EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 303 (FGE.303): Spilanthol from chemical group 30

EFSA Publication; Larsen, John Christian; Nørby, Karin Kristiane; Beltoft, Vibe Meister; Lund, Pia; Binderup, Mona-Lise; Frandsen, Henrik Lauritz

Link to article, DOI:
10.2903/j.efsa.2011.1995

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

European Food Safety Authority

EFSA Journal 2011;9(3):1995

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 303 (FGE.303):

Spilanthol from chemical group 30[1]

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

European Food Safety Authority (EFSA), Parma, Italy

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 requested to evaluate one flavouring substance in the Flavouring Group Evaluation 303, using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The flavouring substance belongs to chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The candidate substance spilanthol [FL-no: 16.121] is a branched chain unsaturated aliphatic amide from chemical group 30.

The substance has been presented with specification of the stereoisomeric composition.

The candidate substance was assigned to structural class III, according to the decision tree approach presented by *Cramer et al.*, 1978.

According to the Flavour Industry spilanthol has been identified in the plant *Spilanthes oleracea*, which is used in some countries as a spice.

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

1 On request from the Commission, Question No EFSA-Q-2010-01502, adopted on 3 February 2011.

2 Panel members Arturo Anadon, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle.

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

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 303 (FGE.303): Spilanthol from chemical group 30. EFSA Journal 2011;9(3):1995. [27 pp.]. doi:10.2903/j.efsa.2011.1995. Available online: www.efsa.europa.eu/efsajournal.htm

Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

Genotoxicity data are not available for the candidate substance spilanthol [FL-no: 16.121]. However, the Panel considers that the lack of genotoxicity data do not preclude the evaluation of this aliphatic amide by using the Procedure.

The candidate substance cannot be anticipated to be metabolised to innocuous products.

According to the default MSDI approach, the candidate substance in this group has an intake in Europe of 24 micrograms/*capita*/day [FL-no: 16.121]. For the candidate substance, this is below the threshold of concern value for structural class III (90 micrograms/person/day).

When the estimated intake was based on the mTAMDI approach it is 830 micrograms/person/day for the candidate substance from structural class III, which is above the threshold of concern for structural III of 90 micrograms/person/day. Therefore more reliable exposure data are required. On the basis of such additional data, the flavouring substance should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

No relevant data on toxicity are available for the candidate substance or the three supporting substances. The only toxicity data available is a 28-day study which is not considered sufficient to evaluate chronic effects of the substance. Accordingly, additional data are required for the candidate substance. According to the practice of the Panel, a minimum requirement to provide an adequate NOAEL for flavourings in the Procedure is a 90-day study.

In order to determine whether the conclusion for the candidate substance can be applied to the material of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the material of commerce have been provided for the flavouring substance.

In conclusion, for the candidate substance spilanthol [FL-no: 16.121] additional data on chemical defined material are required as a 28 day study is not considered sufficient to deriving a NOAEL.

KEY WORDS

Flavouring, food safety, spilanthol, aliphatic amide.

TABLE OF CONTENTS

BACKGROUND

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

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

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

TERMS OF REFERENCE

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

In addition, in letter of 28 January 2010 the Commission requested EFSA to carry out a risk assessment on Spilanthol [FL-no: 16.121] in accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000a):

“The European Commission requests the European Food Safety Authority to carry out a safety assessment of two flavouring substances, Spilanthol and L-methionylglycine, in accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000a) by end 2010”.

The deadline of the Terms of Reference was negotiated to 31 May 2011. L-methionylglycine is evaluated in FGE.305.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 303

1.1. Description

The present Flavouring Group Evaluation 303 (FGE.303), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Annex I of this FGE), deals with one flavouring substance (candidate substance) from chemical group 30 of Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The one candidate substance under consideration in the present evaluation, with its chemical Register name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, and structures is listed in Table 1.

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

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

The flavouring substance spilanthol [FL-no: 16.121] (candidate substance) is a branched chain unsaturated aliphatic amide and is closely related structurally to three flavouring substances (supporting substances) [FL-no: 16.091, 16.093 and 16.094] evaluated at the 65th JECFA meeting (JECFA, 2006d) in the group of "Aliphatic and aromatic amines and amides" and considered by the Panel in FGE.86Rev1. The names and structures of the supporting substances are listed in Table 3, together with their evaluation status.

