EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 303, Revision 1 (FGE.303Rev1): Spilanthol from chemical group 30

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 303, Revision 1 (FGE.303Rev1): 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

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate the flavouring substance spilanthol [FL-no: 16.121] in Flavouring Group Evaluation 303, Revision 1, using the Procedure according to Commission Regulation (EC) No 1565/2000. This revision is made as new 90 days toxicity data have been submitted for spilanthol [FL-no: 16.121]. The substance was considered not to have genotoxic potential. The substance was evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that spilanthol [FL-no: 16.121] does not give rise to safety concern at its level of dietary intake, estimated on the basis of the Maximum Survey-derived Daily Intake (MSDI) approach. Besides the safety assessment of the flavouring substance, the specifications for the material of commerce have also been considered. Adequate specifications including complete purity criteria and identity for the material of commerce have been provided for the candidate substance.

KEY WORDS

flavouring, food safety, spilanthol, aliphatic amide, FGE.303

1 On request from the European Commission, Question No EFSA-Q-2013-00438, adopted on 5 December 2014.

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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 requested to re-evaluate the flavouring substance in the Flavouring Group Evaluation 303Rev1, 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 present revision of FGE.303, FGE.303Rev1 includes a re-evaluation of spilanthol [FL-no: 16.121] as additional data, from a 90-day dietary rat study have become available.

The candidate substance spilanthol [FL-no: 16.121] is a branched chain unsaturated aliphatic amide. The specifications provided specify the stereoisomeric composition. Spilanthol is 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 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.

No *in vitro* or *in vivo* data on genotoxicity are available for the candidate substance spilanthol. However, for the two structurally related substances deca-(2*E*, 4*E*)-dienoic acid isobutyl-amide [FL-no: 16.091] and *N*-cyclopropyl (2*E*,6*Z*)-nonadienamide [FL-no: 16.093] negative genotoxicity studies are available. The Panel therefore concluded that the lack of genotoxicity data for the candidate substance spilanthol [FL-no: 16.121] does not preclude the evaluation of this aliphatic amide using the Procedure.

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

According to the default MSDI approach, spilanthol has an intake in Europe of 24 µg/*capita*/day, which is below the threshold of concern value for structural class III substances of 90 µg/person/day.

When the estimated intake was based on the mTAMDI approach it is 670 µg/person/day for the candidate substance, which is above the threshold of concern for a structural III substance of 90 µg/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.

Since the publication of FGE.303, a 90-day study in rats has become available for spilanthol [FL-no: 16.121], providing a NOAEL of 23.4 mg/kg bw/day. The estimated daily *per capita* intake for the candidate substance of 24 µg corresponds to 0.4 µg/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 5.9×10^4 can be calculated.

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.

Therefore, the Panel concluded that spilanthol [FL-no: 16.121] would not present a safety concern at the estimated level of intake based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA has evaluated the flavouring substance spilanthol [FL-no: 16.121] in the flavouring group evaluation 303 (FGE.303). The opinion was adopted on 3 February 2011. EFSA concluded in its opinion that additional data on the chemically defined material are required as a 28 day study is not considered sufficient to derive a No Observed Adverse Effect Level (NOAEL).

The requested additional data (90-day dietary study in rats) have now been submitted by the applicant.

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substance.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests EFSA to carry out a safety assessment on the following flavouring substance: spilanthol [FL-no: 16.121] in accordance with Commission Regulation (EC) No 1565/2000.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[5] Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p, 1–161.

[6] 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. OJ L 180, 19.7.2000, p. 8–16.

ASSESSMENT

1. History of the Evaluation

In FGE.303, the Panel considered that additional toxicity data were needed for spilanthol [FL-no: 16.121] evaluated through the Procedure, as no adequate toxicity study was available from which a no observed adverse effect level (NOAEL) could be established, neither on spilanthol nor on structurally related substances.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.303	3 February 2011	http://www.efsa.europa.eu/en/efsajournal/pub/1995.htm	1
FGE.303Rev1			1

The present Revision of FGE.303, FGE.303Rev1 includes a re-evaluation of spilanthol [FL-no: 16.121], as a 90-day dietary rat study with the flavouring substance has become available (Bauter, 2012; Flavour Industry, 2013). Additional information on possible neurotoxicity of spilanthol has also been submitted (Kadir et al., 1989; Flavour Industry, 2014). A search in the open literature did not reveal any pertinent new information on spilanthol.

