European Food Safety Authority

The EFSA Journal (2009) 1031, 1-38

SCIENTIFIC OPINION

Flavouring Group Evaluation 43[1]: Thujyl alcohol from chemical group 8

Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2008-047)

Adopted on 26 March 2009

PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked 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 one flavouring substance in the Flavouring Group Evaluation 43 (FGE.43), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. This flavouring substance belongs to chemical group 8, Annex I of the Commission Regulation (EC) No 1565/2000.

The present FGE deals with thujyl alcohol [FL-no: 02.207].

The flavouring substance possesses four chiral centres and has been presented without specification of the stereoisomeric composition.

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Material, Enzymes, Flavrourings and Processing Aids on a request from the European Commission on Flavourings Groep Evaluation 43 (FGE43). *The EFSA Journal* (2009) 1031, 1-36.

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The flavouring substance is classified into structural class I.

The flavouring substance in the present group has been reported to occur naturally in ginger.

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 flavouring substance in this group has intake in Europe 0.012 microgram/capita/day, which is below the threshold of concern value for structural class I (1800 microgram/person/day) substances.

In absence of genotoxicity data there is no indication that the candidate substance in the present FGE possesses genotoxic potential. This would not preclude the evaluation of this substance through the Procedure.

It is anticipated that thujyl alcohol is metabolised to innocuous products.

No toxicity data was available for the candidate substance.

It was considered that on the basis of the default MSDI approach the candidate substance [FL-no: 02.207] would not give rise to safety concerns at the estimated level of intake arising from its use as flavouring substance.

When the estimated intake was based on the mTAMDI it is 1600 microgram/person/day. Thus, the intake was below the threshold of concern for structural class I. The candidate substance having a mTAMDI intake estimate below the threshold of concern for the structural class, is also expected to be metabolised to innocuous products.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity and identity for the material of commerce have been provided for the flavouring substance, except that information on stereoisomerism has not been provided. Thus, the final evaluation of the material of commerce cannot be performed for this substance [FL-no: 02.207] pending further information.

KEYWORDS

Flavourings, safety, thujyl alcohol.

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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 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 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.

ACKNOWLEDGEMENTS

European Food Safety Authority wishes to thank the members of the Working Groups on Flavourings and ac hoc experts for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Riccardo Crebelli, Karl-Heinz Engel, Henrik Frandsen, Jørn Gry, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Catherine Leclercq, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin.

ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 43

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 tjujyl alcohol from chemical group 8, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The flavouring substance (candidate substance) under consideration, as well as the chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) number, structure and specification, is listed in Table 1 and 2.

The candidate substance is structurally related to three flavouring substances (supporting substances). One of these, menthol, was evaluated at the 51st JECFA meeting (JECFA, 1999a) in "Menthol". Two were evaluated at the 63rd meeting (JECFA, 2006a) in the group of "Monocyclic and bicyclic secondary alcohols, ketones and related esters" (see 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 candidate substance thujyl alcohol [FL-no: 02.207] possesses four chiral centres and has been presented without specification of the stereoisomeric composition.

1.3. Natural Occurrence in Food

The candidate substance has been reported to occur in ginger (*Zingiber cassumunar Roxb*. and *Zingiber officinale Roscoe*).

Quantitative data on the natural occurrence in food have been reported: Up to 4000 mg/kg in ginger (*Zingiber officinale Roscoe*) (TNO, 2000).

2. Specifications

Purity criteria for the candidate substance thujyl alcohol [FL-no: 02.207] have been provided by the Flavour Industry (EFFA, 2005f) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the information is not adequate as information on chirality is needed for the candidate substance (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[2] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation 43 (FGE.43) the total annual production volume of the candidate substance for use as flavouring substances in Europe was reported to be 0.1 kg (EFFA, 2005f).

For the three supporting substances the annual volume of production is approximately 130.000 kg in Europe (JECFA, 1999a; JECFA, 2006a). Approximately 98 % is accounted for by menthol [FL-no: 02.015] (128000 kg). Approximately 1100 kg is accounted for by borneol [FL-no: 02.016] and approximately 450 kg is accounted for by fenchyl alcohol [FL-no: 02.038].

On the basis of the annual volume of production reported for the candidate substance, MSDI value for the flavouring has been estimated (Table 2). The estimated MSDI of thujyl alcohol [FL-no: 02.207] from use as a flavouring substance is 0.012 microgram/capita/day (Table 2).

