



EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 300 (FGE.300): One cyclo-aliphatic amide from chemical group 33

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 300 (FGE.300):

One cyclo-aliphatic amide from chemical group 33¹

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 a flavouring substance in the Flavouring Group Evaluation 300 using the Procedure in Commission Regulation (EC) No 1565/2000. The substance was not considered to have genotoxic potential. The substance was evaluated through a stepwise approach (the Procedure) 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 for the substance [FL-no: 16.115] evaluated through the Procedure, no appropriate NOAEL was available and additional data are required. Besides the safety assessment of this flavouring substance, the specifications for the materials of commerce have also been considered. The composition of the stereoisomeric mixture has to be specified.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) 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

1 On request from the Commission, Question No EFSA-Q-2009-00579, adopted on 6 July 2011.

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Flavouring Group Evaluation 300, (FGE.300), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The flavouring substance belongs to chemical group 33, Annex I of the Commission Regulation (EC) No 1565/2000.

The substance cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no: 16.115] possesses three chiral centres and has been presented with specification of the stereoisomeric composition.

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

The substance in the present group has not been reported to occur naturally in food.

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.

According to the default MSDI approach, the flavouring substance in this group has a total intake in Europe of 3 microgram/*capita*/day, which is below the threshold of concern value for structural class III of 90 microgram/person/day.

The results from the available limited genotoxicity studies do not raise a concern for genotoxicity and hence do not preclude the evaluation of the candidate substance in this FGE through the Procedure.

From the data available it is not possible to conclude that the candidate substance in this group cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no. 16.115] would be metabolised to innocuous products at the reported levels of intake as flavouring substance. Therefore the substance was evaluated along the B-side of the Procedure. No toxicity study is available on the candidate or on the supporting substance that can provide an adequate NOAEL to be used in the Procedure, accordingly additional toxicity data are required for the candidate substance or a structurally related substance.

When the estimated intake was based on the mTAMDI approach it was 960 microgram/person/day for this flavouring substance belonging to structural class III. The estimated intake for the candidate substance is above the threshold of concern of 90 microgram/person/day. Thus, for the flavouring substance considered in this Opinion the intake, estimated on the basis of the mTAMDI, exceed the relevant threshold for the structural class, to which the flavouring substance has been assigned. Therefore, for the substance more reliable exposure data is required. On the basis of such additional data, the flavouring substance should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the candidate substance can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including

purity criteria and identity for the materials of commerce have been provided for the flavouring substance. However, the composition of the stereoisomeric mixture has to be specified.

In conclusion, for the flavouring substance cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no: 16.115] the Panel considered that additional data are needed. Information on composition of isomers is missing.

KEYWORDS

Flavouring, safety, cyclo-aliphatic amide, FGE.300.

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1 BACKGROUND

2 Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a
3 Procedure for the establishment of a list of flavouring substances the use of which will be authorised
4 to the exclusion of all other substances in the EU. In application of that Regulation, a Register of
5 flavouring substances used in or on foodstuffs in the Member States was adopted by Commission
6 Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/163/EC (EC,
7 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are
8 divided into 34 chemical groups. Substances within a group should have some metabolic and
9 biological behaviour in common.

10 Substances which are listed in the Register are to be evaluated according to the evaluation programme
11 laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the
12 Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the
13 manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC,
14 2002b).

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

17 TERMS OF REFERENCE

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

23 In addition, in letter of 11 May 2009 the Commission requested EFSA to carry out a risk assessment
24 on cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no: 16.115] in
25 accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000a):

26 “The European Commission requests the European Food Safety Authority to carry out a safety
27 assessment on eighteen new flavouring substances in accordance with Commission Regulation (EC)
28 No 1565/2000 (EC, 2000a), if possible by the end of the authorisation programme, if not within nine
29 months from the finalisation of that programme.”

30 The deadline of the Terms of Reference for cyclopropanecarboxylic acid (2-isopropyl-5-methyl-
31 cyclohexyl)-amide [FL-no: 16.115] was negotiated to 31 May 2011.

