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Flavouring Group Evaluation 33:
Six Tetrahydrofuran Derivatives From Chemical Groups 13, 14, 16 and 26

Scientific Opinion of the Panel on Food Additives,

Flavourings, Processing Aids and Materials in Contact with Food

(Question No EFSA-Q-2008-037)

Adopted on 1 April 2008

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel is asked to evaluate six flavouring substances in the Flavouring Group Evaluation 33 (FGE.33), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These six flavouring substances belong to chemical groups 13, 14, 16 and 26, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation deals with six tetrahydrofuran derivatives.

All six of the flavouring substances have chiral centres and for none of them has the stereoisomeric composition been specified.

All six substances are classified into structural class III.

Four of the six flavouring substances in the present group 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 six flavouring substances in this group have intakes in Europe from 0.0012 to 3.0 microgram/*capita*/day, which are below the threshold of concern value for structural class III (90 microgram/person/day) substances.

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substances using the Procedure.

Overall, it can be concluded that four of the candidate substances [FL-no: 13.120, 13.167, 13.189 and 13.198] will be metabolised to innocuous metabolites. For the remaining two substances [FL-no: 13.182 and 16.054], there are insufficient data available to conclude that they are 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 concluded that the four candidate substances, which are expected to be metabolised to innocuous products, would not give rise to safety concerns at the estimated intakes arising from their use as flavouring substances based on the MSDI approach. For the two candidate substances [FL-no: 13.182 and 16.054] proceding via the B-side of the Procedure scheme no adequate margin of safety could be established and accordingly additional data are required for these two substances.

The mTAMDI values for the six candidate substances from structural class III are all above the threshold of concern for structural class III of 90 microgram/person/day. Therefore, more reliable exposure data are requested. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Specifications including complete purity criteria and identity tests for the materials of commerce have been provided for all six flavouring substances. Information on stereoisomerism is missing for all of them, and accordingly the safety evaluation cannot be finalised.

KEYWORDS

Flavourings, tetrahydrofuran derivatives, food safety.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all others in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/478/EC (EC, 2008). 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, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

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

TERMS OF REFERENCE

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

ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 33

1.1. Description

The present Flavouring Group Evaluation, using the procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I), deals with six tetrahydrofuran derivatives from chemical groups 13, 14, 16 and 26, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

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

The six flavouring substances (candidate substances) are closely related structurally to 11 flavouring substances (supporting substances) evaluated at the 63rd JECFA meeting (JECFA, 2005c) in the group of "Tetrahydrofuran and furanone derivatives" and to seven supporting substances evaluated at the 61st JECFA meeting (JECFA, 2004a) in the group of "Aliphatic and aromatic ethers".

The candidate substances under consideration in the present evaluation are listed in Table 1 and 2a, the hydrolysis products in Table 2b and the supporting substances are listed in Table 3.

1.2. Stereoisomers

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

The six flavouring substances possess one or more chiral centres [FL-no: 13.120, 13.167, 13.182, 13.189, 13.198 and 16.054]. For all substances the stereoisomeric composition has not been specified.

1.3. Natural Occurrence in Food

Four of the six candidate substances have been reported to occur naturally in: malt, dill, lemon, (baked) potato, sage, blackberry, grapefruit juice, mate, quince, brandy, sherry, port and wine (TNO, 2000). Quantitative data on the natural occurrence in food have been reported for three of the six candidate substances. These reports are:

- Linalool oxide(5) acetate [FL-no: 13.189]: up to 100 mg/kg in sage.
- 6-Methylene-2,10,10-trimethyl-1-oxaspiro[4.5]dec-7-ene [FL-no: 16.054]: 0.0006 mg/kg in grapefruit juice, up to 0.11 mg/kg in red wine, up to 0.32 mg/kg in white wine and up to 2 mg/kg in port wine
- 3,6-dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran [FL-no: 13.198]: 884.6 mg/kg in dill blossom, up to 123.8 mg/kg in dill herb.

According to TNO two of the substances, tetrahydrofuryl phenylacetate [FL-no: 13.167] and 2-methyl-3-thioacetoxytetrahydrofuran [FL-no: 13.182] have not been reported to occur naturally in any food items (TNO, 2000).

2. Specifications

Purity criteria for the six substances have been provided by the Flavour Industry (EFFA, 2004j; EFFA, 2004y; EFFA, 2006l; EFFA, 2006m) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the information is insufficient for the six candidate substances as information on stereoisomerism is needed for the six substances [FL-no: 13.120, 13.167, 13.182, 13.189, 13.198 and 16.054] (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, 1999).

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

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

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

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

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 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 Maximised Survey-derived Daily Intake (MSDI) (SCF, 1999) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[1] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

[1] EU figure 375 millions (Eurostat, 1998). 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.

In the present Flavouring Group Evaluation 33 (FGE.33) the total annual production volume of the six candidate substances for use as flavouring substances in Europe was reported to be 31.5 kg (EFFA, 2004j; EFFA, 2004y; EFFA, 2006l; EFFA, 2006m).

For 16 of the 18 supporting substances the total annual volume of production in Europe is approximately 11300 kg (JECFA, 2006a). The annual volume of production in Europe for two of the supporting substances [FL-no: 13.060 and 13.165] was not reported.

On the basis of the annual volumes of production reported for the six candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a). More than 99 % of the total annual volume of production for the candidate substances (EFFA, 2004j; EFFA, 2004y; EFFA, 2006l; EFFA, 2006m) is accounted for by the following three flavourings: 3,6-dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran [FL-no: 13.198], 6-methylene-2,10,10-trimethyl-1-oxaspiro[4.5]dec-7-ene [FL-no: 16.054] and tetrahydrofuryl phenylacetate [FL-no: 13.167]. The estimated daily *per capita* intakes of these candidate substances from use as flavouring substances are 3.0, 0.65 and 0.12 microgram, respectively. The daily *per capita* intakes for each of the remaining substances are equal to or less than 0.012 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 six candidate substances, information on food categories and normal and maximum use levels[2,3,4] were submitted by the Flavour Industry (EFFA, 2004j; EFFA, 2004y; EFFA, 2006l; EFFA, 2006m; EFFA, 2007a). The six candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

[2] ”Normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i).
[3] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).
[4] The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 01.0	Dairy products, excluding products of category 2	All
Category 02.0	Fats and oils, and fat emulsions (type water-in-oil)	All
Category 03.0	Edible ices, including sherbet and sorbet	All except [FL-no: 13.182 and 13.198]
Category 4.1	Processed fruits	All
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 13.167]
Category 05.0	Confectionery	All except [FL-no: 13.198]
Category 06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 13.198]
Category 07.0	Bakery wares	All
Category 08.0	Meat and meat products, including poultry and game	All
Category 09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 13.198]
Category 10.0	Eggs and egg products	None
Category 11.0	Sweeteners, including honey	None
Category 12.0	Salts, spices, soups, sauces, salads, protein products etc.	All
Category 13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 13.198]
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 13.198]
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All
Category 15.0	Ready-to-eat savouries	All except [FL-no: 13.198]
Category 16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All

According to the Flavour Industry the normal use levels for the six candidate substances are in the range of 0.2 - 500 mg/kg food, and the maximum use levels are in the range of 1 - 5000 mg/kg (EFFA, 2002i; EFFA, 2004j; EFFA, 2004y; EFFA, 2006l; EFFA, 2006m; EFFA, 2007a), Table II.1.2, Appendix II.