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 substance, information on food categories and normal and maximum use levels[3,4,5] were submitted by the Flavour Industry (EFFA, 2005f; EFFA, 2007a). The candidate substance is 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] 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.
[3] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).
[4] 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).
[5] 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**	**Flavouring used**
Category 01.0	Dairy products, excluding products of category 2	[FL-no: 02.207]
Category 02.0	Fats and oils, and fat emulsions (type water-in-oil)	[FL-no: 02.207]
Category 03.0	Edible ices, including sherbet and sorbet	[FL-no: 02.207]
Category 04.1	Processed fruits	[FL-no: 02.207]
Category 04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	No
Category 05.0	Confectionery	[FL-no: 02.207]
Category 06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	[FL-no: 02.207]
Category 07.0	Bakery wares	[FL-no: 02.207]
Category 08.0	Meat and meat products, including poultry and game	[FL-no: 02.207]
Category 09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	[FL-no: 02.207]
Category 10.0	Eggs and egg products	No
Category 11.0	Sweeteners, including honey	No
Category 12.0	Salts, spices, soups, sauces, salads, protein products, etc.	[FL-no: 02.207]
Category 13.0	Foodstuffs intended for particular nutritional uses	[FL-no: 02.207]
Category 14.1	Non-alcoholic ("soft") beverages, excluding dairy products	[FL-no: 02.207]
Category 14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	[FL-no: 02.207]
Category 15.0	Ready-to-eat savouries	[FL-no: 02.207]
Category 16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	[FL-no: 02.207]

According to the Flavour Industry the normal use levels for the candidate substance are in the range of 1 - 5 mg/kg food, and the maximum use levels are in the range of 5 to 25mg/kg (EFFA, 2002i; EFFA, 2005f; EFFA, 2007a).

The mTAMDI value for the candidate substance from structural class I is 1600 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

An old paper from 1912 (Hämäläinen, 1912) described the detection of *p*-menthan-2,4-diol glucuronic acid and thujyl alcohol glucuronic acid in the urine of rabbits after oral administration of thujyl alcohol. In support, the structurally related substance thujone (not in the Register) was metabolised to thujyl alcohol, which was detected in the urine as a glucuronide in rabbits. In mice and rats however, thujone was hydroxylated and conjugated before excretion in the urine. No thujyl alcohol was detected in the urine of mice and rats or in human liver microsomes *in vitro*, indicating that oxidation of thujone was the major pathway of metabolism. The supporting bicyclic substances borneol [FL-no: 02.016] and fenchol [FL-no: 02.038] were both found to be metabolised *in vitro* to the corresponding ketone in addition to further hydroxylation by liver microsomes as the major metabolic pathways. However, in the *in vitro* metabolising systems phase II reactions are not

possible. *In vivo* phase II reactions like glucuronide conjugation of the alcohol groups and followed by excretion in the urine are probably the major metabolism pathway of these compounds compared to the formation of the corresponding ketone. The structurally related substances cyclopentanol and cyclohexanol were also found as glucuronides in the urine of rabbits and humans, while minor metabolites were found as sulphate conjugates (candidate substances evaluated in FGE.09 (EFSA, 2004g)). Further hydroxylation was found as the major metabolising pathway *in vivo* for the supporting substance menthol [FL-no: 02.015], but for menthol metabolites the level of conjugation was not addressed. Therefore, the candidate substance thujyl alcohol is expected to be absorbed from the gastrointestinal tract, conjugated with glucuronic acid and excreted in the urine as the major metabolism pathway. Minor metabolism pathways of thujyl alcohol might include ring opening of the three-membered ring and conjugation to *p*-menthan-2,4-diol glucuronic acid, further oxidation of the methyl groups and conjugation of thujyl alcohol or the oxidation products with sulphate before excretion in the urine. Small amounts of thujyl alcohol may also be converted to thujone, but this will probably be of a very minor importance.

It is thus anticipated that thujyl alcohol is metabolised to innocuous products. As thujone is not expected to be a major metabolite of thujyl alcohol *in vivo*, it is concluded that thujone should not be used as a supporting substance for thujyl alcohol with respect to toxicity. The metabolism and toxicity of thujone have been reviewed by the Scientific Committee on Food in 2003 (SCF, 2003d).

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 substance from chemical group 8 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluation of the substance is summarised in Table 2.

Step 1

The candidate substance thujyl alcohol [FL-no: 02.207] is classified into structural class I according to the decision tree approach by Cramer et al. (1978).

Step 2

The candidate substance is anticipated to be metabolised to innocuous products and proceeds via the A-side of the Procedure.

Step A3

The candidate substance assigned to structural class I has an estimated European daily *per capita* intake (MSDI) of 0.012 microgram. This intake is below the threshold of concern of 1800 microgram/person/day for structural class I. Accordingly, the candidate substance does not pose a safety concern when used as a flavouring substance at the estimated level of intake, based on the MSDI approach.