32 The remaining 17 substances in the request of 11 May 2009 were evaluated in other FGEs.

33 ASSESSMENT

34 1. Presentation of the Substances in Flavouring Group Evaluation 300

35 1.1. Description

36 The present Flavouring Group Evaluation 300 (FGE.300), using the Procedure as referred to in the
37 Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure - shown in schematic form
38 in Annex I of this FGE), deals with one cyclo-aliphatic amide from chemical group 33, Annex I of
39 Commission Regulation (EC) No 1565/2000 (EC, 2000a). The flavouring substance,
40 cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no: 16.115] under
41 consideration, as well as the chemical Register name, FLAVIS- (FL-), Chemical Abstract Service-

1 (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-)
2 numbers, structure and specifications, are listed in Table 1.

3 The Panel is aware that there are several other amides in the Register considered in FGE.86 and
4 FGE.94 and evaluated in FGE.304 which show partly structural similarities to the candidate substance.
5 E.g. N-Ethyl-2-isopropyl-5-methylcyclohexane carboxamide [FL-no: 16.013] from FGE.86, N1-(2-
6 methoxy-4-methylbenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide [FL-no: 16.101], N-
7 [(Ethoxycarbonyl)methyl]-p-menthane-3-carboxamide [FL-no: 16.111] and N-3,7-dimethyl-2,6-
8 octadienyl cyclopropylcarboxamide [FL-no: 16.095]) from FGE.94, all evaluated by the JECFA and
9 considered by the Panel and N-p-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117] and N-
10 (2-(pyridine-2-yl)ethyl)-3-p-menthanecarboxamide [FL-no: 16.118] evaluated by the Panel in
11 FGE.304. Of these N-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide [FL-no: 16.095] shows the
12 best structural similarity with the candidate substance and will as the only one of the amides in the
13 Register be used to support the evaluation of the candidate substance in the present FGE.

14 The outcome of the Safety Evaluation is summarised in Table 2a.

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

16 The supporting substance is listed in Table 3, together with its evaluation status.

17 **1.2. Stereoisomers**

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

27 The candidate substance cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-
28 no: 16.115] possess three chiral centres and has been presented with specification of the stereoisomeric
29 composition of two main isomers, however, as Industry has informed that further four other
30 stereoisomers are present, the ratios of these isomers are needed.

31 **1.3. Natural Occurrence in Food**

32 The candidate substance [FL-no: 16.115] has not been reported to occur naturally in any food (TNO,
33 2009).

34 **2. Specifications**

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

37 Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC,
38 2000a), this information is adequate for the candidate substance except the composition of the
39 stereoisomeric mixture has to be specified.

1 3. Intake Data

2 Annual production volumes of the flavouring substances as surveyed by the Industry can be used to
3 calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production
4 figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU
5 population are consumers (SCF, 1999a).

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

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

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

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

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

28 3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

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

36 Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is
37 consumed by 10 % of the population⁴ (Eurostat, 1998). This is derived for candidate substances from
38 estimates of annual volume of production provided by Industry and incorporates a correction factor of
39 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

40 The total annual volume of production of the candidate substance in the present Flavouring Group
41 Evaluation (FGE.300) from use as flavouring substances in Europe has been reported to be

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.

1 approximately 25 kg (Flavour Industry, 2009h), and for the supporting substance to be approximately
 2 500 kg. The daily *per capita* intake for the candidate and supporting substances are 3.0 (Table 2a) and
 3 61 microgram (Table 3), respectively.

4 **3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)**

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

7 The assumption is that a person may consume a certain amount of flavourable foods and beverages per
 8 day.

9 For the candidate substance information on food categories and normal and maximum use levels^{5,6}.
 10 were submitted by the Flavour Industry (Flavour Industry, 2009h). The candidate substance is used in
 11 flavoured food products divided into the food categories, outlined in Annex III of the Commission
 12 Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of
 13 mTAMDI, the reported normal use levels were used. In the case where different use levels were
 14 reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of 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) | Yes |
| 03.0 | Edible ices, including sherbet and sorbet | No |
| 04.1 | Processed fruits | No |
| 04.2 | Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds | Yes |
| 05.0 | Confectionery | No |
| 06.0 | Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery | No |
| 07.0 | Bakery wares | No |
| 08.0 | Meat and meat products, including poultry and game | Yes |
| 09.0 | Fish and fish products, including molluscs, crustaceans and echinoderms | Yes |
| 10.0 | Eggs and egg products | Yes |
| 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 | No |
| 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 | No |

15
 16 According to the Flavour Industry the normal use levels for the candidate substance is in the range of
 17 0.2-10 mg/kg food, and the maximum use levels are in the range of 1.7-20 mg/kg (Flavour Industry,
 18 2009h).