The mTAMDI values for the six candidate substances from structural class III are 24000 microgram/person/day for [FL-no: 13.198], 3900 microgram/person/day for [FL-no: 13.167], 3200 microgram/person/day for [FL-no: 13.120, 13.189 and 16.054] and 420 microgram/person/day for [FL-no: 13.182].

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

A more detailed description of the metabolism data used to support the candidate substances in this FGE is given in Annex III.

The three candidate esters in this flavouring group, tetrahydrofurfuryl phenylacetate [FL-no: 13.167], 2-methyl-3-thioacetoxytetrahydrofuran [FL-no: 13.182] and linalool oxide(5) acetate [FL-no: 13.189], are expected to be hydrolysed to tetrahydrofuran derivatives (tetrahydrofurfuryl alcohol, 2-methyltetrahydrofurfuryl-3-thiol and linalool oxide) and the corresponding carboxylic acids (phenylacetic acid, acetic acid and acetic acid, respectively). Tetrahydrofurfuryl alcohols are anticipated to be oxidised to the corresponding carboxylic acids, which are conjugated with

glucuronic acid and excreted in the urine. The tertiary alcohol, linalool oxide is also anticipated to be directly conjugated with glucuronic acid and excreted in the urine. 2-methyltetrahydrofurfuryl-3-thiol cannot be anticipated to be metabolised to innocuous products due to possible formation of toxic metabolites. The three alicyclic ethers [FL-no: 13.120, 13.198 and 16.054] are expected to principally undergo ring-hydroxylation, conjugation with glucuronic acid, followed by excretion in the urine. Due to a terminal double bond in the substance [FL-no: 16.054], which may give rise to reactive metabolites, and in the absence of counteracting metabolic options in the substance, it cannot be anticipated that [FL-no: 16.054] can be metabolised to innocuous products.

In conclusion, four of the substances [FL-no: 13.120, 13.167, 13.189 and 13.198] are anticipated to be metabolised to innocuous products and two of the substances [FL-no: 13.182 and 16.054] cannot be anticipated to be metabolised to innocuous products.

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 six candidate substances the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the six substances are summarised in Table 2a.

Step 1

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

Step 2

Four of the candidate substances [FL-no: 13.120, 13.167, 13.189 and 13.198] can be concluded to be metabolised to innocuous products and will therefore proceed via the A-side of the Procedure scheme.

Two of the substances [FL-no: 13.182 and 16.054] cannot be anticipated to be metabolised to innocuous products and proceeds via the B-side of the Procedure scheme.

Step A3

The MSDI of the four candidate substances [FL-no: 13.120, 13.167, 13.189 and 13.198] were estimated to be in the range of 0.0012 to 3.0 microgram/*capita*/day, which are below the threshold of concern for structural class III of 90 microgram/person/day.

Based on results of the safety evaluation sequence of the Procedure, these four candidate substances, proceeding via the A-side of the Procedure scheme, do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

Step B3

The MSDI of the two candidate substances [FL-no: 13.182 and 16.054] proceeding, via the B-side, were estimated to be 0.011 and 0.65 microgram/*capita*/day, respectively, which are below the threshold of concern for structural class III of 90 microgram/person/day. Therefore, these substances can proceed to step B4 of the Procedure.

Step B4

For the two candidate substances [FL-no: 13.182 and 16.054] a No Observed Adverse Effect Level (NOAEL) could not be identified for the substances or for structurally related substances and accordingly additional data are required for these substances.

Accordingly, four substances [FL-no: 13.120, 13.167, 13.189 and 13.198] do not pose a safety concern based on the MSDI approach, whereas for two substances [FL-no: 13.182 and 16.054] additional data are required.

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

The mTAMDI values for the six candidate substances from structural class III are 24000 microgram/person/day for [FL-no: 13.198], 3900 microgram/person/day for [FL-no: 13.167], 3200 microgram/person/day for [FL-no: 13.120, 13.189 and 16.054] and 420 microgram/person/day for [FL-no: 13.182]. These mTAMDI values are all above the threshold of concern for a structural class III substance of 90 microgram/person/day.

For all the candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, 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)
13.120	2,5-Dimethyltetrahydrofuran	0.0012	3200	Class III	90
13.167	Tetrahydrofuryl phenylacetate	0.12	3900	Class III	90
13.189	Linalool oxide(5) acetate	0.012	3200	Class III	90
13.198	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran	3.0	24000	Class III	90
13.182	2-Methyl-3-thioacetoxytetrahydrofuran	0.011	420	Class III	90
16.054	6-Methylene-2,10,10-trimethyl-1-oxaspiro[4.5]dec-7-ene	0.65	3200	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 Flavouring Group Evaluation may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates,

although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake 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, 2004j; EFFA, 2004y; EFFA, 2006l; EFFA, 2006m), the combined estimated daily *per capita* intake as flavourings of the six candidate substances assigned to structural class III is 3.8 microgram, which does not exceed the threshold of concern for a substance belonging to structural class III of 90 microgram/person/day.

The six candidate substances from structural class III, to which the Procedure has been applied, are structurally related to seven supporting substances also from structural class III, evaluated by JECFA at its 61st and 63th meeting (JECFA, 2004a; JECFA, 2005c).

The total combined intakes of the six candidate and the seven supporting substances also from structural class III is approximately 40 microgram/*capita*/day which does not exceed the threshold of concern for a compound belonging to structural class III of 90 microgram/person/day, respectively.

8. Toxicity

8.1. Acute Toxicity

Acute toxicity studies are available on one candidate and nine supporting substances. The LD_{50} values range from 1150 to 4500 mg/kg bpdy weight (bw) in rats and 1000 to 8000 mg/kg bw in mice.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

There are no data available on the candidate substances, but there are data on six supporting substances.

The repeated dose studies are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

No developmental or reproductive toxicity studies are available.

8.4. Genotoxicity Studies

In vitro

Data from *in vitro* genotoxicity tests were available for one candidate substance and three supporting substances.

A good quality reverse mutation assay using the candidate substance 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran [FL-no: 13.198] in *Salmonella typhimurium* strains TA1535, TA1537, TA102, TA100 and TA98 gave negative results at concentrations of up to 316 microgram/plate (Stien, 2005a).