1.2. Stereoisomers

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

The candidate substance spilanthol [FL-no: 16.121] can exist as geometrical stereoisomers due to the presence of double bonds. The name spilantol specify the (2E,6Z,8E) geometric stereoisomer (see Table 1). According to Industry, [FL-no: 16.121] exists as a mixture of the geometrical stereoisomers. The stereoisomeric composition has been specified (Flavour Industry, 2009r) (see Table 1).

1.3. Natural Occurrence in Food

According to TNO, the candidate substance spilanthol [FL-no: 16.121] has not been reported to occur naturally in any food items (TNO, 2010).

Spilanthol has been identified in *Spilanthes oleracea*, which according to Flavour Industry is used as a spice in some countries (Molinatorres *et al.*, 1996; Yasuda *et al.*, 1980; Ramsewak *et al.*, 1999).

2. Specifications

Purity criteria for the candidate substance have been provided by the Flavour Industry (Flavour Industry, 2009r) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate 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, 1999a).

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

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

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

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

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

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

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

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

The anticipated total annual volume of production of the candidate substance spilanthol [FL-no: 16.121] in the present Flavouring Group Evaluation (FGE.303) from use as flavouring substance in Europe has been reported to be approximately 200 kg (Flavour Industry, 2009r). For the supporting substances the total annual volume of production is 1000 kg in Europe (Flavour Industry, 2004f).

On the basis of the annual volumes of production reported for the candidate substance, the daily *per capita* intake for the flavouring has been estimated. The estimated daily *per capita* intake of spilanthol [FL-no: 16.121] from use as a flavouring substance is 24 microgram (Table 2a).

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

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 candidate substance spilanthol [FL-no: 16.121] information on food categories and normal and maximum use levels[5,6] was submitted by the Flavour Industry (Flavour Industry, 2009r). 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.

Table 3.1 Use of the Candidate Substance

Food category	Description	Flavouring used
01.0	Dairy products, excluding products of category 2	Yes
02.0	Fats and oils, and fat emulsions (type water-in-oil)	No
03.0	Edible ices, including sherbet and sorbet	Yes
04.1	Processed fruits	Yes
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	No
05.0	Confectionery	Yes
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Yes
07.0	Bakery wares	No
08.0	Meat and meat products, including poultry and game	No
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Yes
10.0	Eggs and egg products	No
11.0	Sweeteners, including honey	No
12.0	Salts, spices, soups, sauces, salads, protein products etc.	Yes
13.0	Foodstuffs intended for particular nutritional uses	No
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	Yes
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	Yes
15.0	Ready-to-eat savouries	Yes
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	Yes

According to the Flavour Industry the normal use levels for the candidate substance are in the range of 0.25 - 10 mg/kg food, and the maximum use levels are in the range of 1 - 25 mg/kg (Flavour Industry, 2009r) (see Table II.1.2, Annex II).

The mTAMDI value is 830 microgram/person/day for the candidate substance from structural class III (see Section 5).

[5] ”Normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

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

Specific information regarding absorption, distribution, metabolism and excretion is not available for the candidate substance.

The candidate substance is like other aliphatic amides anticipated to be absorbed from the gastrointestinal tract. Aliphatic amides are expected to be at least partly hydrolysed (Bray *et al.*, 1949) to polar metabolites which are eliminated in the urine or bile (James, 1974; Schwen, 1982). Hydrolysis of the amide bond is reported as a metabolic pathway for amides e.g. dihydrocapsaicin and piperine *in vivo* in rats. However, complete hydrolysis of the candidate substance to innocuous metabolites cannot be anticipated (Kawada & Iwai, 1985; Bhat & Chandrasekhara, 1987).

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 spilanthol [FL-no: 16.121] from chemical group 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluation of the substance is summarised in Table 2a.

Step 1

The candidate substance spilanthol [FL-no: 16.121] is classified according to the decision tree approach by Cramer *et al.* (Cramer *et al.*, 1978) into structural class III.

