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

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
(Menthol [02.015])	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	0, and 6 concentrations up to 5000 µg/plate	Negative[1]	(Ishidate et al., 1984)	d,l-Menthol was used. The study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97, TA98, TA100, TA1535	3 - 666 µg/plate	Negative[1]	(Zeiger et al., 1988)	d,l-Menthol was used. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 5 - 500 µg/plate	Negative[1]	(Nohmi et al., 1985)	d,l-menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 20 - 500 µg/plate	Negative[1]	(Nohmi et al., 1985)	l-menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 6.4, 32, 160, and 800 µg/plate	Negative[1]	(Andersen and Jensen, 1984b)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.
	Ames test	*E. coli WP2 uvrA* (Trp^-)	100 - 800 µg/plate	Negative	(Yoo, 1986)	l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.
	Ames test	*S. typhimurium* TA97A, TA98, TA100, TA102	0, 5 - 800 µg/plate	Negative[1]	(Gomes-Carneiro et al., 1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.
	Rec assay	*B. subtilis* H17, M45	Up to 10000 µg/disk	Positive	(Yoo, 1986)	l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method (*rec*-assay) has poor predictive value.
	Rec assay	*B. subtilis* H17, M45	20 µg/disk	Negative	(Oda et al., 1979)	l-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (*rec*-assay) has poor predictive value.
	Alkaline elution assay	Rat hepatocytes	0, 0.1 - 1.3 mM (203.2 µg/ml[4])	Negative	(Storer et al., 1996)	The experiment employed *d*-Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.
	Sister chromatid exchange	Chinese hamster ovary cells	5 - 50 amd 0, 2 - 25 µg/ml[3] 0, 16 - 167 µg/ml [2]	Negative[1]	(Ivett et al., 1989)	*d,l*-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1, 10 mM (1563	Negative[1]	(Murthy et al., 1991)	The study is considered valid.

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

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
			µg/ml[4])			
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1, 10 µg/ml	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 and three concentrations up to 200 µg/ml	Negative[3]	(Ishidate et al., 1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.
	Chromosome aberration	Chinese hamster ovary cells	0, 50 - 250 µg/ml	Negative[1]	(Ivett et al., 1989)	d,l-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Chromosome aberration	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/ml[4])	Negative[1]	(Murthy et al., 1991)	The study is considered valid.
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0, 12.5 - 200 µg/ml	Negative[1]	(Myhr and Caspary, 1991)	d,l-Menthol was used. The maximum concentration was selected by a preliminary test The study is considered valid.
(trans-Menthone [07.176])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0, 6.4 - 800 µg/plate	Positive[1]	(Andersen and Jensen, 1984b)	Concentrations were selected based on preliminary experiments. In absence of metabolic activation, menthone was mutagenic only to strain TA1537 at 6.4 and 32 µg/ml (slightly less than 2-fold increase in mutation frequency), but not at higher (toxic) concentrations. Also in absence of metabolic activation, there was a concentration dependent increase in number of TA97 strain revertants (up to 4-fold increase at 600 µg/l). It was stated that metabolic activation did not enhance the mutagenicity of menthone. The study is considered valid.
Cyclopentanol [02.135]	Modified Ames test	*S. typhimurium* G46, TA98, TA100, TA1535, C3076, TA1537, D3052, TA1538 *E. coli* WP2, WP2 *uvrA*$^-$	0, 0.1 - 1000 µg/ml	Negative[1]	(McMahon et al., 1979)	The study was performed with agar plates containing the following concentration gradients: 0.1 - 1, 1 - 10, 10 - 100, and 100 - 1000 µg/ml. The study is considered valid, although tabulated data on cyclopentanol were not presented.
(Cyclohexanone [07.148])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33 - 3333 µg/plate	Negative[1]	(NTP, 2007)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 33 - 10000 µg/plate	Negative[1]	(Haworth et al., 1983)	The highest level tested was the highest of either10000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 3 µmol/plate	Negative[1]	(Florin et al., 1980)	A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03 – 30 µmol/plate. The validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	NR	Positive	(Massoud et al., 1980)	Only an abstract is available. No reporting with respect to metabolic activation. The substance was also tested with *Bacillus subtilis*. With this specie, toxicity was found as well as a positive response. The validity of the study cannot be evaluated because