Three valid, but limited reverse mutation assays were available for three supporting substances [FL-no: 13.007, 13.020 and 13.049]. At concentrations of up to 3600 microgram/plate, two substances

[FL-no: 13.007 and 13.049] gave negative results using *S. typhimurium* strains TA1535, TA1537, TA1538, TA100 and TA98 (Wild et al., 1983). At concentrations of up to 100 mg/plate, one supporting substance [FL-no: 13.020] produced negative results using *S. typhimurium* strains TA100, TA102 and TA98 (Aeschbacher et al., 1989).

A positive result was seen in a sister chromatid exchange (SCE) study on the supporting substance 1,8-cineole [FL-no: 03.001] (Galloway et al., 1987). This study was only positive without S9 activation and at levels of 1,8-cineole of 200 and 500 microgram/ml, which induced cell cycle delay and therefore were cytotoxic. There are several other genotoxicity tests on this substance, including another SCE study (although the concentrations of test substance were much lower in this study), that have given negative results. In the light of these results in several genotoxicity studies at gene and chromosomal level the positive result in the sister chromatid exchange assay by Galloway et al. (1987) is considered not to be of relevance for the overall evaluation. It is therefore concluded that 1,8-cineole is not genotoxic.

Negative results on the structurally related 2-methyltetrahydrofuran [FL-no: 13.158] were obtained in the Ames test with *S. typhimurium* strains TA97, TA98, TA100, TA102, TA1535 and TA1537 (CCRIS, 2002; NTP, 2003g).

In vivo

One *in vivo* genotoxicity study was available on the supporting substance tetrahydrofurfuryl propionate [FL-no: 13.049] with negative results. This micronucleus assay using doses of 316-949 mg/kg bw was not considered valid as only one time point was assessed, no PCE/NCE ratio was given and no positive control was used (Wild et al., 1983).

Conclusion on genotoxicity

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substances using the Procedure.

The available genotoxicity data are summarised in Annex IV, Table IV.4 and IV.5.

9. Conclusions

The six candidate substances are tetrahydrofuran derivatives from chemical groups 13, 14, 16 and 26. The six flavouring substances are structurally related to 18 supporting substances. All six of the candidate substances have chiral centres and for all six, further information on steroeisomerism is required.

The six candidate substances have been classified into structural class III according to the decision tree approach presented by Cramer et al. (1978).

Four of the six candidate substances [FL-no: 13.120, 13.189, 13.198 and 16.054] have been reported to occur naturally at low levels in foods.

According to the default MSDI approach, the six flavouring substances in this group have intakes in Europe of between 0.0012 and 3 microgram/*capita*/day, which are below the threshold of concern for structural class III of 90 microgram/person/day.

The combined intakes of the six flavouring substances do not exceed the threshold of concern for structural class III of 90 microgram/person/day.

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substances using the Procedure.

The available information on metabolism of the six candidate substances evaluated through the Procedure and the 18 supporting substances for this FGE was very limited. Overall, it can be anticipated that four of the candidate substances [FL-no: 13.120, 13.167, 13.189 and 13.198] will be metabolised to innocuous metabolites. For the remaining two substances [FL-no: 13.182 and 16.054], there are insufficient data available to anticipate that they will be metabolised to innocuous products.

It is concluded that the four candidate substances, which are expected to be metabolised to innocuous products, would not give rise to safety concerns at the estimated intakes arising from their use as flavouring substances based on the MSDI approach. For the two candidate substances [FL-no: 13.182 and 16.054], proceeding via the B-side of the Procedure scheme, no adequate margin of safety could be established and accordingly additional data are required for these two substances.

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

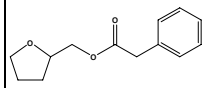
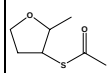
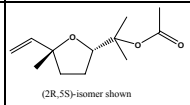
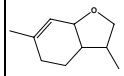
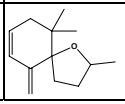
The mTAMDI values for the six candidate substances from structural class III are all above the threshold of concern for structural class III of 90 microgram/person/day. Therefore, more reliable exposure data are requested. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Specifications including complete purity criteria and identity tests for the materials of commerce have been provided for all six flavouring substances. Information on stereoisomerism is missing for all of them, and accordingly the safety evaluation cannot be finalised.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 33

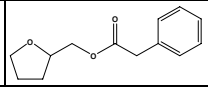
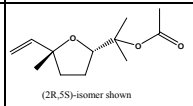
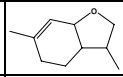
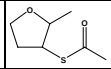
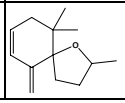
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 33

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
13.120	2,5-Dimethyltetrahydrofuran 6)		1003-38-9	Liquid $C_6H_{12}O$ 100.16	Very slightly soluble 1 ml in 1 ml	91 MS 95 %	1.401-1.407 0.827-0.833	
13.167	Tetrahydrofuryl phenylacetate 6)		5421-00-1	Liquid $C_{13}H_{16}O_3$ 220.26	Insoluble	320-321 MS 99 %	1.512-1.517 1.106-1.112	Register name to be changed to: (tetrahydrofuryl)methyl phenylacetate corresponding to the CASrn in Register.
13.182	2-Methyl-3-thioacetoxytetrahydrofuran 6)			Liquid $C_7H_{12}O_2S$ 160.23	Practically insoluble or insoluble 1 ml in 1 ml	247 MS 95 %	1.490-1.498 1.092-1.100	CASrn to be introduced in the Register 252736-41-7.
13.189	Linalool oxide(5) acetate 6)	 (2R,5S)-isomer shown	56469-39-7	Liquid $C_{12}H_{20}O_3$ 212.29	Practically insoluble or insoluble 1 ml in 1 ml	69 (2 hPa) MS 95 %	1.446-1.452 0.975-0.981	CASrn in Register refers to the (2R,5S)-isomer.
13.198	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran 6)		4315 70786-44-6	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Slightly soluble	183-185 MS 97.8%	1.4807 0.9697	
16.054	6-Methylene-2,10,10-trimethyl-1-oxaspiro[4.5]dec-7-ene 6)		65416-59-3	Solid $C_{13}H_{20}O$ 192.30	Practically insoluble or insoluble 1 ml in 1 ml	58 (0.3 hPa) 49 MS 95 %	n.a. n.a.	CASrn in Register refers to racemate.