Step 2

The candidate substance cannot be anticipated to be metabolised to innocuous products and thus the evaluation proceeds via the B-side of the Procedure scheme.

Step B3

The estimated daily per capita intake of the candidate substance is 24 micrograms, which is below the threshold for its structural class of 90 micrograms/person/day (class III). Accordingly, the evaluation of the substance proceeds to step B4 of the Procedure.

Step B4

For the candidate substance and the three supporting substances, the only available toxicity study is a 28-day oral feeding study in rats with chemical undefined materials. The Panel does not consider this study appropriate for deriving a No Observed Adverse Effect Level (NOAEL) to be used at step B4 of the Procedure for the candidate substance [FL-no: 16.121], and accordingly additional data are required.

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

The estimated intake of the candidate substance spilanthol [FL-no: 16.121] assigned to structural class III, based on the mTAMDI, is 830 micrograms/person/day, which is above the threshold of concern for structural class III of 90 micrograms/person/day.

Thus, for the candidate substance 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)
16.121	Spilanthol	24	830	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

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

On the basis of the reported annual production volume in Europe (Flavour Industry, 2009r), the estimated daily *per capita* intake as flavouring of the candidate substance spilanthol [FL-no: 16.121] belonging to structural class III is 24 micrograms. This value does not exceed the threshold of concern for structural class III of 90 micrograms/person/day.

The candidate substance is structurally related to three supporting substances evaluated by the JEFCA at its 65th meeting (JECFA, 2006b). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for the three supporting substances, deca-(2E,4E)-dienioc acid isobutyl-amide [FL-no: 16.091], N-cyclopropyl (2E,6Z)-nonadienamide [FL-no: 16.093] and N-ethyl (2E,6Z)-nonadienamide [FL-no: 16.094]. The total combined intake of the candidate and supporting substances is approximately 150 micrograms/*capita*/day, which exceed the thresholds of concern for structural class III substances.

However, the Panel agreed that the intake of about 24 micrograms/*capita*/day for the candidate substance is minor compared to the combined intake of about 126 micrograms/*capita*/day of the supporting substances and that at the level of exposure resulting from the use as flavourings, the candidate and supporting substances are expected to be metabolised and would not be expected to saturate the metabolic pathways.

8. Toxicity

8.1. Acute Toxicity

No data are available for the candidate substance or supporting substances.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute toxicity data are available for the candidate substance spilanthol [FL-no: 16.121] but not for the supporting substances of the present flavouring group.

Only a summary is available on a 28-day study in rats. In the study, groups of five male and five female Sprague-Dawley Aai:N(SD)BR rats were maintained on a diet containing 0, 130, 1300 or 13000 ppm gold root extract of unknown purity. As spilanthol comprises approximately 50% of the composition of gold root extract, the effective dietary concentration of spilanthol was about 5.5, 57 and 572 mg/kg body weight (bw)/day for males and 6.5, 64 and 629 mg/kg bw/day for females, respectively. The animals were observed daily for clinical signs and mortality. Individual body weights and food consumption were recorded weekly. On day 29 of the study, blood was sampled from all animals for haematological and clinical chemistry analysis, and gross necropsis were performed on all rats. During the study, no deaths or clinical signs of toxicity were observed in any test group. The authors concluded that the NOAEL for spilanthol was 572 mg/kg bw/day based on the assumption of the concentration above (Moore, 2002). This result was used at the JECFA evaluation of three supporting substances [FL-no: 16.091, 16.093 and 16.094]. However, the Panel does not consider this study appropriate for deriving a NOAEL for chronic effects to be used at step B4 of the Procedure for these substances, and accordingly additional data are required. According to the practice of the Panel, a minimum requirement to provide an adequate NOAEL for flavourings in the Procedure is a 90-day study.

A search in open literature did not reveal further toxicity data on the candidate substance.

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

8.3. Developmental / Reproductive Toxicity Studies

No data on developmental toxicity and reproductive toxicity are available for the candidate substance or supporting substances.