2. Presentation of the Substances in Flavouring Group Evaluation 303, Revision 1

2.1. Description

The present Flavouring Group Evaluation 303, Revision 1 (FGE.303Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Appendix A 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, 2000).

The structural formula of the candidate substance together with its chemical name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, is listed in Table 1.

The outcome of the safety evaluation of the candidate substance, spilanthol [FL-no: 16.121] is summarised in Table 4 and the hydrolysis products of the candidate substance are listed in Table 5.

Spilanthol is a branched chain unsaturated aliphatic amide and is structurally closely related to three flavouring substances [FL-no: 16.091, 16.093 and 16.094] evaluated at the 65th JECFA meeting (JECFA, 2006b) in the group of 'Aliphatic and aromatic amines and amides' and considered by the Panel in FGE.86Rev1. Since the publication of FGE.303, two of the JECFA evaluated substances, *N*-cyclopropyl (2E,6Z)-nonadienamide [FL-no: 16.093] and *N*-ethyl (2E,6Z)-nonadienamide [FL-no: 16.094] are no longer supported for use as flavouring substances in the European Union (DG SANCO, 2012) The names and structures of the supporting flavouring substance and the two structurally related substances are listed in Table 6, together with their JECFA evaluation status.

2.2. 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.).

Spilanthol [FL-no: 16.121] can exist as geometrical stereoisomers due to the presence of double bonds. The name spilanthol specifies the (2*E*,6*Z*,8*E*) stereoisomer (see Table 1). Information on the proportions of the geometrical isomers has been provided (Flavour Industry, 2009) (see Table 1).

2.3. Natural Occurrence in Food

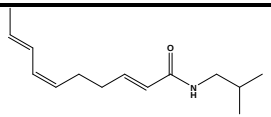
According to TNO, the candidate substance spilanthol [FL-no: 16.121] has not been reported to occur naturally in any food items (TNO, 2010). Spilanthol has been identified in *Spilanthes oleracea*, which according to Flavour Industry is used as a spice in some countries (Yasuda et al., 1980; Molinatorres et al., 1996; Ramsewak et al., 1999).

3. Specifications

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

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), this information is adequate for the candidate substance (see Section 2.2 and Table 1).

Table 1: Specification Summary of the Substance in the FGE.303Rev1

FL-no JECFA-no	Name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [(a)] Solubility in ethanol [(b)]	Boiling point, °C [(c)] Melting point, °C ID test Assay minimum	Refrac. Index [(d)] Spec.gravity [(e)]	Specification comments
16.121 2077	Spilanthol		4668 25394-57-4	Liquid $C_{14}H_{23}NO$ 221.35	Insoluble Soluble	140-160 (13 Pa) IR NMR MS 74 %	1.491-1.541 0.945-0.945	Synonym: (2*E*,6*Z*,8*E*)-*N*-(2-Methylpropyl)-2,6,8-decatrienamide. Secondary compounds: 16.7 % (2*E*,6*E*,8*E*)-, 5.8 % (2*E*,6*E*,8*Z*)-, 0.9 % (2*Z*,6*Z*,8*E*)-, 0.3 % (2*E*,6*E*,8*E*)-, 0.8 % (2*Z*,6*Z*,8*Z*)-isomer, 1.6 % other isomers.

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

4. 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 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, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

4.1. 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, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 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[7] (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).

The anticipated annual volume of production of the candidate substance spilanthol [FL-no: 16.121] in the present Flavouring Group Evaluation (FGE.303Rev1) from use as flavouring substance in Europe has been reported to be approximately 200 kg (Flavour Industry, 2009). For the supporting substance [FL-no: 16.091] the annual volume of production is 93 kg in Europe (IOFI, 2013).

[7] 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.

Based on the annual volume of production reported for the candidate substance, the daily *per capita* intake in the EU from use as a flavouring substance is 24 µg (Table 4).

4.2. 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.

Information on food categories and normal and maximum use levels[8,9] for spilanthol [FL-no: 16.121] was submitted by the Flavour Industry (Flavour Industry, 2009). 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, 2000), as shown in Table 2. For the present calculation of the 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 2: 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, 2009) (see Table B.1.2, Annex B).