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

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
						of lack of experimental information.
	Cytogenetic assay	Human leukocytes	0.1 - 10 mM	Inconclusive [3]	(Collin, 1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.
	Chromosomal aberration	Human lymphocytes	0, 0.005 - 0.1 µg/ml	Positive	(Dyshlovoi et al., 1981)	Article is not in English. Only an abstract available in English. The validity of the study cannot be evaluated.
	Gene mutation (HPRT)	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative[1]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Chromosomal aberration	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative[1]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Sister chromatic exchange	Chinese hamster ovary cells	0, 7.5 µg/ml	Positive[3] Negative[2]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Mutation	Mouse lymphoma L5178Y $Tk^{+/-}$ cells	312.5–5000 µg/ml	Negative	(NTP, 2007)	
Cyclohexanol [02.070]	Ames test	*S. typhimurium* TA98, TA1535, TA1537, TA1538	500 - 10000 µg/plate[3] 500 - 15000 µg/plate[2]	Negative[1]	(Barsky, 1976)	The highest concentrations showed cytotoxicity. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 10 - 3333 µg/plate	Negative[1]	(Haworth et al., 1983)	The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. Both rat and hamster liver S9 were used. The test was run twice. The study is considered valid.
	Chromosomal aberration	Human leukocytes	0.1 - 10 mM	Inconclusive [3]	(Collin, 1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.
(Cyclohexyl acetate [09.027])	DNA damage	*B. subtilis* H17(rec^+), M45 (rec^-)	19 mg/disc	Negative[1]	(Yoo, 1986)	
(Cyclohexyl butyrate [09.230])	DNA damage	*B. subtilis* H17(rec^+), M45 (rec^-)	19 mg/plate	Negative[1]	(Oda et al., 1979)	
(Cycopentanone [07.149])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.5 - 2500 mg/plate	Negative[1]	(Florin et al., 1980)	
(2,2,6-Trimethyl cyclo-hexanone [07.045])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	4.2 - 3600 mg/plate	Negative[1]	(Florin et al., 1980)	
Methyl 3-oxo-2-pentyl-1-	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535,TA1537	5 mg/plate	Negative[1]	(Thompson, 2000)	Valid study in compliance with the OECD Guideline -471.

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

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
cyclopentylacetate [09.520]	Reverse mutation	*E. coli* WP2 *uvrA*	5 mg/plate	Negative[1]	(Wagner and Klug, 2000)	Valid study in compliance with the OECD Guideline -471.
	Forward mutation Test	Mouse lymphoma cells *L5178y*	200 & 300 µg/L 300 µg/L	Positive[3] Positive[2]	(Ross and Harris, 1979b)	Pre-GLP study - not possible to assess the reliability of these studies.
	Forward mutation Test	Mouse lymphoma cells *L5178y*	100 - 325 µg/L	Negative[1]	(Cifone, 2001)	Valid study and in compliance with OECD Guideline 476.
(Carveol [02.062])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	560 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
(Carvyl acetate [09.215])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	333 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
(L-menthyl (R,S)-3-hydroxybutyrate)	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative[1,6]	(Morimoto, 2005)	The JECFA evaluated the racemate of L-menthyl (R,S)-3-hydroxybutyrate.
	Reverse mutation	*E. coli* WP2uvrA	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative[1,6]	**(Morimoto, 2005)**	

NA: Not applicable.

NR: Not reported.

[1] With and without S9 metabolic activation.

[2] With S9 activation.

[3] Without S9 activation.

[4] Calculated based on molecular weight of menthol = 156.3 g/mol.

[5] Marked differential toxicity was seen at dose levels above 25 µmol/plate. No observations were noted at lower dose levels.

[6] Modified preincubation method.

In vivo mutagenicity/genotoxicity data are available for two candidate substance of the present FGE and for three supporting substances evaluated by JECFA at the 51st and 59th meetings. Supporting substances are listed in brackets.

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

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Menthol [02.015])	Host mediated mutation assay	*S. typhimurium* TA1530 and G46; *S. cerevisiae* D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Equivocal	(Food and Drug Research Laboratories, Inc., 1975a)	Negative results, with exception of the combination *S. typhimurium* TA1530 – 5000 mg/kg bw and *S. cerevisiae* D3 – 1150 mg/kg bw/day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels.
	In vivo cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	Oral DL_{50} was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.
	In vivo micronucleus assay	B6C3F1 male mouse bone marrow cells	Intra peritonal	0, 250 - 1000 mg/kg bw/day, during 3 days	Negative	(Shelby et al., 1993)	d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	In vivo dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	This study is considered valid.
(trans-Menthone [07.176])	*In vivo* SMART assay	*D. melanogaster* – flr3 x mwh cross	Whole body	0, 1.3 µl/disk	Positive	(Franzios et al., 1997)	Somatic Mutation and Recombination Test. Only one dose level (1.29 µl/disk; slightly higher than the LD_{50}) was tested. A two-fold increase in mutation frequency as compared to control was observed. Menthone was not recombinogenic. The validity of this study is unclear.
(Cyclohexanone [07.148])	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/ml	Negative	(Goncharova, 1970)	Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.
Cyclohexanol [02.070]	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/ml	Negative	(Goncharova, 1970)	The validity of the study cannot be evaluated.
	In vivo micronucleus test	NMRI mouse bone marrow	Oral	500 - 1500 mg/kg bw	Negative	(Gelbke, 1991)	The study is considered valid. The negative result of this study is of

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

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
							limited relevance, since no bone marrow toxicity could be detected. Testing at higher dose levels might not have been possible due to observed general toxicity at the highest dose.
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Micronucleus test	ICR mice	Intraperitonal	280, 560 & 1120 mg/kg bw	Negative	(Gudi and Krsmanovic, 1998)	Valid study in compliance with the OECD Guideline 474.
	Unscheduled DNA Synthesis	Rat hepatocytes	Intraperitonal	333.3 & 1000 mg/kg bw	Negative	(Durward, 2001)	Valid study in compliance with the OECD Guideline 486.

NR: Not reported.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
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
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