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

The estimated intake for the candidate substance thujyl alcohol [FL-no: 02.207] in structural class I based on the mTAMDI is 1600 microgram/person/day. For the substance the mTAMDI is below the threshold of concern of 1800 microgram/person/day. 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)
02.207	Thujyl alcohol	0.012	1600	Class I	1800

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

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

The candidate substance is structurally related to three supporting substances evaluated by JEFCA at its 51st and 63rd meetings (JECFA, 1999a; JECFA, 2006a). The total combined intake (in Europe) of the candidate substance and the supporting substances all assigned to structural class I is approximately 16200 microgram/capita/day, which exceeds the threshold of concern for the corresponding structural class (1800 microgram/ person/day).

However, the major contribution to the total combined intake of flavouring substances assigned to structural class I (99 %) is provided by menthol [FL-no: 02.015] (16000 microg/capita/day).

The estimated intake of menthol [FL-no: 02.015] corresponds to 0.27 mg/kg bw/day. This represents 7 % of the acceptable daily intake (ADI) of 4 mg/kg bw/day for menthol established at the 51st JECFA meeting (JECFA, 1999a).

Excluding the major contributor, menthol, the total combined intake (in Europe) for the candidate substance and the two supporting substances belonging to structural class I is approximately 190 microgram/capita/day, which does not exceed the threshold of concern for the corresponding structural class (1800 microgram/person/day).

8. Toxicity

8.1. Acute Toxicity

No acute toxicity studies are available for the candidate substance thujyl alcohol. Two supporting substances [FL-no: 02.015 and 09.269] were tested for acute toxicity in the mouse and rat. The LD_{50} values ranged from 940 mg/kg body weight (bw) to over 5000 mg/kg bw. The magnitudes of the LD_{50} values indicate that the oral acute toxicity is rather low for the supporting substances.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

No studies on subacute, subchronic, chronic or carcinogenicity are available for the candidate substance thujyl alcohol. For two supporting substances [FL-no: 02.015 and 02.016] there are toxicity data available.

All long-term toxicity studies are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

No studies on developmental or reproductive toxicity are available for the candidate substance thujyl alcohol. For one supporting substance [FL-no: 02.015] there are several studies.

All developmental and reproductive toxicity studies are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

No genotoxicity studies are available for the candidate substance thujyl alcohol. Genotoxicity data are available for two supporting substances [FL-no: 02.015 and 02.016]:

There are genotoxicity data for the supporting substance menthol [FL-no: 02.015], which gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes. With the same substance equivocal results in an *in vivo* host mediated mutation assay were observed at high dose levels and negative results in several Ames tests, a TK+/- mouse lymphoma assay, sister chromatid exchange (SCE) tests in Chinese hamster ovary (CHO) cells and human lymphocytes, and chromosomal aberration assays with human embryonic lung cells, human lymphocytes and CHO cells. Negative results were also reported in two *in vivo* micronucleus and chromosomal aberration assays. 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.

Borneol [FL-no: 02.016] was consistently tested negative in the Ames assay when a variety of *Salmonella typhimurium* strains including TA97, TA98, TA100, TA1535, TA1537 and TA1538 were incubated with up to 5,000 µg/plate with or without metabolic activation. Borneol showed no mutagenic activity when tested in *Escherichia coli* WP2 uvrA at concentrations up to 3,200 µg/plate. In the Rec assay, borneol was reported to induce growth inhibition in *Bacillus subtilis* strain M45- when tested at concentrations up to 10 mg/disc. This test has very limited relevance for the genotoxicity evaluation.

Conclusion on genotoxicity

The genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substance using the Procedure.

The *in vitro / in vivo* studies available are summarised in Annex IV, Table IV.4 and IV.5.

9. Conclusions

The candidate substance, thujyl alcohol [FL-no: 02.207], is a bicyclic secondary alcohol. Although it possesses four chiral centres, it has been presented without specification of the stereoisomeric composition.

The candidate substance has been reported to occur naturally in ginger. It is allocated to structural class I.

The intake of thujyl alcohol, estimated according to the default MSDI approach, is 0.012 microgram/capita/day, which is below the threshold of concern for structural class I (1800 microgram/person/day).

The candidate substance is structurally related to three supporting substances [FL-no: 02.015, 02.016, 02.038] which were evaluated by the JEFCA at its 51st and 63rd meetings. The total combined daily per capita intakes (in Europe) of the candidate substance and supporting substances, all from structurally class I, is 16200 microgram, which exceeds the threshold of concern for the corresponding structural class (1800 microgram/ person/day). However, the major contribution to the total combined intake of flavouring substances assigned to structural class I (99 %) is provided by menthol [FL-no: 02.015] (16000 microg/capita/day). The estimated intake of menthol [FL-no: 02.015] corresponds to 0.27 mg/kg bw/day. This represents 7 % of the ADI of 4 mg/kg bw/day for menthol established at the 51st JECFA meeting. Excluding the major contributor, menthol, the total combined intake (in Europe) for the candidate substance and the two supporting substances belonging to structural class I is approximately 190 microgram/capita/day, which does not exceed the threshold of concern for the corresponding structural class (1800 microgram/person/day).

