SCIENTIFIC OPINION

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

Spilanhol from chemical group 30

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

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 spilanhol [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 spilanhol 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

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1 On request from the Commission, Question No EFSA-Q-2010-01502, adopted on 3 February 2011.
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 Görtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Specijers, Harriet Wallin and EFSA’s staff member Kim Rygaard Nielsen for the preparatory work on this scientific Opinion.


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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.
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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.


“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 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 population⁴ (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).

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

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).

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\(^5\) "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; Schweng, 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 & Chandrasekhar, 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>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>MSDI (µg/capita/day)</th>
<th>mTAMDI (µg/person/day)</th>
<th>Structural class</th>
<th>Threshold of concern (µg/person/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.121</td>
<td>Spilanthol</td>
<td>24</td>
<td>830</td>
<td>Class III</td>
<td>90</td>
</tr>
</tbody>
</table>

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 JECFA 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>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Mol.formula</th>
<th>Mol.weight</th>
<th>Solubility 1</th>
<th>Solubility in ethanol 2</th>
<th>Boiling point, °C 3</th>
<th>Melting point, °C</th>
<th>ID test</th>
<th>Refract. Index 4</th>
<th>Spec.gravity 5</th>
<th>Specification comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.121</td>
<td>Spilanthol</td>
<td><img src="image" alt="Spilanthol Structural Formula" /></td>
<td>4668</td>
<td>25394-57-4</td>
<td></td>
<td>Liquid</td>
<td>C_{14}H_{23}NO</td>
<td>221.35</td>
<td>Not soluble</td>
<td>Soluble</td>
<td>140-160 (13 Pa)</td>
<td>IR, NMR, MS</td>
<td>74 %</td>
<td>1.4911-1.5411</td>
<td>0.9452-0.9468</td>
<td>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.</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>MSDI 1) (μg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>Outcome on the named compound [ 4) or 5]</th>
<th>Outcome on the material of commerce [6), 7), or 8]</th>
<th>Evaluation remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.121</td>
<td>Spilanthol</td>
<td><img src="image" alt="Spilanthol" /></td>
<td>24</td>
<td>Class III</td>
<td>Additional data required 6)</td>
<td>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. 7)</td>
<td>No conclusion can be drawn due to lack of information on the purity of the material of commerce. 8)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>SCF status 1)</th>
<th>JECFA status 2)</th>
<th>CoE status 3)</th>
<th>EFSA status</th>
<th>Structural class 4)</th>
<th>Procedure path (JECFA) 5)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6,8-Triendecanoic acid</td>
<td></td>
<td></td>
<td>Not evaluated as flavouring substance</td>
<td>Not evaluated as flavouring substance</td>
<td>Not evaluated as flavouring substance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.002</td>
<td>Isobutylamine</td>
<td></td>
<td>No safety concern a)</td>
<td>Class I 1) A3: Intake below threshold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1583</td>
<td></td>
<td></td>
<td>Category A b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>JECFA no Specification available</th>
<th>MSDI (EU) 1) (µg/capita/day)</th>
<th>SCF status 2)</th>
<th>JECFA status 3)</th>
<th>CoE status 4)</th>
<th>EFSA Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.091</td>
<td>Deca-(2E,4E)-dienoic acid isobutylamide</td>
<td><img src="image" alt="Deca-(2E,4E)-dienoic acid isobutylamide" /></td>
<td>1598</td>
<td>18836-52-7</td>
<td>1598 JECFA specification (JECFA, 2005d)</td>
<td>6.1</td>
<td>No safety concern a)</td>
<td>EFSA conclusion: B4-No, additional data required (EFSA, 2008ar).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.093</td>
<td>N-Cyclopropyl (2E,6Z)-nonadienamide</td>
<td><img src="image" alt="N-Cyclopropyl (2E,6Z)-nonadienamide" /></td>
<td>1597</td>
<td>608514-55-2</td>
<td>1597 JECFA specification (JECFA, 2005d)</td>
<td>61</td>
<td>No safety concern a)</td>
<td>EFSA conclusion: B4-No, additional data required (EFSA, 2008ar).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.094</td>
<td>N-Ethyl (2E,6Z)-nonadienamide</td>
<td><img src="image" alt="N-Ethyl (2E,6Z)-nonadienamide" /></td>
<td>1596</td>
<td>608514-56-3</td>
<td>1596 JECFA specification (JECFA, 2005d)</td>
<td>61</td>
<td>No safety concern a)</td>
<td>EFSA conclusion: B4-No, additional data required (EFSA, 2008ar).</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 products7 (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous8 (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).
Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

Step 1.
Decision tree structural class

Step 2.
Can the substance be predicted to be metabolised to innocuous products?