1) *Solubility in water, if not otherwise stated.*
2) *Solubility in 9 5% ethanol, if not otherwise stated.*
3) *At 1013.25 hPa, if not otherwise stated.*
4) *At 20°C, if not otherwise stated.*
5) *At 25°C, if not otherwise stated.*
6) *Stereoisomeric composition not specified.*

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

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.120	2,5-Dimethyltetrahydrofuran		0.0012	Class III A3: Intake below threshold	4)	7)	
13.167	Tetrahydrofuryl phenylacetate		0.12	Class III A3: Intake below threshold	4)	7)	
13.189	Linalool oxide(5) acetate	 (2R,5S)-isomer shown	0.012	Class III A3: Intake below threshold	4)	7)	
13.198	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran		3.0	Class III A3: Intake below threshold	4)	7)	
13.182	2-Methyl-3-thioacetoxytetrahydrofuran		0.011	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
16.054	6-Methylene-2,10,10-trimethyl-1-oxaspiro[4.5]dec-7-ene		0.65	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

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

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

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

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

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

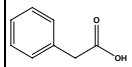
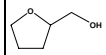
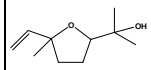
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

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

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

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

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.038	Phenylacetic acid 1007		No safety concern d) Category B c)	Class I A3: Intake below threshold	
13.020	Tetrahydrofurfuryl alcohol 1443		No safety concern e) Category B c)	Class III A3: Intake below threshold	
13.140	Linalool oxide (5-ring) 1454		No safety concern e)	Class II A3: Intake below threshold	
13.160	2-Methyltetrahydrofuran-3-thiol 1090		No safety concern d)	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	

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, Class II = 540, Class III = 90 µg/person/day.

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

a) (SCF, 1995).

b) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 2002c).

e) (JECFA, 2005c).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

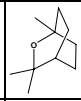
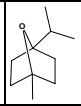
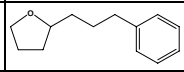
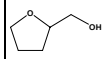
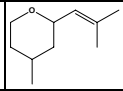
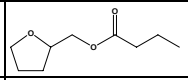
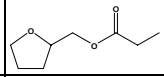
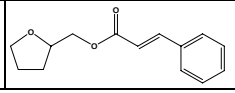
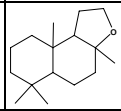
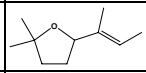
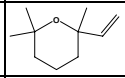
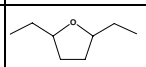
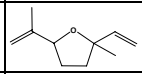
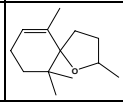
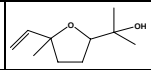
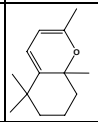
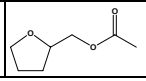
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
03.001	1,8-Cineole		2465 182 470-82-6	1234 JECFA specification (JECFA, 2003b)	1200	No safety concern a) Category B b)	
03.007	1,4-Cineole		3658 11225 470-67-7	1233 JECFA specification (JECFA, 2003b)	3.9	No safety concern a)	
13.007	2-(3-Phenylpropyl)tetrahydrofuran		2898 489 3208-40-0	1441 JECFA specification (JECFA, 2005b)	0.0009	No safety concern c) Category A b)	
13.020	Tetrahydrofurfuryl alcohol		3056 2029 97-99-4	1443 JECFA specification (JECFA, 2005b)	33	No safety concern c) Category B b)	
13.037	2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran		3236 2269 16409-43-1	1237 JECFA specification (JECFA, 2003b)	3.8	No safety concern a) Category B b)	
13.042	4,5-Dihydro-2-methylfuran-3(2H)-one		3373 2338 3188-00-9	1448 JECFA specification (JECFA, 2005b)	20.5	No safety concern c) Category B b)	
13.048	Tetrahydrofurfuryl butyrate		3057 11841 2217-33-6	1444 JECFA specification (JECFA, 2005b)	0.009	No safety concern c)	
13.049	Tetrahydrofurfuryl propionate		3058 11843 637-65-0	1445 JECFA specification (JECFA, 2005b)	0.051	No safety concern c)	
13.060	Tetrahydrofurfuryl cinnamate		3320 11821 65505-25-1	1447 JECFA specification (JECFA, 2005b)	ND	No safety concern c)	

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
13.072	1,5,5,9-Tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane		3471 10514 3738-00-9	1240 JECFA specification (JECFA, 2003b)	1.2	No safety concern a)	
13.090	2,2-Dimethyl-5-(1-methylprop-1-enyl)tetrahydrofuran		3665 10937 7416-35-5	1452 JECFA specification (JECFA, 2005b)	9.4	No safety concern c)	
13.094	2,6,6-Trimethyl-2-vinyltetrahydropyran		3735 10976 7392-19-0	1236 JECFA specification (JECFA, 2003b)	0.012	No safety concern a)	
13.095	2,5-Diethyltetrahydrofuran		3743 11882 41239-48-9	1453 JECFA specification (JECFA, 2005b)	0.009	No safety concern c)	
13.097	Anhydrolinalool oxide (5)		3759 11944 13679-86-2	1455 JECFA specification (JECFA, 2005b)	0.9	No safety concern c)	
13.098	Theaspirane		3774 10515 36431-72-8	1238 JECFA specification (JECFA, 2003b)	1.7	No safety concern a)	
13.140	Linalool oxide (5-ring)		3746 11876 1365-19-1	1454 JECFA specification (JECFA, 2005b)	72.5	No safety concern c)	
13.165	6,7,8,8a-Tetrahydro-2,5,5,8a-tetramethyl-5H-1-benzopyran		3822 5552-30-7	1239 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
13.166	Tetrahydrofurfuryl acetate		3055 2069 637-64-9	1442 JECFA specification (JECFA, 2005b)	0.6	No safety concern c) Category B b)	

ND) No intake data reported.

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, 2004a).

b) (CoE, 1992).

c) (JECFA, 2005c).

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, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

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

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

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

- can the flavourings be predicted to be metabolised to innocuous products[5] (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[6] (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.