8.4. Genotoxicity Studies

No *in vitro* or *in vivo* data are available for the candidate substance spilanthol. However, for two of the supporting substances [FL-no: 16.091 and 16.093] negative genotoxicity studies are available. The Panel therefore also considers that for the candidate substance spilanthol [FL-no: 16.121] the lack of genotoxicity data does not preclude the evaluation of this aliphatic amide using the Procedure.

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

9. Conclusions

The candidate substance spilanthol [FL-no: 16.121] is a branched chain unsaturated aliphatic amide from chemical group 30.

The substance has been presented with specification of the stereoisomeric composition.

The candidate substance was assigned to structural class III, according to the decision tree approach presented by Cramer *et al.*, 1978.

According to the Flavour Industry spilanthol has been identified in the plant *Spilanthes oleracea,* which is used in some countries as a spice.

Genotoxicity data are not available for the candidate substance spilanthol [FL-no: 16.121]. However, the Panel considers that the lack of genotoxicity data do not preclude the evaluation of this aliphatic amide by using the Procedure.

The candidate substance cannot be anticipated to be metabolised to innocuous products.

According to the default MSDI approach, the candidate substance in this group has an intake in Europe of 24 micrograms/*capita*/day [FL-no: 16.121]. For the candidate substance, this is below the threshold of concern value for structural class III (90 micrograms/person/day).

When the estimated intake was based on the mTAMDI approach it is 830 micrograms/person/day for the candidate substance from structural class III, which is above the threshold of concern for structural III of 90 micrograms/person/day. Therefore more reliable exposure data are required. On the basis of such additional data, the flavouring substance should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

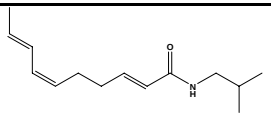
No relevant data on toxicity are available for the candidate substance or the three supporting substances. The only toxicity data available is a 28-day study which is not considered sufficient to evaluate chronic effects of the substance. Accordingly, additional data are required for the candidate substance. According to the practice of the Panel, a minimum requirement to provide an adequate NOAEL for flavourings in the Procedure is a 90-day study.

In order to determine whether the conclusion for the candidate substance can be applied to the material of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the material of commerce have been provided for the flavouring substance.

In conclusion, for the candidate substance spilanthol [FL-no: 16.121] additional data on chemical defined material are required as a 28 day study is not considered sufficient to deriving a NOAEL.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 303

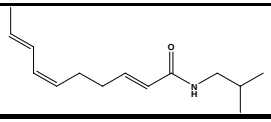
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 303

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
16.121	Spilanthol		4668 25394-57-4	Liquid $C_{14}H_{23}NO$ 221.35	Not soluble Soluble	140-160 (13 Pa) IR NMR MS 74 %	1.4911-1.5411 0.9452-0.9468	Synonym: (2E,6Z,8E)-N-(2-Methylpropyl)-2,6,8-decatrienamide. Mixture of isomers: 74.007 % (2E,6Z,8E)-, 16.669 % (2E,6E,8E)-, 5.759 % (2E,6E,8Z)-, 0.884 % (2Z,6Z,8E)-, 0.334 % (2E,6E,8E)-, 0.764 % (2Z,6Z,8Z)-isomer, 1.553 % other isomers.

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

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

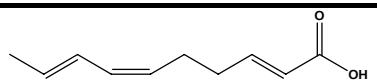
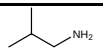
Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
16.121	Spilanthol		24	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required	6)	

1) *EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.*
2) *Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.*
3) *Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.*
4) *No safety concern based on intake calculated by the MSDI approach of the named compound.*
5) *Data must be available on the substance or closely related substances to perform a safety evaluation.*
6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).*
7) *Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.*
8) *No conclusion can be drawn due to lack of information on the purity of the material of commerce.*

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE SUBSTANCES (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

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

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
	2,6,8-Triendecanoic acid		Not evaluated as flavouring substance	Not evaluated as flavouring substance	Not evaluated as flavouring substance.
11.002	Isobutylamine 1583		No safety concern a) Category A b)	Class I A3: Intake below threshold	

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

2) No safety concern at estimated levels of intake.

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

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

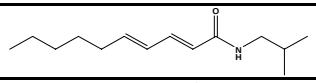
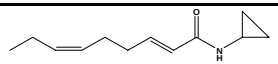
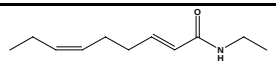
5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (JECFA, 2008d).

b) (CoE, 1992).