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).

1 The mTAMDI value is 960 microgram/person/day for the candidate substance from structural class III
2 (see Section 5).

3 For detailed information on use levels and intake estimations based on the mTAMDI approach, see
4 Section 6 and Annex II.

5 **4. Absorption, Distribution, Metabolism and Elimination**

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

8 Simple aliphatic amides, such as formamide, acetamide, propionamide, n-butyramide and n-
9 valeramide were reported to undergo hydrolysis in rabbits after oral administration. The extent of
10 hydrolysis increased with increasing chain-length and ranged from 28 to 97 % of the dose. Complete
11 hydrolysis was reported for phenylacetamide in rabbits. For the aliphatic amides increased hydrolysis
12 was seen with increased chain-lengths following incubation with rabbit liver extracts and liver slices
13 (Bray et al., 1949).

14 Aliphatic and aromatic amides are expected to be partly metabolised to polar metabolites which are
15 eliminated in the urine or bile (James, 1974; Schwen, 1982). Hydrolysis of the amide bond has been
16 reported as a metabolic pathway for the amides dihydrocapsaicin and piperine *in vivo* in rats (Kawada
17 and Iwai, 1985; Bhat & Chandrasekhara, 1987).

18 Like other aliphatic and aromatic amides the candidate substance is anticipated to be absorbed from
19 the gastrointestinal tract and at least partly hydrolysed. However, due to the lack of specific
20 information on hydrolysis and metabolism and given the limited knowledge on hydrolysis of amides,
21 it cannot be anticipated that the candidate substance is metabolised to innocuous products.

22 For more detailed information, see Annex III.

23 **5. Application of the Procedure for the Safety Evaluation of Flavouring Substances**

24 The application of the Procedure is based on intakes estimated on the basis of the MSDI approach.
25 Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its
26 corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure.
27 In these cases the Panel requires more precise data on use and use levels. For comparison of the intake
28 estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

29 For the safety evaluation of the candidate substance from chemical group 33 the Procedure as outlined
30 in Annex I was applied, based on the MSDI approach. The stepwise evaluation of the substance is
31 summarised in Table 2a.

32 Step 1

33 The candidate substance cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-
34 no: 16.115] is classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978)
35 into structural class III.

36 Step 2

37 Step 2 requires consideration of the metabolism of the candidate substances. The candidate substance
38 [FL-no: 16.116], cannot be anticipated to be metabolised to innocuous products and thus the
39 evaluation proceeds via the B-side of the Procedure.

40 Step B3

1 The estimated daily *per capita* intake of the candidate substance [FL-no: 16.115] is 3.0 microgram,
 2 which is below the threshold for its structural class of 90 microgram/person/day (class III).
 3 Accordingly, the evaluation of the substance proceeds to step B4 of the Procedure.

4 Step B4

5 No appropriate toxicity study could be identified to provide a No Observed Adverse Effect Level
 6 (NOAEL) for the candidate substance cyclopropanecarboxylic acid (2-isopropyl-5-methyl-
 7 cyclohexyl)-amide [FL-no: 16.115]. For the supporting substance N-3,7-dimethyl-2,6-octadienyl
 8 cyclopropylcarboxamide [FL-no: 16.095] a NOAEL can be established from a 28-day feeding study in
 9 rats. However, the Panel does not accept the use of a 28-day study for deriving a NOAEL to be used in
 10 the Procedure. Accordingly, additional toxicity data are required.

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

13 The estimated intake for the candidate substance [FL-no: 16.115] assigned to structural class III, based
 14 on the mTAMDI, is 960 microgram/person/day, which is above the threshold of concern of 90
 15 microgram/person/day.

16 Thus, for the candidate substance [FL-no: 16.115] further information is required. This would include
 17 more reliable intake data and then, if required, additional toxicological data.

18 For comparison of the MSDI and mTAMDI values, see Table 6.1

19

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

| FL-no | Proposed name for Registration | MSDI ($\mu\text{g}/\text{capita}/\text{day}$) | mTAMDI ($\mu\text{g}/\text{person}/\text{day}$) | Structural class | Threshold of concern ($\mu\text{g}/\text{person}/\text{day}$) |
|--------|---|--|--|---------------------|--|
| 16.115 | Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide | 3.0 | 960 | Class III | 90 |

20 **7. Considerations of Combined Intakes from Use as Flavouring Substances**

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

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

31 The combined intake of the candidate and supporting substance, both from structural class III, in
 32 Europe is estimated to be 64 micorgram/capita/day (3 and 61 microgram/capita/day, respectively).
 33 This value is below the threshold of concern for a structural class III substance of 90
 34 microgram/person/day.