[5] *"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).*

[6] *"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).*

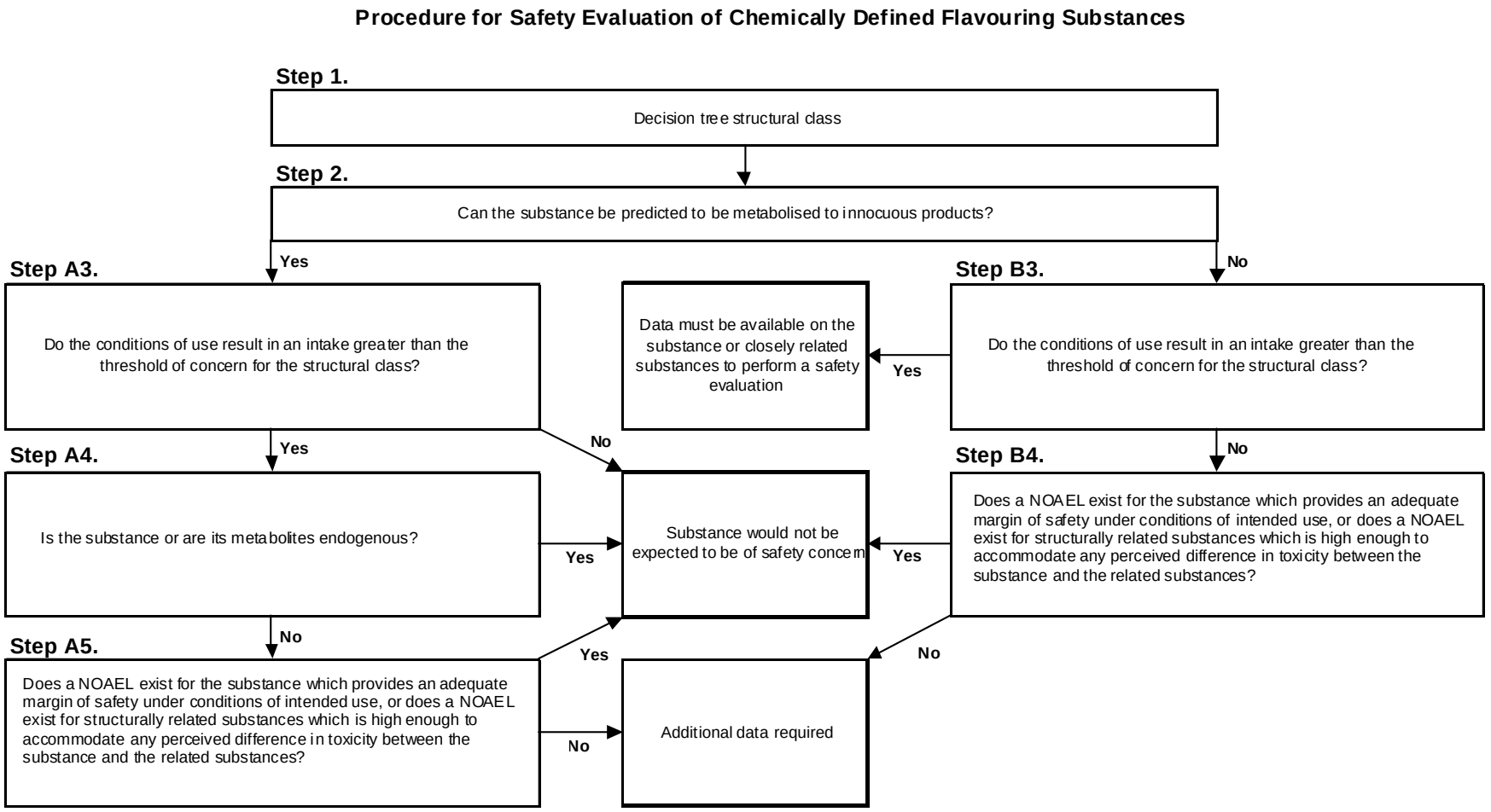


Figure I.1 ***Procedure for Safety Evaluation of Chemically Defined Flavouring Substances***

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 (EC, 2000a) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

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

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

The "normal and maximum use levels" are provided by Industry for the six candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2. Normal and Maximum use levels (mg/kg) for candidate substances in FGE.33 (EFFA, 2004y; EFFA, 2004j; EFFA, 2006l; EFFA, 2006m; EFFA, 2007a)

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
13.120	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25
13.167	7 35	5 25	10 50	7 35	7 35	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
13.182	0,5 2,5	0,2 1	- -	0,5 2,5	- -	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
13.189	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25
13.198	50 250	50 250	- -	25 125	- -	- -	- -	100 500	25 125	- -	- -	- -	500 5000	- -	- -	50 250	- -	50 250
16.054	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25

II.2. mTAMDI Calculations

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

consumes the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

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

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

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

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

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

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

The mTAMDI values (see Table II.2.3) are presented for each of the six flavouring substances in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (EFFA,

2004j; EFFA, 2004y; EFFA, 2006l; EFFA, 2006m; EFFA, 2007a). The mTAMDI values are only given for highest reported normal use.

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
13.120	2,5-Dimethyltetrahydrofuran	3200	Class III	90
13.167	Tetrahydrofuryl phenylacetate	3900	Class III	90
13.189	Linalool oxide(5) acetate	3200	Class III	90
13.198	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran	24000	Class III	90
13.182	2-Methyl-3-thioacetoxytetrahydrofuran	420	Class III	90
16.054	6-Methylene-2,10,10-trimethyl-1-oxaspiro[4.5]dec-7-ene	3200	Class III	90

ANNEX III: METABOLISM

III.1. Absorption, Distribution, and Excretion

Alicyclic Ethers

After the oral administration of 200 mg/kg bw 1,8-cineole [FL-no: 03.001] (synonym: eucalyptol) to rabbits, peak plasma concentration of the parent compound occurred within 30 minutes and reached a maximum plasma level of 840 µg/dl while the plasma level of the principal unconjugated metabolite, (+)-2-endo-hydroxy-1,8-cineole, peaked at 2400 µg/dl within one hour post exposure and then decreased slowly between two and six hours. Peak plasma levels (1250 µg/dl) of the major conjugated metabolite, the glucuronide of (+)-2-*exo*-hydroxy-1,8-cineole, occurred within 1.5 to 2 hours after dosing (Miyazawa et al., 1989).

When 4, 20 or 40 µl of rosemary oil as a oil/water emulsion, containing 39 % 1,8-cineole (approximately equivalent to 52, 260 and 520 mg/kg bw of 1,8-cineole, respectively) were administered orally to mice, blood levels of the parent compound reached a peak level 5 minutes following the exposure. At 260 mg/kg bw, blood levels remained fairly constant over the following 90 minutes. At 520 mg/kg bw, the peak blood concentration dropped to 60 % of the maximum value and remained in that range for the following 80 minutes (Kovar et al., 1987). These results indicate that at doses up to 200 mg/kg, 1,8-cineole is rapidly absorbed into the blood, and eliminated by conjugation to polar metabolites. At higher doses, metabolism appears to be slower, due to saturation of the metabolic pathway.

In humans, data are available for the inhalation route. In four healthy volunteers exposed for 20 minutes to air passing over 4 ml of 1,8-cineole via a closed breathing circuit, 1,8-cineole showed biphasic elimination from the blood. The peak blood concentration was reached within 15 minutes in all the subjects, attaining similar values (about 460-1100 ng/ml), indicating small interindividual differences in the absorption phase. The mean half-life for distribution was 6.7 minutes, whereas the half-life for elimination is 104.6 minutes. However, 1,8-cineole distribution seemed to be affected by the body composition of the volunteer (Jäger et al., 1996).