The mTAMDI value is 670 µg/person/day for the candidate substance from structural class III.

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

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

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 7 and Annex B.

5. 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 and 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 and Iwai, 1985; Bhat and Chandrasekhara, 1987) therefore the substance should be evaluated via the B side of the Procedure.

6. 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 4.

Step 1

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

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.

Step B3

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

Step B4

For the candidate substance spilanthol [FL-no: 16.121], a NOAEL of 23.4 mg/kg bw/day from a multiple dose 90-day oral toxicity study in rats could be derived (Bauter, 2012). The estimated daily *per capita* intake of 24 µg corresponds to 0.4 µg/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 5.9×10^4 can be calculated**.** The Panel agrees that this provides a sufficient safety margin and that the flavouring substance can be concluded at step B4 of the Procedure to be of no safety concern.

7. Comparison of the Estimated Intake Based on the MSDI and the mTAMDI Approaches

When the estimated intake is based on the mTAMDI approach, the value for the structural class III substance, spilanthol is 670 µg/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 3.

Table 3: Estimated Intakes Based on the MSDI and the mTAMDI Approaches

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	670	Class III	90

8. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and the 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, 2009), the estimated daily *per capita* intake as flavouring of spilanthol [FL-no: 16.121] belonging to structural class III is 24 µg. This value does not exceed the threshold of concern for structural class III of 90 µg/person/day.

The candidate substance is structurally related to one supporting flavouring substance evaluated by the JEFCA at its 65th meeting (JECFA, 2006a). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for the supporting substance, deca-(2*E*,4*E*)-dienioc acid isobutyl-amide [FL-no: 16.091]. The total combined intake of the candidate and supporting substance is 35 µg/*capita*/day, which does not exceed the thresholds of concern for structural class III substances.

9. Toxicity

9.1. Acute Toxicity

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

The substance was earlier demonstrated to be toxic to *Anopheles* larvae as well as to adult houseflies (Jacobson, 1957 as quoted in (Kadir et al., 1989)). Later it was further shown to be acute toxic (LD_{50} 2.46 µg/g insect) also to adult American cockroach (*Periplaneta Americana* L.) (Kadir et al., 1989). In the latter study spilanthol shows a higher toxicity than three conventional insecticides representing various classes, namely carbaryl, bioresmethrin and lindane. Electrophysiological studies indicated

immediate hyperexitation followed by complete inhibition of the cockroach central nerve activity (Kadir et al., 1989).

On the basis of the absence of clinical signs indicating neurotoxicity in the 90-day study the Panel considered the acute toxicity studies using insect's surface exposure in acetone solution directly on the chitin exoskeleton as not being of relevance to humans.

9.2. 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 mg/kg 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 new 90-day dietary study is now available on spilanthol [FL-no: 16.121].

A 90-day study was performed with spilanthol [FL-no: 16.121] (Bauter, 2012). The study was performed according to OECD guideline (TG 408). Four groups of rats (10/sex/dietary intake level) of male and female CRL Sprague-Dawley CD® IGS rats were fed a diet designed to provide 0 (dietary control), 180, 360 and 1200 mg/kg of spilanthol in the feed (Bauter, 2012). These dietary levels correspond to the measured daily intake of 0, 11.8, 23.4 and 80.3 mg/kg bw for males and 0, 14.3, 27.9 and 92.5 mg/kg bw for females. Spilanthol was stable during the course of the study with overall average concentrations of 92.4, 95.3 and 99.5 %, respectively for 180, 360 and 1200 mg/kg dietary levels. Clinical observations of toxicity were performed on day 0 and weekly throughout the study until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed during week 12 after overnight fast. Urine was collected during the 15 hours prior to the blood draw. At termination of the study all survivors were sacrificed and subject to full necropsy.

No substance-related mortalities, no gross observations and no macroscopic findings at sacrifice were attributed to spilanthol in the diet. Decreases in food consumption of approximately 20 % were observed at all dose levels. In the male top dose group this was accompanied by a decreased body weight and decreased body weight gain. Dose-dependent decreases in food consumption in females did not result in reduced body weight or body weight gain. No adverse effects were observed in clinical chemistry or urinalysis. The only microscopic finding related to spilanthol was a minimal hypertrophy of the submandibular salivary gland acini in males and females of the top dose only.