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)	EFSA Comments
16.091	Deca-(2E,4E)-dienoic acid isobutyl-amide		18836-52-7	1598 JECFA specification (JECFA, 2005d)	6.1	No safety concern a)	EFSA conclusion: B4-No, additional data required (EFSA, 2008ar).
16.093	N-Cyclopropyl (2E,6Z)-nonadienamide		608514-55-2	1597 JECFA specification (JECFA, 2005d)	61	No safety concern a)	EFSA conclusion: B4-No, additional data required (EFSA, 2008ar).
16.094	N-Ethyl (2E,6Z)-nonadienamide		608514-56-3	1596 JECFA specification (JECFA, 2005d)	61	No safety concern a)	EFSA conclusion: B4-No, additional data required (EFSA, 2008ar).

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, 2008d).

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer *et al.*, 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 micrograms/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[7] (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[8] (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.

[7] *"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).*

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

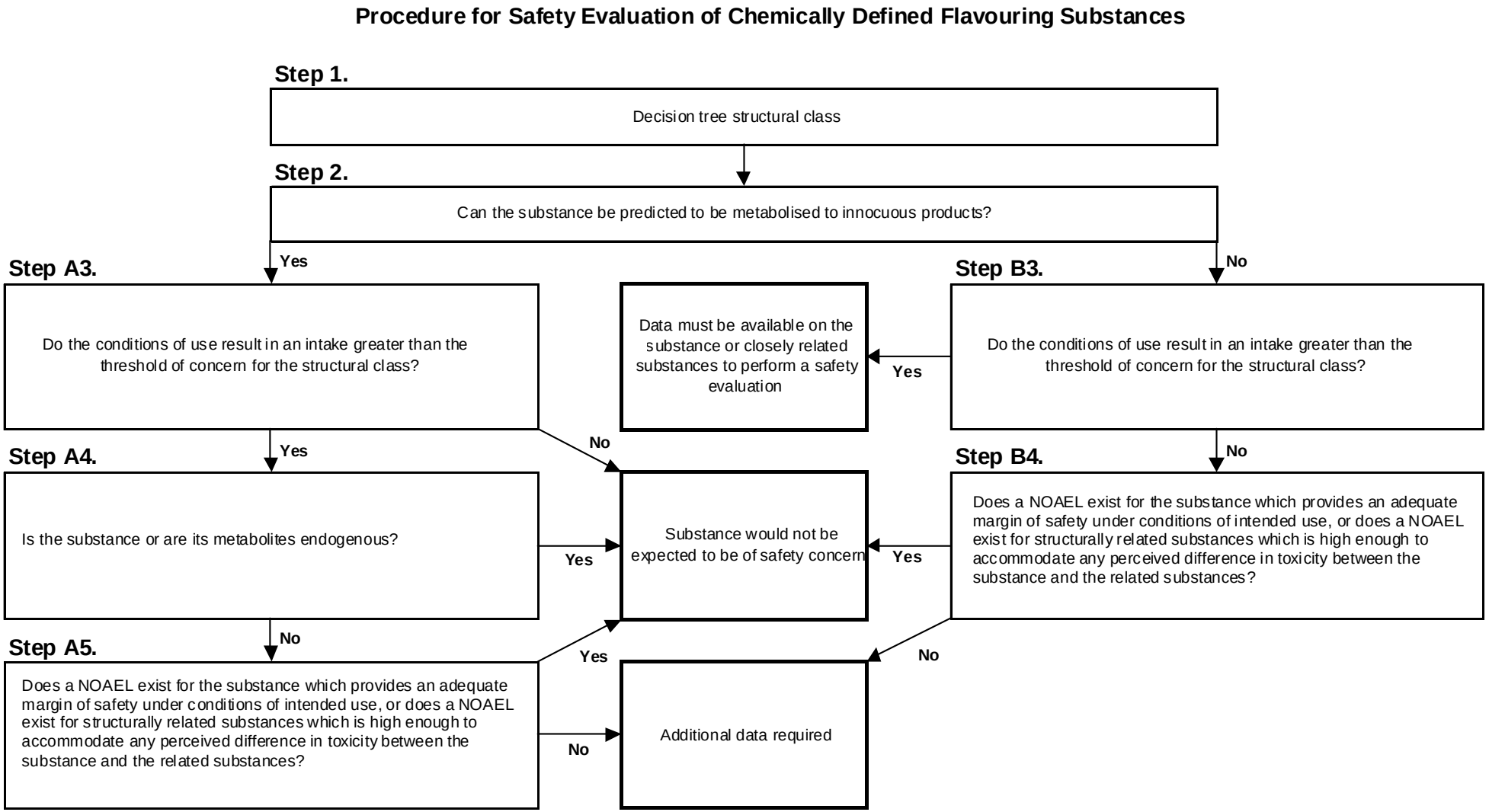


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

The "normal and maximum use levels" are provided by Industry for the one candidate substance, spilanthol [FL-no: 16.121] in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substance in FGE.303 (Flavour Industry, 2009r).

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
16.121	1,25	-	1,25	0,25	-	10	1	-	-	0,5	-	-	0,75	-	0,5	1	10	1
	2,5	-	2,5	1	-	17,5	3	-	-	1,5	-	-	1,5	-	1,5	3	25	3

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

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

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

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

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

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

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for the candidate substance in the present flavouring group, for which Industry has provided use and use levels (Flavour Industry, 2009r). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
16.121	Spilanthol	830	Class III	90

ANNEX III: METABOLISM