1 **8. Toxicity**

2 **8.1. Acute Toxicity**

3 Data available for the candidate and supporting substances reports that the oral LD₅₀ value, in rats, was
4 greater than 2000 mg/kg body weight (bw).

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

6 **8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies**

7 No data has been submitted, and no data on the candidate substance [FL-no: 16.115] was found when
8 the open literature was searched.

9 The supporting substance [FL-no: 16.095] has been tested in a 28-day study in rats.

10 Abstract cited from the JECFA (JECFA, 2008b):

11 “A 28-day dietary toxicity study was conducted in male and female CRL:CD (SD) IGS BR rats that
12 were fed a diet containing N-3,7-dimethyl-2,6-octadienylcyclopropylcarboxamide at concentrations of
13 0, 11, 110 or 1100 mg/kg. These dietary levels corresponded to measured daily intakes of 0, 0.92, 9
14 and 92 mg/kg bw and 0, 0.98, 10 and 97 mg/kg bw for males and females, respectively. Whereas 5
15 males and 5 females were included at the low- and mid-dose levels, the control and high-dose groups
16 consisted of 10 males and 10 females.

17 The test and control diets were presented to their respective groups on day 0 of the study. All the low-
18 and mid-dose animals and half of the control and high-dose animals (non-recovery groups 1–8) were
19 exposed to their test or control diet for at least 28 days, prior to necropsy on day 31. The remaining
20 control and high-dose animals (groups 9–12) constituted the recovery group and were exposed to the
21 test or control diet for 28 days, then were fed unsupplemented feed for an additional 14 days before
22 necropsy on day 45.

23 Prior to initial dosing and again on day 28, all rats were weighed and examined for visual impairment.
24 In addition, all animals were observed daily for general health, symptoms of toxicity and behavioural
25 changes. All rats were subjected to detailed weekly observation, including body weight and food
26 consumption. Functional observational battery and motor activity tests were performed on test groups
27 1–8 at week 4 post-initiation and on the recovery group animals at week 6. Blood for haematological
28 and clinical biochemistry analysis was collected from groups 1–8 at week 5 and from groups 9–12 at
29 week 7. Animals providing blood samples were fasted 24 hours prior to collection, and an urine
30 sample was also collected from each animal. At the conclusion of the test period, gross necropsies
31 were performed on all study rats, and selected organs and tissues were evaluated histologically in the
32 control and high-dose groups.

33 There were no test substance–related mortalities or clinical effects. A significant increase in food
34 efficiency was reported during an unspecified measurement interval in low-dose males compared with
35 controls; however, as this was transient and not dose related, it was not considered to be
36 toxicologically significant. Urinalysis revealed no significant findings for any of the test groups as
37 compared with controls. Haematology and clinical biochemistry revealed increases in mean cell
38 volume in the low-dose and non–recovery group high-dose females. Low-dose females also exhibited
39 an increase in mean cell haemoglobin compared with controls. Red blood cell counts for the 1100
40 mg/kg recovery group females were statistically significantly increased compared with control values,
41 but this occurred towards the end of the recovery period and therefore was not considered to be
42 associated with the administration of the test material. Sorbitol dehydrogenase levels were increased in
43 the 11 mg/kg and non–recovery group 1100 mg/kg males; however, this was attributed to the
44 unusually low levels of sorbitol dehydrogenase in control rats (Everds, 2005; Merkel, 2005).

1 Compared with controls, significant increases in the organ to body weight and the organ to brain
2 weight ratios of the thymus were reported in the high-dose non-recovery group males. In females, a
3 significant increase in the organ to body weight ratio of the liver was reported in the high-dose non-
4 recovery group compared with controls. Macroscopic examination revealed gross lesions of the liver,
5 lung, spleen, uterus, caecum, lymph nodes and kidneys in both sexes of animals and at varying dose
6 levels; however, incidence of these lesions did not reach statistical significance compared with
7 controls. Moreover, there were no underlying microscopic abnormalities associated with any of the
8 lesions, or the microscopic changes were considered to be incidental and unrelated to the presence of
9 the test material in the feed. Given the absence of any microscopic abnormalities related to the dietary
10 administration of the test material, the relative organ weight variations were determined to be
11 clinically irrelevant (Funk, 2005; Merkel, 2005).”