Overall, considering both sexes, there were no significant changes in absolute and/or relative organ weights in the low and mid dosed groups compared with control as a result of test substance exposure. However, decreases in absolute but also relative adrenal gland- and liver weights were observed in

male rats in a dose-dependent manner reaching statistically significant at the highest dose. In addition, in this group statistically significant decreases in absolute heart and kidney weights and an increased brain-to-body weight ratio compared with control were seen. However, none of these effects were correlated with clinical or histopathological findings, therefore, they were considered non-adverse. In general, substance administration appeared to affect males more than females.

Based on the fact that no statistically significant changes in absolute or relative organ weights were seen in the low and mid dose groups, and based on the hypertrophy of salivary gland in both sexes in the highest dose, but not in the mid dose group, the NOAEL for spilanthol in the diet is set to 360 mg/kg feed (mid dose), which corresponds to calculated intakes of 23.4 mg/kg bw/day in males and 27.9 mg/kg bw/day in females (Bauter, 2012).

Although specific functional neurological tests were not conducted, a large set of observations were made twice daily in the 90-day study including among others occurrence of secretions, excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiration pattern), changes in gate, posture, response to handling, and the presence of clonic or tonic movements etc. No treatment related effects were identified at these observations.

Repeated dose toxicity data are summarised in Table 7.

9.3. Developmental / Reproductive Toxicity Studies

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

9.4. Genotoxicity Studies

No *in vitro* or *in vivo* data on genotoxicity 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 concluded that the lack of genotoxicity data for spilanthol [FL-no: 16.121] does not preclude the evaluation of this aliphatic amide using the Procedure.

Genotoxicity data are summarised in Table 8.

CONCLUSION

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

This revision is made due to the submission of new toxicological data from a 90 days study with the substance spilanthol [FL-no: 16.121].

The substance has been presented with specification of the stereoisomeric composition and it is assigned to structural class III, according to the decision tree approach presented by Cramer et al., 1978. Spilanthol has been identified in the plant *Spilanthes oleracea*, which is used in some countries as a spice according to the Flavour Industry.

No *in vitro* or *in vivo* data on genotoxicity are available for the candidate substance spilanthol. However, for the two structurally related substances [FL-no: 16.091 and 16.093] negative genotoxicity studies are available. The Panel therefore 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.

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

According to the default MSDI approach, spilanthol has an intake in Europe of 24 µg/*capita*/day, which is below the threshold of concern value for structural class III (90 µg/person/day).

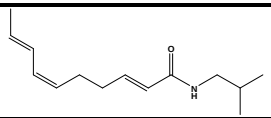
When the estimated intake is based on the mTAMDI approach it is 670 µg/person/day for the candidate substance from structural class III, which is above the threshold of concern for structural III of 90 µg/person/day. Therefore more reliable exposure data are required. On the basis of such additional data, the flavouring substance should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

Since the publication of FGE.303, a 90-day study in the rat has become available for the candidate substance spilanthol [FL-no: 16.121], providing a NOAEL of 23.4 mg/kg bw/day. The estimated daily *per capita* intake for the candidate substance of 24 µg corresponds to 0.4 µg/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 5.9×10^4 can be calculated.

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.

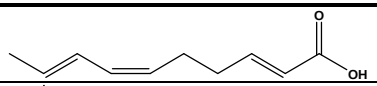
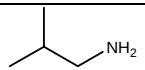
Therefore, the Panel concluded that spilanthol [FL-no: 16.121] would not present a safety concern at the estimated level of intake based on the MSDI approach.

Table 4: Summary of Safety Evaluation Applying the Procedure

FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/capita/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d),(e)]	Outcome on the material of commerce [(f),(g),(h)]	Evaluation remarks
16.121 2077	Spilanthol		24	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

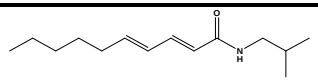
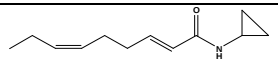
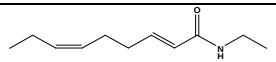
(a): EU MSDI: Amount added to food as flavour in (kg / year) × 10E9 / (0.1 x population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita/day.
(b): Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): 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.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.