The genotoxicity data available do not preclude the evaluation of the candidate substance through the Procedure.

The candidate substance thujyl alcohol is expected to be absorbed from the gastrointestinal tract, conjugated with glucuronic acid and excreted in the urine. It is anticipated that thujyl alcohol is metabolised to innocuous products.

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

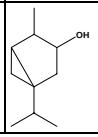
It was considered that on the basis of the default MSDI approach the candidate substance [FL-no: 02.207], to which the Procedure could be applied, would not give rise to safety concerns at the estimated levels of intake arising from its use as flavouring substance.

When the mTAMDI method was used, the intake was estimated to be 1600 microgram/person/day for the candidate substance. This is below the threshold of concern of 1800 microgram/person/day (class I). The candidate substance having an mTAMDI intake below the threshold of concern for the corresponding structural class, is also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the candidate substance can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including purity and identity for the materials of commerce have been provided for the flavouring substance. However information on stereoisomerism has not been provided for the substance. Thus, the final evaluation of the material of commerce cannot be performed for this substance [FL-no: 02.207] pending further information.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 43

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 43

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
02.207	Thujyl alcohol 6)		4079 21653-20-3	Solid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble 1 ml in 1 ml	100 (16 hPa) 28 NMR 95 %	1.460-1.466 0.919-0.925	CASrn in the Register refers to (1S,3S,4R,5R) thujyl alcohol.

1) Solubility in water, if not otherwise stated.

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

3) At 1013.25 hPa, if not otherwise stated.

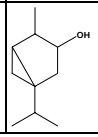
4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

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

Table 2: 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
02.207	Thujyl alcohol		0.012	Class I A3: Intake below threshold	4)	7)	

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.

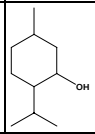
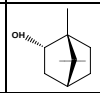
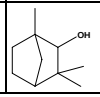
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

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 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).
02.016	Borneol		2157 64 507-70-0	1385 JECFA specification (JECFA, 2005b)	130	No safety concern c) Category B b)	
02.038	Fenchyl alcohol		2480 87 1632-73-1	1397 JECFA specification (JECFA, 2005b)	55	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, 2000a).

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[6] (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[7] (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.

[6] *"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).*

[7] *"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).*

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.