12 **8.3. Developmental / Reproductive Toxicity Studies**

13 No data has been submitted, and no data on the candidate substance [FL-no: 16.115] was found when
14 the open literature was searched.

15 **8.4. Genotoxicity Studies**

16 *In vitro* data are available for both the candidate and the supporting substance.

17 *Candidate substance [FL-no: 16.115]*

18 No genotoxic potential was observed when cyclopropanecarboxylic acid (2-isopropyl-5-methyl-
19 cyclohexyl)-amide [FL-no: 16.115] was incubated with *Salmonella typhimurium* strains TA98,
20 TA100, TA102, TA1535 and TA1537 with or without metabolic activation at concentrations up to
21 1000 µg/plate in two separate experiments using the plate incorporation method and the preincubation
22 method. The authors noted that in the plate incorporation method with and without metabolic
23 activation, the 1000 µg/plate concentration of the candidate substance was cytotoxic to the bacteria
24 (August, 2007).

25 *Supporting substance [FL-no: 16.095]*

26 N-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide [FL-no: 16.095] was tested in a bacterial
27 reverse mutation test using *S. typhimurium* strains TA97a, TA98, TA100 and TA1535 and *E. coli*
28 strain WP2uvrA with and without metabolic activation. It was concluded to be negative for the
29 induction of mutagenicity (Next Century Incorporated, 2004).

30 The results from the available limited genotoxicity studies do not raise a concern for genotoxicity and
31 hence do not preclude the evaluation of the candidate substance in this FGE through the Procedure.

32 Genotoxicity data are summaries in Annex IV, Table IV.4.

33 **9. Conclusions**

34 The candidate substance cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-
35 no: 16.115] possesses three chiral centres and has been presented with specification of the
36 stereoisomeric composition.

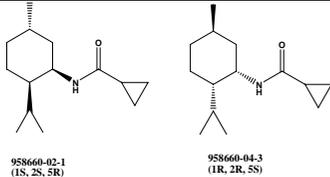
37 The substance is assigned into structural class III, according to the decision tree approach presented by
38 Cramer et al., 1978.

39 The substance in the present group has not been reported to occur naturally in food.

- 1 According to the default MSDI approach, the flavouring substance in this group has a total intake in
2 Europe of 3 microgram/*capita*/day, which is below the threshold of concern value for structural class
3 III of 90 microgram/person/day.
- 4 The results from the available limited genotoxicity studies do not raise a concern for genotoxicity and
5 hence do not preclude the evaluation of the candidate substance in this FGE through the Procedure.
- 6 From the data available it is not possible to conclude that the candidate substance in this group
7 cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no. 16.115] would be
8 metabolised to innocuous products at the reported levels of intake as flavouring substance. Therefore
9 the substance was evaluated along the B-side of the Procedure. No toxicity study is available on the
10 candidate or on the supporting substance that can provide an adequate NOAEL to be used in the
11 Procedure, accordingly additional toxicity data are required for the candidate substance or a
12 structurally related substance.
- 13 When the estimated intake was based on the mTAMDI approach it was 960 microgram/person/day for
14 this flavouring substance belonging to structural class III. The estimated intake for the candidate
15 substance is above the threshold of concern of 90 microgram/person/day. Thus, for the flavouring
16 substance considered in this opinion the intake, estimated on the basis of the mTAMDI, exceed the
17 relevant threshold for the structural class, to which the flavouring substance has been assigned.
18 Therefore, for the substance more reliable exposure data is required. On the basis of such additional
19 data, the flavouring substance should be reconsidered along the steps of the Procedure. Following this
20 procedure additional toxicological data might become necessary.
- 21 In order to determine whether the conclusion for the candidate substance can be applied to the
22 materials of commerce, it is necessary to consider the available specifications. Specifications including
23 purity criteria and identity for the materials of commerce have been provided for the flavouring
24 substance. However, the composition of the stereoisomeric mixture has to be specified.
- 25 In conclusion, for the flavouring substance cyclopropanecarboxylic acid (2-isopropyl-5-methyl-
26 cyclohexyl)-amide [FL-no: 16.115] the Panel considered that additional data are needed. Information
27 on composition of isomers is missing.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 300