III.2. Metabolism

Alicyclic esters

The two candidate esters in this flavouring group [FL-no: 13.167 and 13.189] are expected to be hydrolysed to tetrahydrofuran alcohols and the corresponding carboxylic acids. In animals, the hydrolysis of esters is catalysed by classes of enzymes known as carboxylesterases or esterases (Heyman, 1980). The thioester [FL-no: 13.182] is hydrolysed by lipases and esterases to the corresponding carboxylic acid, acetic acid and thiol, 2-methyltetrahydrofurfuryl-3-thiol (Kurooka et al., 1976)

The hydrolysis of the three candidate tetrahydrofuran derivative esters give rise to three different types of alcohols/thiol:

Tetrahydrofurfuryl alcohol, a primary alcohol which is anticipated to be oxidised to the corresponding carboxylic acid which is then in turn conjugated and excreted in the urine.

5(2-Hydroxyisopropyl)-2-methyl-2-vinyltetrahydrofuran [FL-no: 13.096] (linalool oxide), a tertiary alcohol, which is anticipated to be directly conjugated with glucuronic acid and excreted in the urine.

A metabolism study on elemol[7] [FL-no: 02.149], a structurally related substance to linalool evaluated in FGE.18 (EFSA, 2006b), indicates that the substance is absorbed from the gastrointestinal tract and mainly excreted after conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of the terminal double bond in neither elemol nor in linalool would be anticipated (EFSA, 2006b).

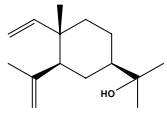
2-Methyl tetrahydrofurane-3-thiol, like other thiols, may follow a combination of pathways including S-oxidation, oxidative desulphuration and dealkylation, alkylation, and conjugation with glutathione (GSH) and/or glucuronic acid. The majority of thiols are readily ionised at physiological pH to the nucleophilic thiolate anion giving rise to their reactivity. Thiols may form mixed disulphides, reacting with endogenous thiols present either in small hydrophilic molecules (i.e. GSH or cysteine, leading to products easily excreted in the urine), or in cellular macromolecules, as for instance in the catalytic site of many enzymes, resulting in adverse effect induction. Among conjugating reactions, thiol S-methylation catalysed by thiol-S-methyl-transferases, is a quite common pathway of biotransformation for simple aliphatic and aromatic thiols, followed by S-oxygenation to water-soluble methyl-sulphoxides and/or sulphones. Alternatively, thiols are enzymatically oxidised to reactive unstable sulphenic (R-S-OH) acid, which can be further oxidised to sulphinic ($R\text{-}SO_2H$) acid or react with excess thiol (preferentially GSH), yielding the corresponding disulphide. These latter can be either reduced back to thiols (enzymatically by thiol-transferase or chemically by exchange with GSH or endogenous thiols), or be oxidised to thio-sulphenic, sulphinic and sulphonic ($R\text{-}SO_3H$) acid. This oxidation cycle followed by reduction could eventually deplete glycogen, due to NADPH production, deplete GSH and alter the cellular redox status. This condition has been associated, at least partially, with toxic effects induced by some sulphur-containing compounds (FGE.08) (EFSA, 2008).

Alicyclic Ethers

In humans and other animals, alicyclic ethers, such as the supporting substances 1,4-cineole [FL-no: 03.007] and 1,8-cineole [FL-no: 03.001], have been shown to be oxidised via P450 isoenzymes to yield polar hydroxylated metabolites, which are conjugated and excreted or further oxidised and excreted. Cleavage of the ether is, at most, a very minor metabolic pathway (Hiroi et al., 1995; Miyazawa et al., 2001a; Miyazawa et al., 2001b; Miyazawa & Shindo, 2001).

The metabolism of 1,8-cineole [FL-no: 03.001] has been studied in various animal species. It has been reported that 1,8-cineole principally undergoes ring hydroxylation to form 2- or 3-hydroxy-1,8-cineole, which are subsequently excreted as the glucuronic acid conjugates (Williams, 1959) (see Figure III.1). Indeed, following the gavage administration of 800 mg 1,8-cineole/kg bw to male albino rats, major metabolites included 2- and 3-hydroxy-1,8-cineole and their conjugates and 1,8-dihydroxy-10-carboxy-p-menthane, which were hypothesised to be formed by the oxidation of the metabolite p-menthane-1,8-diol formed by cleavage of the ether linkage (Madyastha & Chadha, 1986).

These results are consistent with a more recent study, in which 1,8-cineole [FL-no: 03.001] metabolism in microsomes from male Hooded Wistar rats and humans was studied (Pass et al., 2001). To determine the effect on the efficiency of biotransformation, expressed as the intrinsic clearance (V_{max}/K_m) of the reaction, caused by possible induction of metabolism, rats were pretreated daily for six days with a mixture of terpenes (255 mg 1,8-cineole/kg bw; 4 mg p-cymene/kg bw; 34

7 

mg/limonene/kg bw; 103 mg α-pinene/kg bw) by gavage, or 80 mg/kg bw of phenobarbital (PB). Liver microsomes prepared from pretreated and control rats, as well human liver microsomes pooled from seven male patients were incubated with 5 – 200 µM 1,8-cineole. Intrinsic clearance values were as follows: 27.5; 258.2; 1824.7 and 11.6 µl·mg protein^{-1}·minute^{-1} in microsomes from control, terpene-treated, PB-treated rats and humans, respectively. The efficiency in 1,8-cineole metabolism was similar in control rat and human microsomes, whereas terpenes and, at a higher extent, PB-induced rat microsomes metabolised 1,8-cineole more efficiently. This result suggests that terpenes are able to induce their own metabolism, in which CYP2B1 (induced by PB) is very likely involved (Pass et al., 2001). Although with differences in their relative amounts, qualitatively the various liver microsomes produced the same hydroxylated metabolites. Control rat microsomes produced 3-hydroxy-1,8-cineole as the major metabolite, followed by 2- and 9-hydroxycineole. Microsomes from terpene-treated rats produced similar amounts of 2- and 3-hydroxy-1,8-cineole and lesser amounts of 9-hydroxy-1,8-cineole. Of the six metabolites detected in the microsomes from PB-treated rats, 2-hydroxy-1,8-cineole was the major metabolite, followed by 3- and 9-hydroxy-1,8-cineole, whereas the remaining three metabolites consisted of trace amounts of 7-hydroxy-1,8-cineole, 9-cineolic acid and one unknown hydroxycineole metabolite. 2-Hydroxy-1,8-cineole was the major metabolite from pooled human liver microsomes, while 9-hydroxy-1,8-cineole was the minor metabolite. The authors concluded that in rats and humans oxidation was preferred at the aliphatic ring carbons over methyl substituents (Pass et al., 2001).