Table 5: Evaluation Status of Hydrolysis Products of Candidate Substance

FL-no	EU Register name JECFA no	Structural formula	SCF status [(a)] JECFA status [(b)] CoE status [(c)] EFSA status	Structural class [(d)] Procedure path (JECFA) [(e)]	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 (JECFA, 2008) Category A (CoE, 1992)	Class I A3: Intake below threshold	

(a): 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.
(b): No safety concern at estimated levels of intake.
(c): Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.
(d): Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
(e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

Table 6: Summary of Safety Evaluation of Supporting Substance

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (µg/*capita*/day)	SCF status [b] JECFA status [c] CoE status [d]	EFSA Comments
16.091	Deca-(2E,4E)-dienoic acid isobutyl-amide		4148 18836-52-7	1598 JECFA specification (JECFA, 2005).	11	No safety concern (JECFA, 2008)	No safety concern at the estimated level of intake based on the MSDI approach.
16.093	*N*-Cyclopropyl (2E,6Z)-nonadienamide		4087 608514-55-2	1597 JECFA specification (JECFA, 2005).	-	No safety concern (JECFA, 2008)	No longer supported by Industry (DG SANCO, 2012).
16.094	*N*-Ethyl (2E,6Z)-nonadienamide		4113 608514-56-3	1596 JECFA specification (JECFA, 2005).	-	No safety concern (JECFA, 2008)	No longer supported by Industry (DG SANCO, 2014).

(a): EU MSDI: Amount added to food as flavouring substance in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita/day.
(b): 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.
(c): No safety concern at estimated levels of intake.
(d): Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

Table 7: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels mg/kg bw/day	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
Spilanthol [16.121]	Rats, M, F 5	Oral	M: 5.5, 57, 572 F: 6.5, 64, 629	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.
	Rats, M, F 3/20	Diet	M:11.8/ 23.4, 80.3 F: 14.3, 27.9, 92.5	90 days	23.4	(Bauter, 2012)	OECD Guideline study (408).

Table 8: 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	*S.typhimurium* TA98, TA100, TA102, TA1535, TA1537	5 to 1500 µg/plate(c)	Negative(a)	(King, 2003)	
	Reverse Mutation	*S.typhimurium* TA98, TA100, TA102, TA1535, TA1537	5 to 5000 µg/plate(d)	Negative(b)	(King, 2003)	
(*N*-Cyclopropyl (2E,6Z)-nonadienamide)	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	Up to 5000 µg/plate	Negative(a)	(Bowles, 2003)	
	Reverse Mutation	*E.coli WP2 uvrA*$^-$	Up to 5000 µg/plate	Negative(a)	(Bowles, 2003)	

*: Supporting or structurally related substance
(a): With and without S9 metabolic activation.
(b): With metabolic activation.
(c): Toxic and precipitates at 1500 µg/plate.
(d): Toxic and precipitates at 5000 µg/plate.

DOCUMENTATION PROVIDED TO EFSA

1. Bauter MR, 2012. Final report. Spilanthol: A 90-day dietary study in rats. Product Safety Labs. Study no. 33621. October 23, 2012. Unpublished report submitted by Flavour Industry to FLAVIS Secretariat.

2. Flavour Industry, 2014. Unpublished information submitted by Flavour Industry to EFSA and forwarded to FLAVIS Secretariat. A-303Rev1 [FL-no: 16.121]. FLAVIS/8.251.

3. Takasago, 2013. Takasago International Corporation. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 30 (Annex I of 1565/2000/EC) Spilanthol as Evaluated by EFSA in FGE.303. Addendum to FGE.303. 19.03.2013. FLAVIS/8.196.

REFERENCES

Bauter MR, 2012f. Final report. Spilanthol: A 90-day dietary study in rats. Product Safety Labs. Study no. 33621. October 23, 2012. Unpublished report submitted by Flavour Industry to FLAVIS Secretariat.

Bhat BG and Chandrasekhara N, 1987. Metabolic disposition of piperine in the rat. Toxicology 44, 99–106.

Bowles AJ, 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 HG, James SP, Thorpe WV, Wasdell MR and Wood PB, 1949. The fate of certain organic acids and amides in the rabbit. 9. Lower aliphatic amides. Biochemical Journal 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 GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetics Toxicology 16(3), 255–276.

DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/02 2012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.

DG SANCO (Directorate General for Health and Consumer Affairs), 2014. Request for a re-revaluation of the flavouring substance FL 16.091 from FGE.86Rev1. 30.01.2014.