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 300

| FL-no | Proposed name for Registration | Structural formula | FEMA no CoE no CAS no | Phys.form Mol.formula Mol.weight | Solubility 1) Solubility in ethanol 2) | Boiling point, °C 3) Melting point, °C ID test Assay minimum | Refrac. Index 4) Spec.gravity 5) | Specification comments |
|--------|---|---|-----------------------------|---|--|--|---|---|
| 16.115 | Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide |  <p>958660-02-1 (1S, 2S, 5R)</p> <p>958660-04-3 (1R, 2R, 5S)</p> | 4558 958660-02-1 | Solid C ₁₄ H ₂₅ NO 223.36 | Insoluble Soluble | 166 MS 98 % | n.a. n.a. | Two CASrn assigned 958660-02-1 (1S,2S,5R) and 958660-04-3 (1R,2R,5S). Min assay is sum of isomers: Two main isomers and four other stereoisomers. Composition of mixture to be specified. |

1) Solubility in water, if not otherwise stated.

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

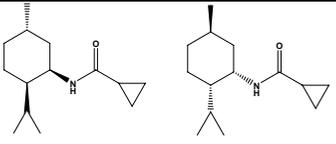
3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

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

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

| FL-no | Proposed name for Registration | Structural formula | MSDI 1) ($\mu\text{g}/\text{capita}/\text{day}$) | Class 2) Evaluation procedure path 3) | Outcome on the named compound [4) or 5] | Outcome on the material of commerce [6), 7), or 8)] | Evaluation remarks |
|--------|---|---|---|---|--|--|--------------------|
| 16.115 | Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide |  <p>958660-02-1 (1S, 2S, 5R)</p> <p>958660-04-3 (1R, 2R, 5S)</p> | 3.0 | Class III B3: Intake below threshold, B4: No adequate NOAEL | Additional data required | 7 | |

1) EU MSDI: Amount added to food as flavour in (kg / year) $\times 10E9 / (0.1 \times \text{population in Europe} (= 375 \times 10E6) \times 0.6 \times 365) = \mu\text{g}/\text{capita}/\text{day}$.

2) Thresholds of concern: Class I = 1800 $\mu\text{g}/\text{person}/\text{day}$, Class II = 540 $\mu\text{g}/\text{person}/\text{day}$, Class III = 90 $\mu\text{g}/\text{person}/\text{day}$.

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

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

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

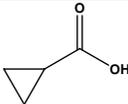
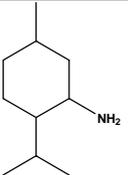
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

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

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

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

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

| FL-no | EU Register name JECFA no | Structural formula | SCF status 1) JECFA status 2) CoE status 3) EFSA status | Structural class 4) Procedure path (JECFA) 5) | Comments |
|-------------|-------------------------------------|---|--|--|---|
| Not in Reg. | Cyclopropanecarboxylic acid |  | Not evaluated as a flavour | - | Not evaluated as a flavouring substance |
| Not in Reg. | 2-Isopropyl-5-methyl-cyclohexylamin |  | Not evaluated as a flavour | - | Not evaluated as a flavouring substance |

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.

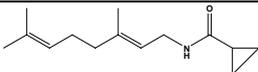
3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

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

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

| FL-no | Chemical name | Structural formula | FEMA no CoE no CAS no | JECFA no Specification available | MSDI (EU) 1) (µg/capita/day) | SCF status 2) JECFA status 3) CoE status 4) | Comments |
|--------|--|---|-----------------------------|-------------------------------------|---------------------------------|---|----------|
| 16.095 | N-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide |  | 4267 744251-93-2 | 1779 | 61 | No safety concern a) | |

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, 2008b).