The metabolism of 1,8-cineole [FL-no: 03.001] was studied *in vivo* in rabbits treated by gavage with 200 mg/kg bw. The major metabolites were identified as 2- and 3-hydroxy-1,8-cineole (Miyazawa et al., 1989). When rat and human liver microsomes and recombinant human CYPs (i.e. c-DNA expressed in insect cells) were incubated *in vitro* with 1,8-cineole, it was oxidised at high rates to 2-exo-hydroxy-1,8-cineole (see Figure III.1) (Miyazawa et al., 2001b; Miyazawa & Shindo, 2001). As indicated by results obtained with recombinant CYPs, P450 inducers (PB and pregnenolone 16-alpha-carbonitrile), and specific P450 inhibitors, the reaction in humans is mainly catalysed by CYP3A2 and 3A4 in rat and human liver microsomes, respectively (Miyazawa et al., 2001a; Miyazawa et al., 2001b). Earlier studies also indicate that CYP3A family is induced by 1,8-cineole. Hepatic microsomes prepared from male Sprague-Dawley rats intraperitoneally injected 300 mg 1,8-cineole/kg bw once a day for five days showed increased levels of 2B1 and 3A2 expression and in their related enzymatic activities (Hiroi et al., 1995).

Hepatic microsomes from beta-naphthoflavone- or PB-pretreated female Wistar rats were used to investigate the inhibitory effects of 1,8-cineole [FL-no: 03.001] on the marker activities of CYP1A1 (ethoxyresorufin-O-deethylase, EROD), 1A2 (methoxyresorufin-O-demethylase, MROD), and 2B1 (pentoxyresorufin-O-depenylase, PROD). 1,8-Cineole caused no or negligible inhibition on EROD and MROD (up to 150 µM), while CYP2B1 activity was decreased in the presence of 1,8-cineole. The competion with the specific probe substrate for CYP2B1 indicates that also this isoform is involved in 1,8-cineole metabolism in the rat (De-Oliveira et al., 1999).

Oxidation of 1,4-cineole [FL-no: 03.007] was studied in rat and human liver microsomes as well as with recombinant human CYPs: in all cases the major identified metabolite was 2-exo-hydroxy-1,4-cineole. Based on the results obtained with single recombinant isoforms, on the effects of specific CYP inhibitors and antibodies and on the data from correlation studies, CYP3A4 was identified as the CYP mainly responsible for 1,4-cineole oxidation. Similarly, CYP3A2 was active in the rat (Miyazawa et al., 2001a).

In rabbits, 1,4-cineole [FL-no: 03.001] is metabolised by ring- and side chain hydroxylation. Urinary metabolites collected over three days following administration of 10,000 mg 1,4-cineole/rabbit include

the ring hydroxylation product 3,8-dihydroxy-1,4-cineole, the side chain hydroxylation product 9-hydroxy-1,4-cineole and its corresponding carboxylic acid, 1,4-cineole-9-carboxylic acid. Other metabolites included 8,9-dihydroxy-1,4-cineole and 1,4-cineole-8-en-9-ol. No evidence of ether cleavage was observed at this dose level (Asakawa et al., 1988).

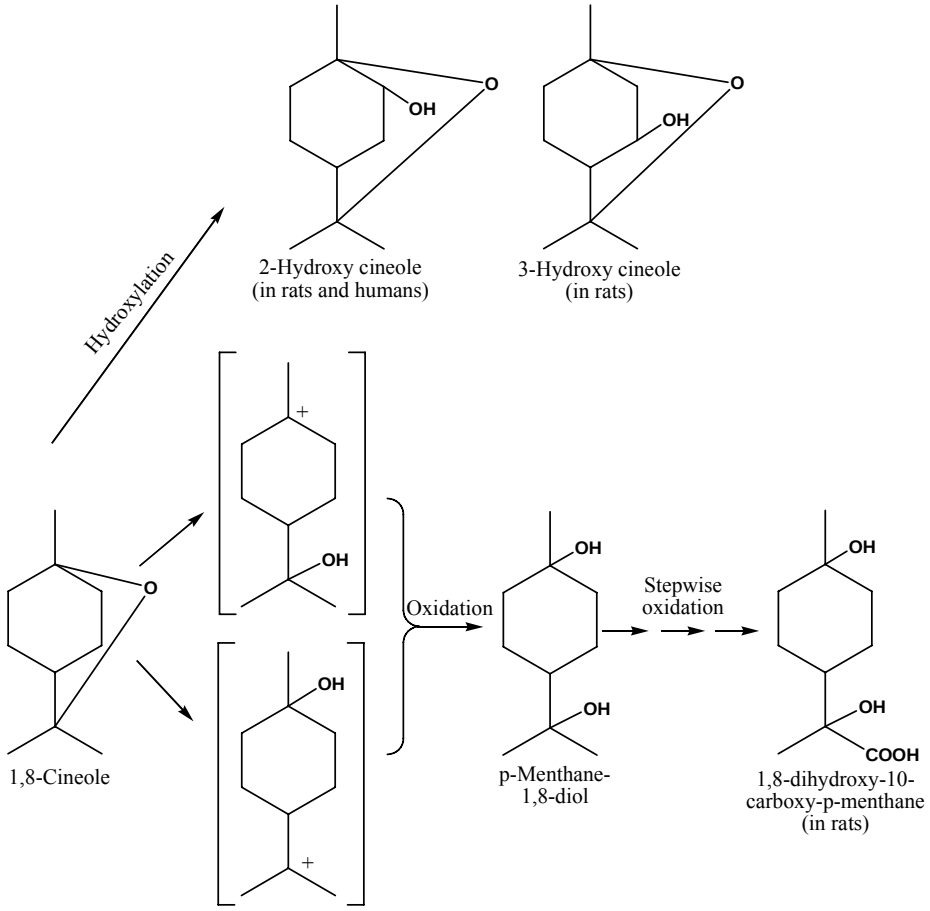


Figure III.1 *Metabolism of 1,8-cineole (eucalyptol) in rats and humans.*

III.3. Conclusions for Absorption, Distribution, Metabolism, and Excretion

In conclusion, alicyclic ethers principally undergo ring-hydroxylation by CYP-450, conjugation with glucuronic acid followed by excretion in the urine. The data available indicate that the four candidate substances [FL-no: 13.120, 13.167, 13.189 and 13.198] are absorbed, distributed, metabolised to inoccuous products, and excreted. For the remaining two candidate substances [FL-no: 13.182 and 16.054] it cannot be anticipated that they are metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 13, 14, 16 and 26 and for nine supporting substances evaluated by JECFA at the 61st and 63rd meeting (supporting substances are listed in brackets).