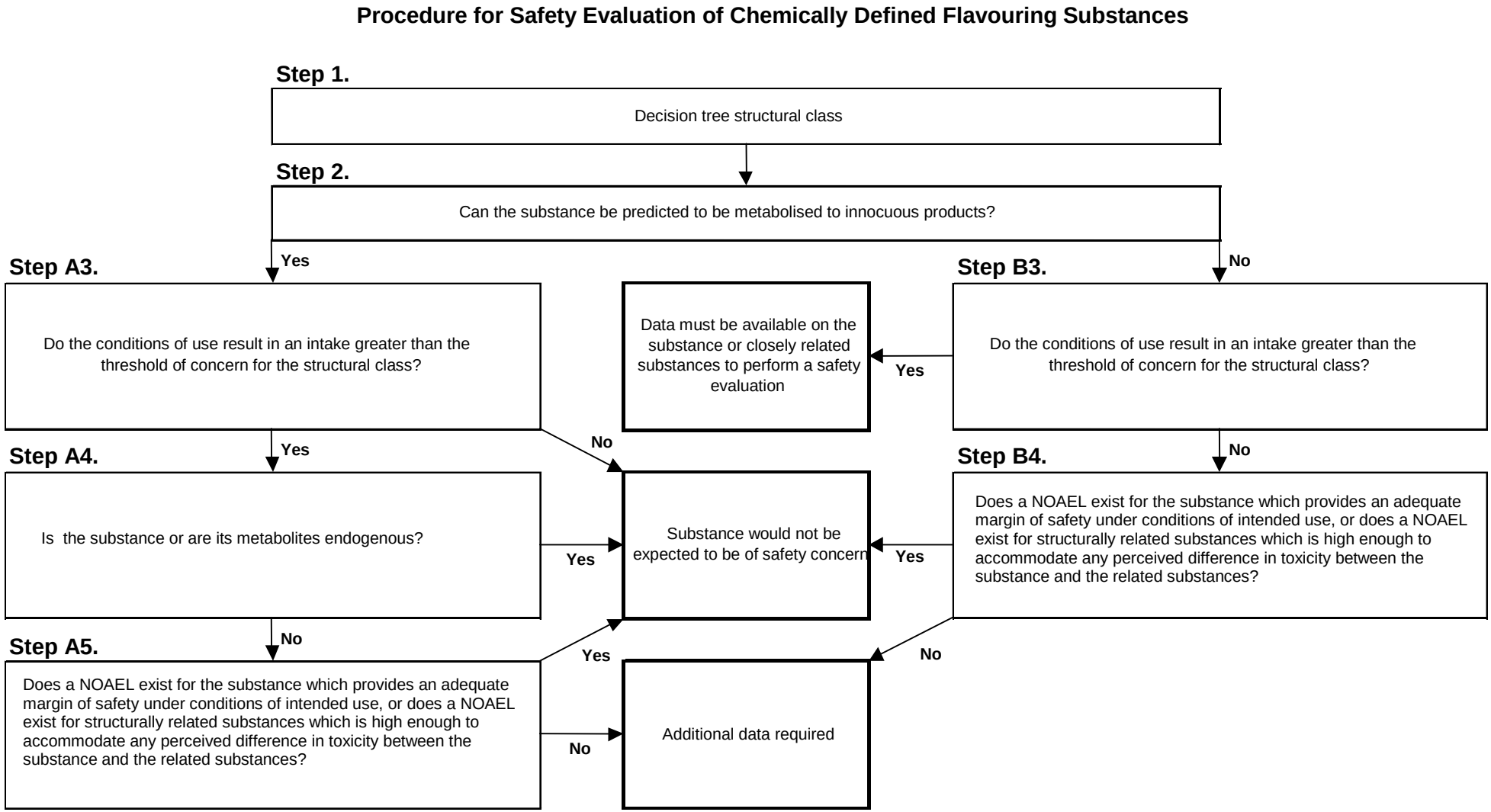


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 candidate substance 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.43 (EFFA, 2005f; 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**
02.207	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

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 flavouring substance in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (EFFA, 2005f; 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)
02.207	Thujyl alcohol	1600	Class I	1800

ANNEX III: METABOLISM

One old publication on the biotransformation of the candidate substance thujyl alcohol is available. Data on biotransformation is available for the supporting substances borneol, fenchol and menthol and the structurally related substances thujon (not in Register), cyclohexanol and cyclopentanol (both evaluated in FGE.09 as candidate substances (EFSA, 2004g).

III.1. Absorption, Distribution, and Excretion

Mice and rats were treated orally with 40 mg/kg alpha-thujon or 40 mg/kg beta-thujon in propylene glycol. Urine was collected for 18 hours in metabolic cages. Metabolites, mainly hydroxylated, of thujon were detected in the urine indicating absorption from the gastrointestinal tract, although the amounts of the thujon dose recovered were not stated (Höld et al., 2001). It is anticipated that the candidate substance thujol will be absorbed from the gastrointestinal tract and excreted in the urine.

III.2. Metabolism

Thujyl alcohol

In an old publication from 1912 rabbits were treated daily with 0.5 to 2.5 g thujyl alcohol through gavage, and urine was collected. The duration of treatment was not described. Two metabolites were detected in the urine. One metabolite, *p*-menthan-2,4-diol glucuronic acid, resulting from opening of the three-membered ring. The formation of the p-menthane-2,4-diol as an artefact raised from thujyl alcohol during acidic cleavage of the urinary conjugate was considered unlikely, as this was excluded based on experimental data. The second metabolite was thujyl alcohol glucuronic acid. The ratio or amounts of the excreted metabolites were not given (for information on the metabolites, see page 159 and 180) (Hämäläinen, 1912).

Thujon

After oral administration to male rabbits of a mixture of alpha- and beta-thujon (ratio 9:1) at a dose level of approximately 650 – 800 mg/kg bw, two metabolites were detected in the urine, 3-alpha-thujyl alcohol and 3-beta-thujyl alcohol, which were excreted as glucuronides (Ishida et al., 1989b). Rabbit liver cytosol with NADPH (1 mM) was incubated with alpha-thujon (0.2 microM) for 1 hour at 37°C. The metabolism products detected were the reduction products *R*-thujyl alcohol and *S*-thujyl alcohol (Höld et al., 2000).

Male albino Swiss-Webster mice and male albino rats were treated orally with alpha-thujon (40 mg/kg) or beta-thujon (40 mg/kg) in propylene glycol. Urine was collected for 18 hours. Four metabolites, 2-*R*-hydroxy-alpha-thujon (78 %), 4-hydroxy-alpha-thujon (15 %), 7-hydroxy-alpha-thujon (3 %) and 4,10-dehydro-thujon (4 %), and three metabolites, 4-hydroxy-alpha-thujon (56 %), 4,10-dehydro-thujon (31 %) and 7-hydroxy-alpha-thujon (12 %), were found in the urine after treatment with alpha-thujon in mice and rats, respectively. Three metabolites, 7-hydroxy-beta-thujon (84 %), 4-hydroxy-alpha-thujon (9 %) and 4,10-dehydro-thujon (7 %), and three metabolites, 4-hydroxy-alpha-thujon (82 %), 7-hydroxy-beta-thujon (10 %) and 4,10-dehydro-thujon (8 %), were found in the urine after treatment with beta-thujon in mice and rats, respectively (Höld et al., 2001).