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 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

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

- can the flavourings be predicted to be metabolised to innocuous 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

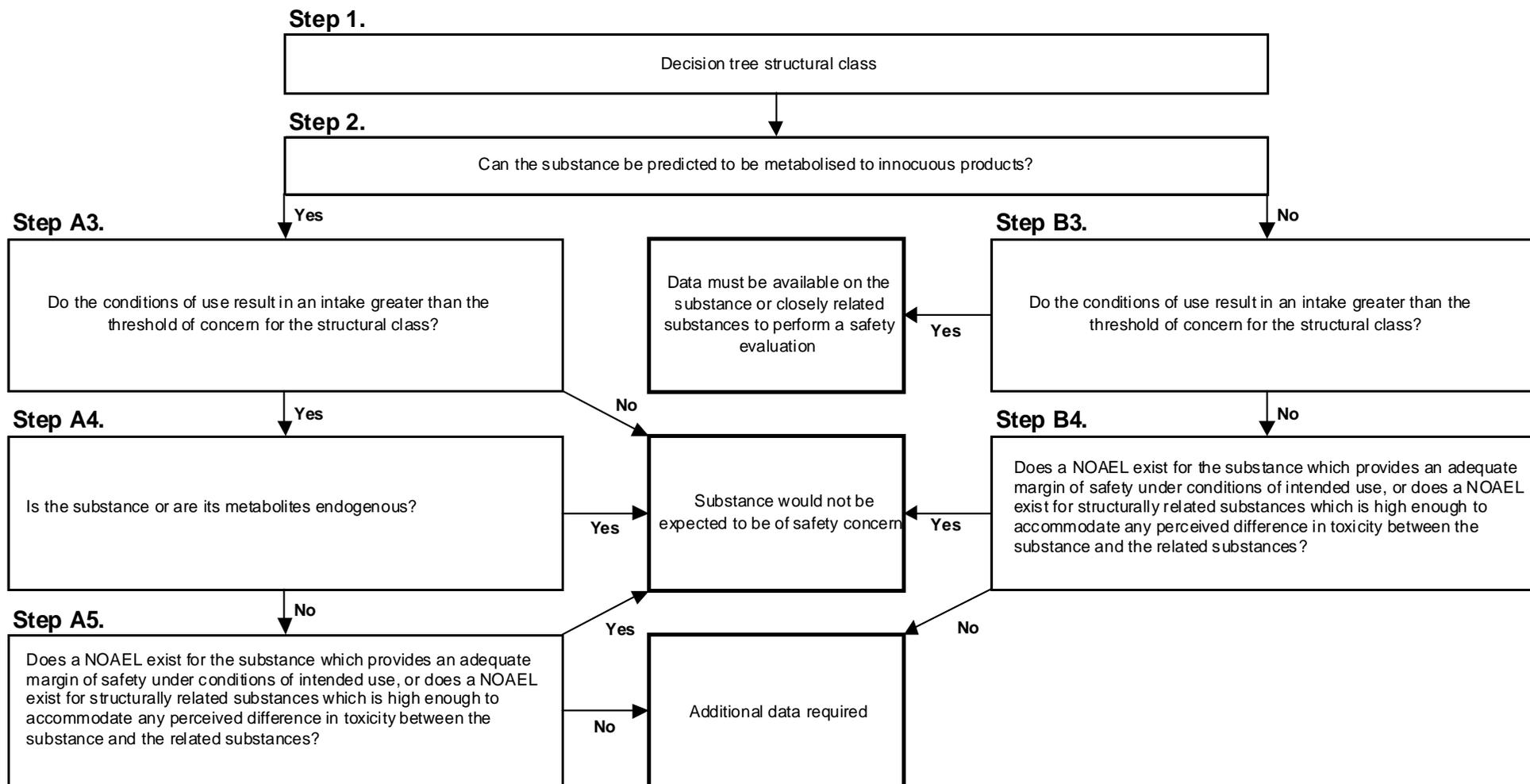


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances.

1 **ANNEX II: USE LEVELS / mTAMDI**

2 **II.1 Normal and Maximum Use Levels**

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

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

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

8

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

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.300 (Flavour Industry, 2009h)

| 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 |
| 16.115 | 2 | 2 | - | - | 2 | - | - | - | 2 | 2 | 4 | - | 10 | - | 0,2 | - | 8 | - |
| | 6 | 8 | - | - | 8 | - | - | - | 8 | 8 | 10 | - | 20 | - | 1,7 | - | 20 | - |

11 **II.2 mTAMDI Calculations**

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

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

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

1

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

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

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

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

14

1 The mTAMDI value (see Table II.2.3) is presented for the flavouring substance in the present flavouring
 2 group (Flavour Industry, 2009h). The mTAMDI value is only given for the highest reported normal use
 3 levels.