Specific information regarding absorption, distribution, metabolism and excretion is not available for the candidate substance.

The candidate substance is like other aliphatic amides anticipated being absorbed from the gastrointestinal tract. Aliphatic amides are expected to be at least partly hydrolysed (Bray *et al.*, 1949) to polar metabolites which are eliminated in the urine or bile (James, 1974; Schwen, 1982). Hydrolysis of the amide bond is reported as a metabolic pathway for amides e.g. dihydrocapsaicin and piperine *in vivo* in rats, however, complete hydrolysis of the candidate substance cannot be anticipated (Kawada & Iwai, 1985; Bhat & Chandrasekhara, 1987).

ANNEX IV: TOXICITY

ACUTE TOXICITY

No oral acute toxicity data are available for the candidate substance of the present Flavouring Group Evaluation from chemical group 30, nor for the supporting substances evaluated by the JECFA at the 65th meeting (JECFA, 2006d).

SUBACUTE, SUBCRONIC, CHRONIC AND CARCINOGENIC TOXICITY STUDIES

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for the candidate substance of the present Flavouring Group Evaluation from chemical group 30 but not for the supporting substances evaluated by the JECFA at the 65th meeting (JECFA, 2006d).

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
Spilanthol [16.121]	Rats, M, F 5	Oral	M: 5.5, 57, 572 mg/kg bw/day F: 6.5, 64, 629 mg/kg bw/day	28 days	572	(Moore, 2002)	The study is not considered valid. The study has not been available. Only a short summary has been submitted by Industry. The JECFA evaluation of this study at the 65th meeting has also been considered but the Panel did not agree with the JECFA that the study is appropiate for deriving a NOAEL.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

No developmental and reproductive toxicity data are available for the candidate substance of the present Flavouring Group Evaluation from chemical group 30 or for the supporting substances evaluated by the JECFA at the 65th meeting (JECFA, 2006d).

GENOTOXICITY (*IN VITRO*)

No *in vitro* mutagenicity/genotoxicity data are available for the candidate substance of the present Flavouring Group Evaluation from chemical group 30 but for two supporting substances evaluated by the JECFA at the 65th meeting (JECFA, 2006d).