Mouse liver microsomes and NADPH (1 mM) were incubated with alpha-thujon (0.2 µM) for 1 hour at 37°C. Metabolism in mouse liver microsomes did not result in the formation of thujyl alcohol. Five different metabolites were detected, all containing an alcohol substituent and a ketone

group. The major metabolite was identified as 7-hydroxy-alpha-thujon and two minor metabolites as the diastereomers (or diastereoisomers) of 4-hydroxy-thujon (Höld et al., 2000). Mouse and rat liver microsomes and NADPH (1 mM) were incubated with alpha-thujon and beta-thujon (0.2 µM) for 1 hour at 37°C. Five metabolites, 7-hydroxy-alpha-thujon (66 %), 4-hydroxy-beta-thujon (9 %), 2-*R*-hydroxy-alpha-thujon (9 %), 4-hydroxy-alpha-thujon (8 %) and 7,8-dehydro-alpha-thujon (2 %), and three metabolites, 7-hydroxy-alpha-thujon (38 %), 4-hydroxy-alpha-thujon (34 %) and 7,8-dehydro-alpha-thujon (28 %), were detected after incubation with alpha-thujon for mice and rats, respectively. Five metabolites, 4-hydroxy-beta-thujon (51 %), 7-hydroxy-beta-thujon (32 %), 2-*R*-hydroxy-beta-thujon (15 %), 4-hydroxy-alpha-thujon (1 %) and 7,8-dehydro-beta-thujon (1 %), and four metabolites, 4-hydroxy-beta-thujon (62 %), 7-hydroxy-beta-thujon (16 %), 4-hydroxy-alpha-thujon (16 %) and 7,8-dehydro-beta-thujon (6 %), were detected after incubation with beta-thujon in mice and rats, respectively (Höld et al., 2001).