EC (European Commission), 2000. 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.

EFFA (European Flavour and Fragrance Association), 2002. 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 (European Flavour and Fragrance Association), 2004. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

EFSA (European Food Safety Authority), 2004. 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. Available online: http://www.efsa.europa.eu/en/events/event/afc040712-m.pdf/.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online].

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

Flavour Industry, 2013. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-303Rev1 [FL-no: 16.121]. FLAVIS/8.196.

Flavour Industry, 2014. Unpublished information submitted by Flavour Industry to EFSA and forwarded to FLAVIS Secretariat. A-303Rev1 [FL-no: 16.121]. FLAVIS/8.251.

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

IOFI (International Organization of the Flavor Industry), 2013. Global poundage survey 2010. IOFI Global Poundage Survey Committee, Geneva, Switzerland 2013, pp. 1–291.

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 (Joint FAO/WHO Expert Committee on Food Additives), 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 (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. 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 (Joint FAO/WHO Expert Committee on Food Additives), 1999. 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 (Joint FAO/WHO Expert Committee on Food Additives), 2005. 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 (Joint FAO/WHO Expert Committee on Food Additives), 2006a. 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 (Joint FAO/WHO Expert Committee on Food Additives), 2006b. 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 (Joint FAO/WHO Expert Committee on Food Additives), 2008. Sixty-ninth meeting, Geneva, 17-26 June 2008. Summary and conclusions issued 4 July 2008.

Kadir HA, Zakaria MB, Kechil AA and Azirun MS, 1989. Toxicity and electrophysical effects of *Spilanthes acmella murr*. extracts on *Periplaneta americana L*. Pesticide Science 25(4), 329–335.

Kawada T and Iwai K, 1985. *In vivo* and *in vitro* metabolism of dihydrocapsaicin, a pungent principle of hot pepper, in rats. Agricultural and Biological Chemistry 49 (2), 441–448.

King MT, 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 and Rio RE, 1996. Biochem-syst-ecol. Oxford, U.K.: Elsevier Science Ltd. 24(1), 43–47. (Only abstract).

Moore GE, 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 RS, Erickson AJ and Nair MG, 1999. Bioactive *N*-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry 51, 729–732.

SCF (Scientific Committee for Food), 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 (Scientific Committee for Food), 1999. 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 to 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. Federation Proceedings 41, 1558.

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2010. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 12.2/12.3. Zeist, The Netherlands. TNO Triskelion, 1963–2010.

Yasuda I, Takeya K and Itokawa H, 1980. The geometric structure of spilanthol. Chemical and Pharmaceutical Bulletin 28(7), 2251–2253.

APPENDIX A: 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, 2000), named the 'Procedure', is shown in schematic form in Figure A.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, 1996; JECFA, 1997; JECFA, 1999).

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

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

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[10] (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[11] (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.

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

[11] '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, 1997).