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Deca-(2E,4E)-dienoic acid isobutyl-amide [16.091])	Reverse Mutation	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537	5 to 1500 µg/plate[3]	Negative[1]	(King, 2003)	
	Reverse Mutation	*S.typhimurium* TA98, TA100, TA102, TA1535, TA1537	5 to 5000 µg/plate[4]	Negative[2]	(King, 2003)	
(N-Cyclopropyl (2E,6Z)-nonadienamide [16.093])	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	Up to 5000 µg/plate	Negative[1]	(Bowles, 2003)	
	Reverse Mutation	*E.coli WP2 uvrA*$^-$	Up to 5000 µg/plate	Negative[1]	(Bowles, 2003)	

1 With and without S9 metabolic activation.

2 With metabolic activation.

3 Toxic and precipitates at 1,500 µg/plate.

4 Toxic and precipitates at 5,000 µg/plate.

GENOTOXICITY (*IN VIVO*)

No *in vivo* mutagenicity/genotoxicity data are available for the candidate substance of the present Flavouring Group Evaluation from chemical group 30 nor for the supporting substances evaluated by the JECFA at the 65th meeting (JECFA, 2006d).

REFERENCES

Bhat, B.G., Chandrasekhara, N., 1987. Metabolic disposition of piperine in the rat. Toxicology 44, 99-106.

Bowles, A.J., 2003. Reverse mutation assay (ames-test) using *Salmonella typhimurium* and *Escherichia coli*. SafePharm Laboratories Ltd., Shardlow, United Kingdom. Project No. 1543/077. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bray, H.G., James, S.P., Thorpe, W.V., Wasdell, M.R., Wood, P.B., 1949. The fate of certain organic acids and amides in the rabbit. 9. Lower aliphatic amides. Biochem. J. 45(4), 467-471.

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

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

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

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

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

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

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

EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

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

EFSA, 2008ar. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 86: Consideration of aliphatic and aromatic amines and amides evaluated by JECFA (65th meeting) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 22 May 2008. EFSA-Q-2008-070.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.

Flavour Industry, 2004f. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-86.

Flavour Industry, 2009r. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-303

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

James, R., 1974. Appendix 4, animal studies-summary of results. N-Ethyl-2-isopropyl-5-methylcyclohexane. Unpublished report to the Flavor Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

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

JECFA, 2005d. Compendium of food additive specifications. Addendum 13. Joint FAO/WHO Expert Committee of Food Additives 65th session. Geneva, 7-16 June 2005. FAO Food and Nutrition paper 52 Add. 13.

JECFA, 2006b. Evaluation of certain food additives. Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 934. Geneva, 7-16 June 2005.

JECFA, 2006d. Safety evaluation of certain food additives and contaminants. Sixty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 56. IPCS, WHO, Geneva.

JECFA, 2008d. Joint FAO/WHO Expert Committee on Food Additives. Sixty-ninth meeting, Geneva, 17-26 June 2008. Summary and conclusions issued 4 July 2008.

Kawada, T., Iwai, K., 1985. *In vivo* and *in vitro* metabolism of dihydrocapsaicin, a pungent principle of hot pepper, in rats. Agric. Biol. Chem. 49 (2), 441-448.

King, M.T., 2003. Mutagenicity study of HR 03/G05015 in the *Salmonella typhimurium*/mammalian microsome reverse mutation assay (Ames-test). Project No. AM00103N. King & Harnasch GmbH. Unpublished report to the Flavor and Extract Manufacturers Association (FEMA). Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA.

Molinatorres, J., Salgado Garciglia, R., Ramirez Chavez, E., Rio, R.E., 1996. Biochem-syst-ecol. Oxford, U.K.: Elsevier Science Ltd. 24(1), 43-47. (Only abstract).

Moore G.E., 2002. 28-dietary toxicity study in rodents. Study No. 11326. Product Safety Labs, East Brunswick, New Jersey, USA. Unpublished report to the Flavor Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Ramsewak, R.S., Erickson, A.J., Nair, M.G., 1999. Bioactive N-isobutylamides from the flower buds of Spilanthes acmella. Phytochemistry 51, 729-732.

SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF, 1999a. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Schwen, R., 1982. Excretion and tissue distribution of 14C after an oral dose of capsaicin analog, N-(vanillyl)-[1(14C)]-nonanamide. Fed. Proc., 41, 1558.

TNO, 2010. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Yasuda, I., Takeya, K., Itokawa, H., 1980. The geometric structure of spilanthol. Chem. Pharm. Bull. 28(7), 2251-2253.

ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CoE	Council of Europe
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear Magnetic Resonance
No	Number
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
WHO	World Health Organisation