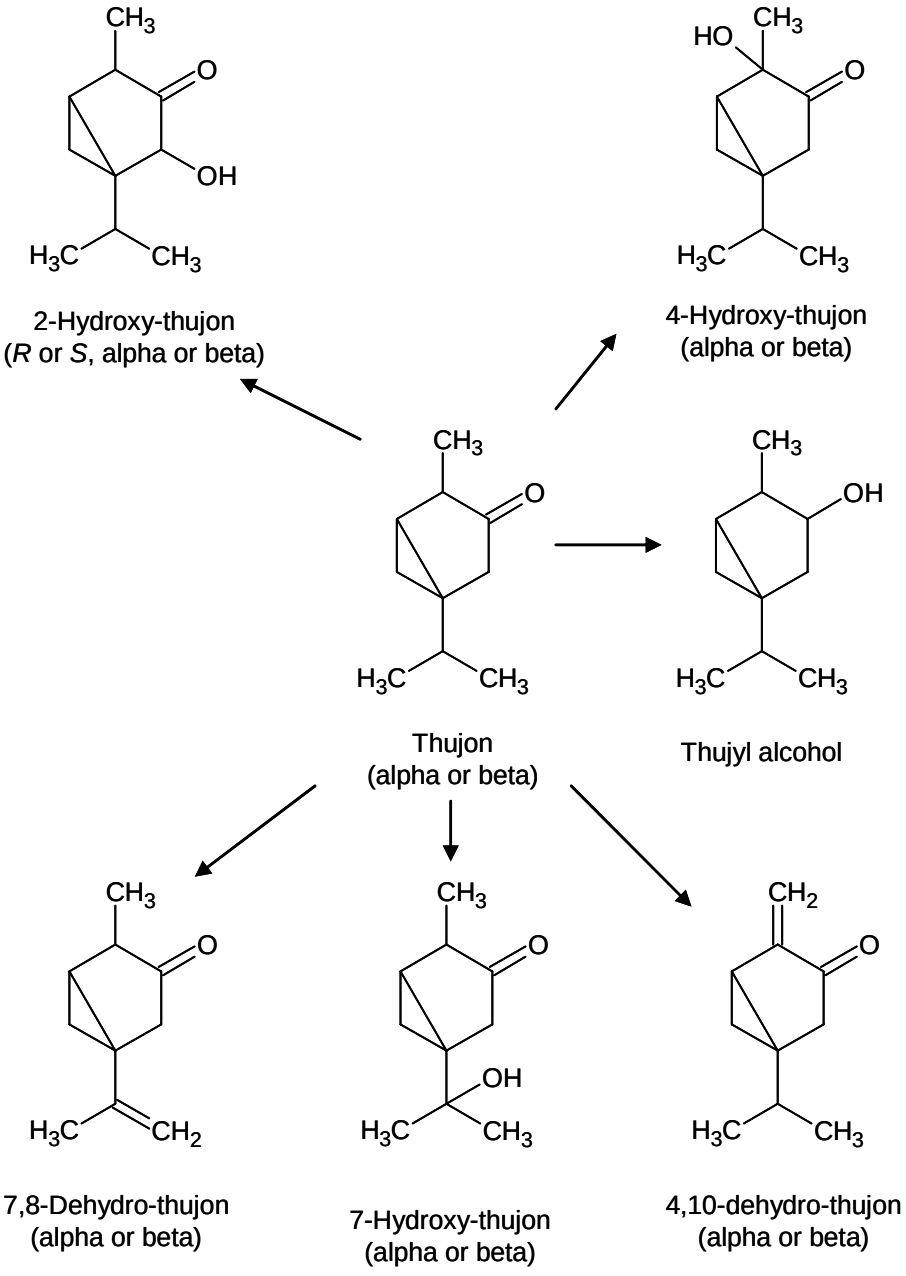


Figure III.1: *Metabolic pathway for thujon in rabbits, mice, rats and humans (in vitro or in vivo) as suggested by Höld et al. (2000) and Höld et al. (2001). Note that the metabolite thujyl alcohol is only found in rabbits.*

Pooled human microsomes (1 mg) or human P450 3A4 supersomes (baculovirus infected cells containing human P450 3A4) (1 mg) and NADPH (1 mM) were incubated with alpha-thujon or beta-thujon (0.2 microM) in phosphate buffer (100 mM) for 1 hour at 37°C. The same metabolites were detected for the microsomes and the supersomes. The major metabolites were 7-hydroxy-alpha-thujon (56-58 %), 4-hydroxy-alpha-thujon (15-29 %) and 7,8-dehydro-alpha-thujon (13-29 %) for alpha-thujon and 4-hydroxy-beta-thujon (60-66 %), 7-hydroxy-beta-thujon (10-35 %) and 4-hydroxy-alpha-thujon (2-24 %) for beta-thujon (Höld et al., 2001).

No thujyl alcohol or conjugates of thujyl alcohol were observed in rat and mice *in vivo* or in rats, mice and humans *in vitro* indicating that in thujon metabolism oxidation is more important than reduction (Höld et al., 2001).

Borneol

Rat liver microsomes, 0.1 M potassium phosphate buffer (pH 7.4) and NADPH (1 mM) were incubated with borneol (325 µM) for 30 minutes at 37°C. Four metabolites were observed in the incubation mixture, which were not present in the control incubations. One of the metabolites of borneol was confirmed–to be the oxidation product bornanone (= camphor), the corresponding ketone of borneol. The structure of the three other metabolites was not completely identified. One metabolite was proposed to be a result of de-methylation of borneol, while the two other/remaining metabolites were proposed to be hydroxylated borneol (Zhang et al., 2008).

Fenchol

The metabolism of fenchol (100 µM) was studied in rat and human liver microsomes in potassium phosphate buffer (100 mM, pH 7.4) containing NADPH (0.5 mM). Incubations were carried out at 37°C for 30 minutes. Fenchol was oxidised to fenchone, 6-*oxo*-hydroxyfenchol and 10-hydroxyfenchol by liver microsomes of phenobarbital-treated rats. Fenchol was oxidised to fenchone by human liver microsomal P450 enzymes (Miyazawa and Gyoubu, 2007).

Menthol

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. In the rat urine, no menthone was detected (Madhava et al., 1988).

Cyclopentanol and cyclohexanol

The secondary alcohols cyclopentanol and cyclohexanol 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, which are conjugated with glucuronic acid and excreted (Elliott et al.,

1959; James & 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 also the same metabolic pathways are 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).

A small fraction of the substances cyclopentanol and cyclohexanol 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, given by gavage to rabbits (1.7-2.3 mmol/kg) and rats (1.8-2.5 mmol/kg), in which 1-3 % of the dose was found in the urine as sulphate conjugates (James & Waring, 1971). As cyclopentanol and cyclohexanol, thujyl alcohol can possibly be conjugated to sulphate as a minor metabolite.

Other secondary alicyclic saturated alcohols

Oxidation of alkyl groups has been observed for menthol, neo-dihydrocarvyl acetate, menthyl formate and for the substance 3,3,5- trimethylcyclohexan-1-one (Truhaut et al., 1970; Yamaguchi et al., 1994). This indicates that oxidation of the methyl groups of thujyl alcohol might occur.