TABLE IV.1: ACUTE TOXICITY

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
3,6- Dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran [13.198]	Rat	F	Oral	< 2000	(Vaeth, 2005a)	
(Tetrahydrofurfuryl alcohol [13.020])	Rat	NR	Oral	4500	(Gajewski & Alsdorf, 1949)	
(2-Methyltetrahydrofuran-3-one [13.042])	Mouse	M, F	Oral	1860	(Moran & Easterday, 1980)	
(2,2-Dimethyl-5-(1-methylpropen-1-yl)tetrahydrofuran [13.090])	Rat	M, F	Oral	3900	(Cooper & Good, 1979)	
(2,5-Diethyltetrahydrofuran [13.095])	Rat	M, F	Oral	3800 3754	(Burdock & Ford, 1990j) (Reagan & Becci, 1984i)	
(Linalool oxide [13.140])	Rat	NR M, F M, F	Oral	1150 2210 1924	(Moreno, 1977acr) (Colaianni, 1967) (Reagan & Becci, 1984j)	
(5-Isopropenyl-2-methyl-2-vinyltetrahydrofuran [13.097])	Mouse	NR	Oral	1000-2000	(Bonetti, 1983)	
(1,4-Cineole [03.007])	Rat	NR	Oral	3100	(Moreno, 1981c)	
(1,8-Cineole [03.001])	Rat	M, F	Oral	2480 1680	(Bär & Griepentrog, 1967; Jenner et al., 1964) (Brownlee, 1940)	
(2,2,6-Trimethyl-6-vinyltetrahydropyran [13.094])	Rat	M, F	Oral	2700-2800	(Sauer-Freeman, 1980)	
	Mouse	M, F	Oral	4000-8000	(Roure Bertrand Dupont, 1979)	

NR Not reported.

Subacute / subchronic / chronic / carcinogenic toxicity data are not available for any of the candidate substances of the present flavouring group evaluation from chemical group 13, 14, 16 and 26 but for six supporting substances evaluated by JECFA at the 61st and 63rd meeting. The supporting substances are listed in brackets.

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

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

Chemical Name [FL-no]	Species; Sex No./Group*	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(2-(3-Phenylpropyl)tetrahydrofuran [13.007])	Rat; M, F 10-16	Oral	Average dietary intake: M: 43 mg /kg bw/day F: 48.6 mg /kg bw/day	90 days	M: 43 F: 48.6	(Posternak et al., 1969)	
(Tetrahydrofurfuryl alcohol [13.020])	Rat; M, F 10	Oral	0, 6, 60, 300 mg /kg bw/day	7 days	M: 332 F: 312	(Arts & Lina, 2003)	
	Rat; M, F 10	Oral	0, 6, 60, 300 mg /kg bw/day	28 days	60	(Arts & Lina, 2003)	
(4,5-Dihydro-2-methylfuran-3(2H)-one [13.042])	Rat; M, F 46	Oral	-	90 days	M: 92 F: 91	(Shellenberger, 1970e)	
(1,8-Cineole [03.001])	Rat; M, F 12/4	Gavage	0, 150, 300, 600, 1200 mg/kg bw/day	28 days	300 (M) 1200 (F)	(NTP, 1987c)	
	Rat: M, F 12/4	Diet	0, 375, 750, 1500, 3000 mg/kg bw/day	28 days	NE (M) 1500 (F)	(NTP, 1987c)	
	Mice; M, F 12/4	Gavage	0, 150, 300, 600, 1200 mg/kg bw/day	28 days	1200	(NTP, 1987d)	
	Mice; M, F 12/4	Diet	0, 375, 750, 1500, 3000 mg/kg bw/day	28 days	562.5 (M) 1125 (F)	(NTP, 1987d)	
	Mice; M 52/2	Gavage	0, 32 mg/kg bw/day	560 days	32	(Roe et al., 1979)	1,8-cineole in a mixture also containing chloroform and peppermint oil.
(Tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran [13.037])	Rat; M, F 20-32/1	Diet	-	90 days	2514 (M) 2805 (F)	(Posternak et al., 1969)	
(Cycloionone [13.165])	Rat; M, F 10/1	Diet	36.6 (M) 33.7 (F)	14 days	36.6 (M) 33.7 (F)	(Wnorowski, 1997e)	
	Rat; M, F 10/4	Gavage	0, 30, 120, 400, 1200 mg/kg bw/day	28 days	120	(Wnorowski, 1998)	

NE Not established.

* *Total number of test groups does not include control animals.*

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental and reproductive toxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 13, 14, 16 and 26 or for any supporting substance evaluated by JECFA at the 61st and 63rd meeting.

In vitro mutagenicity/genotoxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 13, 14, 16 and 26 and for four supporting substances evaluated by JECFA at the 61st and 63rd meeting. Supporting substances are listed in brackets.

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

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2-(3-Phenylpropyl)tetrahydrofuran [13.007])	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100 and TA98	Up to 3600 microg/plate	Negative[1]	(Wild et al., 1983)	
(Tetrahydrofurfuryl alcohol [13.020])	Reverse mutation	*S. typhimurium* TA100, TA102 and TA98	1–102 100 microg/plate	Negative[1, 2]	(Aeschbacher et al., 1989)	
(Tetrahydrofurfuryl proprionate [13.049])	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538 and TA100	Up to 3600 microg/plate	Negative[3]	(Wild et al., 1983)	
3,6- Dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran [13.198]	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA102, TA100 and TA98	0, 3.16, 10, 31.6, 100, 316 microg/plate	Negative[1]	(Stien, 2005a)	
(1,8-Cineole [03.001])	Reverse mutation	*S. typhimurium* TA100, TA102, TA98 and TA97	250-2500 microg/plate	Negative[1]	(Gomes-Carneiro et al., 1998)	
	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA100 and TA98	3.3-3333 microg/plate	Negative[1, 2]	(Haworth et al., 1983)	
	Sister chromatid exchange	Chinese hamster ovary cells	50-500 microg/ml 600-800 microg/ml	Positive[3] Negative[4]	(Galloway et al., 1987)	
	Sister chromated exchange	Chinese hamster ovary cells K-1	10, 33.3 and 100 micromol/l (1.5, 5.1 and 15.4 microg/ml)	Negative[3]	(Sasaki et al., 1989)	
	Chromosomal aberrations	Chinese hamster ovary cells	479-663 microg/ml 630-810 microg/ml	Negative[3] Negative[4]	(Galloway et al., 1987)	
	DNA repair	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	18 microg/disk	Negative	(Oda et al., 1979)	
	DNA repair	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	< 20 microl/disk (20000 microg/disk)	Negative	(Yoo, 1986)	

[1] *With or without metabolic activation.*

[2] *Modified pre-incubation method.*

[3] *Without metabolic activation.*

[4] *With metabolic activation.*

No *in vivo* mutagenicity/genotoxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 13, 14, 16 and 26 but for one supporting substance evaluated by JECFA at the 61st and 63rd meeting. Supporting substance is listed in brackets.

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

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Tetrahydrofurfuryl propionate [13.049])	Micronucleus formation	Male and female mouse bone marrow	Intraperitoneally	316, 632, 949 mg/kg bw	Negative	(Wild et al., 1983)	

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