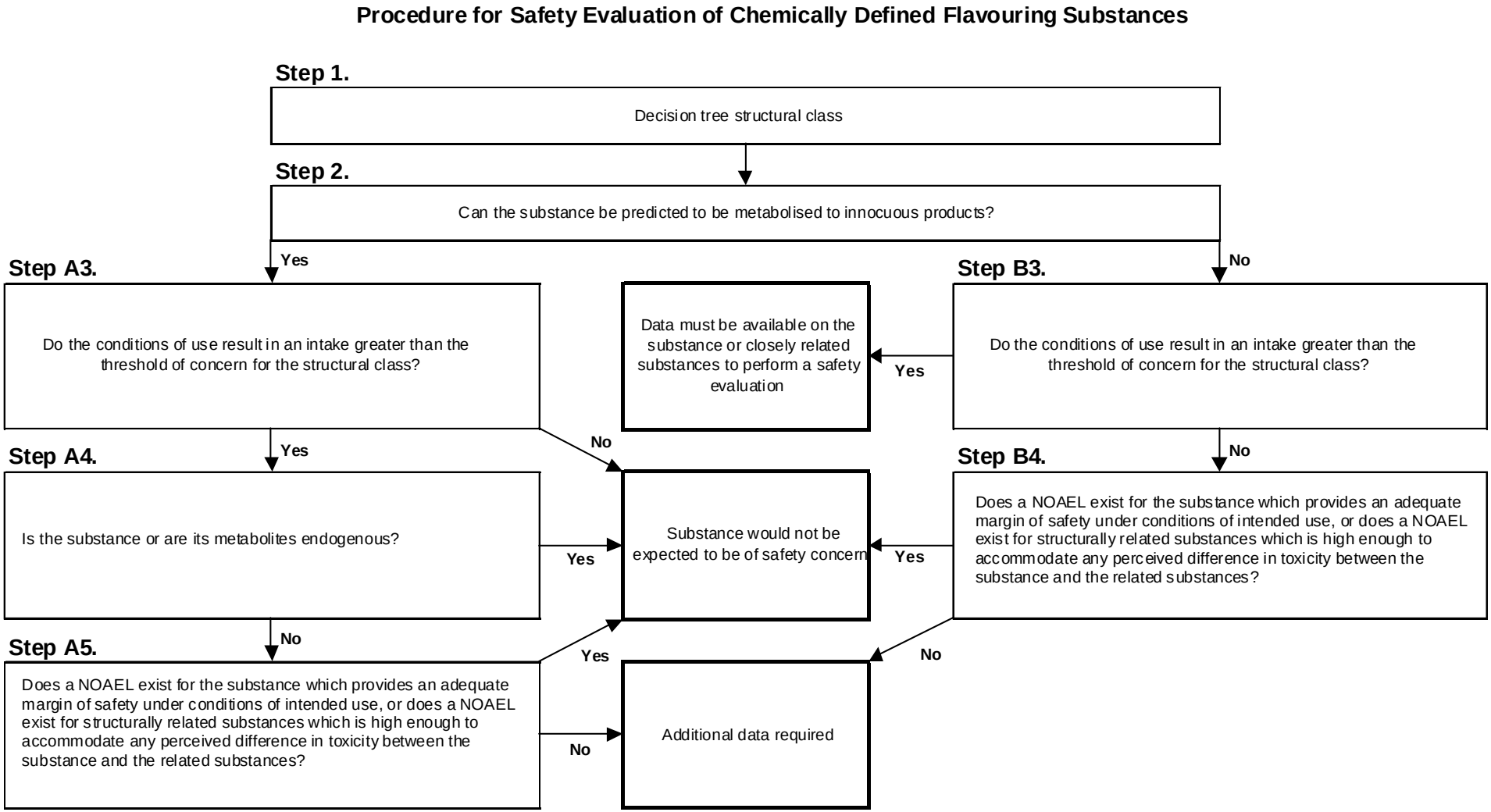


Figure A.1: Procedure for safety evaluation of chemically defined flavouring substances

APPENDIX B: USE LEVELS / MTAMDI

B.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table B.1.1) in which the candidate substances are used, Flavour Industry reports a 'normal use level' and a 'maximum use level' (EC, 2000). 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, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

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

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 B.1.2).

B.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 B.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table B.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)

Table B.1.2 Normal and Maximum use levels (mg/kg) for the candidate substance in FGE.303, Revision 1 (Flavour Industry, 2009).

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	0.5	1
	2.5	-	2.5	1	-	17.5	3	-	-	1.5	-	-	1.5	-	1.5	3	1.5	3

Table B.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) 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 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, 2000) and reported by the Flavour Industry in the following way (see Table B.2.2):

Beverages (SCF, 1995) correspond to food category 14.1

Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16]

Exception a (SCF, 1995) corresponds to food category 5 and 11

Exception b (SCF, 1995) corresponds to food category 15

Exception c (SCF, 1995) corresponds to food category 14.2

Exception d (SCF, 1995) corresponds to food category 12

Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

The candidate substance **[**FL-no. 16.121] is also anticipated to be used in chewing gum, which is not covered by any of the food categories in 1565/2000. Normal/maximum use levels for chewing gum are reported to be 10/25 mg/kg for [FL-no: 16.121]. For chewing gum, the intake estimate is 2 g/day. Under the assumptions that all of the flavouring substance is released from the chewing gum and that the intake estimate is 2 g chewing gum/day, the calculation of the mTAMDI of the candidate substance based on the 16 food categories and the use of chewing gum sum up to 670 µg/person/day, see Table B.2.3.

Table B.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	670	Class III	90

ABBREVIATIONS

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	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
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
WHO	World Health Organisation