III.3 Conclusion

An old paper describe the detection of *p*-menthan-2,4-diol glucuronic acid and thujyl alcohol glucuronic acid in the urine of rabbits after oral administration of thujyl alcohol. In support, the substance thujone is metabolised to thujyl alcohol which is detected in the urine as a glucuronide in rabbits. In mice and rats however, the thujone is hydroxylated and conjugated before excretion in the urine. No thujyl alcohol is detected in the urine of mice and rats or in human liver microsomes *in* vitro, indicating that oxidation of thujone is the major pathway of metabolism. The supporting bicyclic substances borneol and fenchol were both found to be metabolised *in vitro* to the corresponding ketone in addition to further hydroxylation by liver microsomes as the major metabolic pathways. However, in the *in vitro* metabolising systems phase II reactions are not possible. *In vivo* phase II reactions like glucuronide conjugation of the alcohol groups and followed by excretion in the urine is probably the major metabolism pathway of these compounds compared to the formation of the corresponding ketone. The supporting substances cyclopentanol and cyclohexanol are also found as glucuronides in the urine of rabbits and humans, while minor metabolites are found as sulphate conjugates. Further hydroxylation was found as the major metabolising pathway *in vivo* for the supporting substance menthol, but for the menthol metabolites the level of conjugation was not addressed. Therefore, the candidate substance thujyl alcohol is expected to be absorbed from the gastrointestinal tract, conjugated with glucuronic acid and excreted in the urine as the major metabolism pathway. Minor metabolism pathways of thujyl alcohol might be ring opening of the three membered ring to *p*-menthan-2,4-diol glucuronic acid, further oxidation of the methyl groups and conjugation of thujyl alcohol or the oxidation products with sulphate before excretion in the urine. Small amounts of thujyl alcohol may also be converted to thujone, but this will probably be of a very minor importance.

It is thus anticipated that thujyl alcohol is metabolised to innocuous products.

ANNEX IV: TOXICITY

No oral acute toxicity data are available for the candidate substance of the present flavouring group evaluation from chemical group 8, but for two supporting substances evaluated by JECFA.

TABLE IV.1: ACUTE TOXICITY

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)	
(Fenchyl acetate [09.269])	Rat	NR	Oral	>5000	(Moreno, 1975s)	

No subacute / subchronic / chronic / carcinogenic toxicity data are available for the candidate substance of the present flavouring group evaluation from chemical group 8 but for two supporting substances evaluated by JECFA.

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
(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	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.
(Borneol [02.016])	Dog; NR 1/3	Gavage	526 mg/kg bw /day	31 days	526 4	(Miller et al., 1933)	
	Dog; NR 1/5	Diet	500 mg/kg bw /day	37 days	<500	(Miller et al., 1933)	
	Dog; NR 1/3	Diet	1300 mg/kg bw /day	90 days	<1300[3]	(Miller et al., 1933)	

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] *Animals were gradually introduced to the final dose level of 1,300 mg/kg bw per day over a 2-month period.*

No developmental and reproductive toxicity data are available for the candidate substance of the present flavouring group evaluation from chemical group 8 but for one supporting substance evaluated by JECFA.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw /day), Including information of possible maternel toxicity	Reference	Comments
(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)	

F: Female.

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

No *in vitro* mutagenicity/genotoxicity data are available for the candidate substance of the present flavouring group evaluation from chemical group 8 but for two supporting substances evaluated by JECFA.

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

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 & 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/disc	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.

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

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
	Rec assay	*B. subtilis* H17, M45	20 µg/disc	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-Menthol 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 µg/ml[4])	Negative[1]	(Murthy et al., 1991)	The study is considered valid.
	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-Menthol 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 & Caspary, 1991)	d,l-Menthol was used. The maximum concentration was selected by a preliminary test The study 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 of lack of experimental information.

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

Chemical Name	Test system	Test Object	Concentration	Result	Reference	Comments
	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, 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 chromatid 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.
(Borneol [02.016])	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA97	1 mg/ml (1000 µg/ml)	Negative[1]	(Azizan & Blevins, 1995)	
	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 5 mg/plate (5000 µg/plate)	Negative	(Simmon et al., 1977)	
	DNA repair	*Bacillus subtilis* M45 and H17	Up to 10 mg/disc	Positive	(Yoo, 1986)	
	Mutation test	*Escherichia coli* WP2 uvrA (trp-)	0.4-3.2 mg/plate	Negative	(Yoo, 1986)	

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.*

No *in vivo* mutagenicity/genotoxicity data are available for the candidate substance of the present flavouring group evaluation from chemical group 8 but for one supporting substance evaluated by JECFA.

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
(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 DL50 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	Intraperitoneal	0, 250 - 1000 mg/kg bw/day, during 3 day	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.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
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
NADPH	
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
TAMDI	Theoretical Added Maximum Daily Intake
WHO	World Health Organisation