Yes

Step A3.
Do the conditions of use result in an intake greater than the threshold of concern for the structural class?

Yes

Step A4.
Is the substance or are its metabolites endogenous?

Yes

No

Yes

Step A5.
Does a NOAEL exist for the substance which provides an adequate margin of safety under conditions of intended use, or does a NOAEL exist for structurally related substances which is high enough to accommodate any perceived difference in toxicity between the substance and the related substances?

Yes

No

Substance would not be expected to be of safety concern

Step B3.
Do the conditions of use result in an intake greater than the threshold of concern for the structural class?

Yes

No

Step B4.
Does a NOAEL exist for the substance which provides an adequate margin of safety under conditions of intended use, or does a NOAEL exist for structurally related substances which is high enough to accommodate any perceived difference in toxicity between the substance and the related substances?

Yes

No

Additional data required

Figure 1.1 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)

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

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).

<table>
<thead>
<tr>
<th>FL-no</th>
<th>01.0</th>
<th>02.0</th>
<th>03.0</th>
<th>04.1</th>
<th>04.2</th>
<th>05.0</th>
<th>06.0</th>
<th>07.0</th>
<th>08.0</th>
<th>09.0</th>
<th>10.0</th>
<th>11.0</th>
<th>12.0</th>
<th>13.0</th>
<th>14.1</th>
<th>14.2</th>
<th>15.0</th>
<th>16.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.121</td>
<td>1.25</td>
<td>-</td>
<td>0.25</td>
<td>-</td>
<td>10</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>0.75</td>
<td>-</td>
<td>0.5</td>
<td>1</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.5</td>
<td>1</td>
<td>17.5</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>3</td>
<td>25</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

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 e: 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)

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

Table II.2.3 Estimated intakes based on the mTAMDI approach

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>mTAMDI (µg/person/day)</th>
<th>Structural class</th>
<th>Threshold of concern (µg/person/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.121</td>
<td>Spilanthol</td>
<td>830</td>
<td>Class III</td>
<td>90</td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Species; Sex No./Group</th>
<th>Route</th>
<th>Dose levels</th>
<th>Duration</th>
<th>NOAEL (mg/kg bw/day)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spilanthol [16,121]</td>
<td>Rats, M, F 5</td>
<td>Oral</td>
<td>M: 5.5, 57, 572 mg/kg bw/day F: 6.5, 64, 629 mg/kg bw/day</td>
<td>28 days</td>
<td>572</td>
<td>(Moore, 2002)</td>
<td>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 appropriate for deriving a NOAEL.</td>
</tr>
</tbody>
</table>

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*)

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Test System</th>
<th>Test Object</th>
<th>Concentration</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Deca-(2E,4E)-dienoic acid isobutyl-amide [16.091])</td>
<td>Reverse Mutation</td>
<td><em>Salmonella typhimurium</em> TA98, TA100, TA102, TA1535, TA1537</td>
<td>5 to 1500 µg/plate&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Negative&lt;sup&gt;2&lt;/sup&gt;</td>
<td>(King, 2003)</td>
<td></td>
</tr>
<tr>
<td>(N-Cyclopropyl (2E,6Z)-nonadienamide [16.093])</td>
<td>Reverse Mutation</td>
<td><em>Salmonella typhimurium</em> TA98, TA100, TA102, TA1535, TA1537</td>
<td>5 to 5000 µg/plate&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Negative&lt;sup&gt;2&lt;/sup&gt;</td>
<td>(King, 2003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse Mutation</td>
<td><em>Salmonella typhimurium</em> TA98, TA100, TA1535, TA1537</td>
<td>Up to 5000 µg/plate</td>
<td>Negative&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(Bowles, 2003)</td>
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</tr>
<tr>
<td></td>
<td>E.coli WP2 uvrA</td>
<td></td>
<td>Up to 5000 µg/plate</td>
<td>Negative&lt;sup&gt;2&lt;/sup&gt;</td>
<td>(Bowles, 2003)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> With and without S9 metabolic activation.  
<sup>2</sup> With metabolic activation.  
<sup>3</sup> Toxic and precipitates at 1,500 µg/plate.  
<sup>4</sup> 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


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


### ABBREVIATIONS

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