Table II.2.3 Estimated intakes based on the mTAMDI approach

| FL-no | EU Register name | mTAMDI (µg/person/day) | Structural class | Threshold of concern (µg/person/day) |
|--------|---|---------------------------|------------------|---|
| 16.115 | Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide | 960 | Class III | 90 |

4

1 ANNEX III: METABOLISM

2 III.1. Introduction

3 The present FGE consists of one cyclo-aliphatic amide from chemical group 33: the candidate substance
4 cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no: 16.115].

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

7 III.2. Absorption, Distribution, Metabolism and Excretion (ADME)

8 The JECFA (2006) in its evaluation of a group of aliphatic and aromatic amines and amides used as
9 flavouring substances evaluated the ADME of a few amides.

10 The following text on absorption, distribution and elimination of amides is taken from the JECFA (JECFA,
11 2006a):.

12 “Studies on selected members of the group indicate that amides *per se* are rapidly absorbed and
13 metabolised”.

14 “Male Sprague-Dawley rats given a single oral dose of 4 mg/kg bw of *N*-(vanillyl)-[1-¹⁴C]nonamide
15 (nonanyl 4-hydroxy-3-methoxybenzylamide) excreted 17.9%, 45.9% and 22.7% of the radiolabel in the
16 urine, faeces and expired CO₂, respectively, within 72 hours, although most of the radiolabel was excreted
17 within the first 24 hours. Bile duct-cannulated rats excreted 11.4%, 3.7%, 11.7% and 65.1% of the radiolabel
18 in the urine, faeces, expired CO₂ and bile, respectively. In fasted rats, peak blood levels of radiolabel
19 occurred 10 minutes after administration. By 72 hours after dosing, the highest concentration of radiolabel
20 was found in fat, liver and adrenal gland. These results indicate that nonanyl 4-hydroxy-3-
21 methoxybenzylamide is rapidly absorbed and that appreciable quantities undergo enterohepatic circulation
22 and partial conversion to CO₂ (Schwen, 1982)”.

23 “Groups of male albino Wistar rats were given piperine at a dose of 170 mg/kg bw by gavage or 85 mg/kg
24 bw by intraperitoneal injection, and urine and faeces were collected every 24 hours for 12 days. Urine and
25 faeces from rats fed a control diet for 10 days were collected for 3 days before treatment and used as control
26 samples. When given by either route, about 3% of the unchanged dose was detected in faeces over 5 days,
27 indicating that 97% of the piperine was absorbed. Peak excretion in the faeces occurred on day 1 after
28 intraperitoneal injection and on 3 days after gavage. No unchanged piperine was detected in urine after
29 administration by either route; however, there was increased excretion of conjugated glucuronides, sulphates
30 and phenols, with maxima on days 1-4. Overall, 91-97% of the administered dose was accounted for. After
31 treatment the animals were killed at various intervals, when blood was collected from the heart, and the liver,
32 kidney, spleen and gut (stomach, small intestine, caecum and large intestine) were removed. By 30 minutes
33 after ingestion of piperine, 29% was detected in the gut (22% in stomach and 6% in small intestine). By 48
34 hours, 1% was detected in stomach, and 2-3% in the caecum and large intestine, indicating that 97% had
35 been absorbed. A similar pattern was reported in rats intraperitoneally injected with piperine, although some
36 of the values differed (data not reported). Between 1 and 10 hours after treatment, only traces of piperine
37 administered by either route were detected in blood. Between 0.5 and 24 hours after treatment,
38 intraperitoneally administered piperine was detected in the liver (2.12-0.4%) and kidney (0.04-0.2%).
39 Similarly, orally administered piperine was detected in the liver (0.25-0.12%) and kidney (0.03-0.17%) up to

1 24 hours after treatment. No piperine was detected after 48 hours in any of the tissues examined (Bhat and
2 Chandrasekhara, 1986a)".

3 "A group of male albino Wistar rats were given 175 mg/kg bw piperine by gavage. After 1 hour, some of the
4 rats, including a group of untrated rats that served as controls, received a bile duct cannula, and bile was
5 collected for 6 hours. Urine was collected from the remaining rats for 4 days and pooled, while urine
6 collected for 4 days before dosing served as control samples. No unchanged piperine was detected in urine.
7 Piperine was detected in the bile (about 1% of the original dose) within 6 hours, and various metabolites
8 (piperonylic acid, vanillic acid and piperonyl alcohol) were excreted in urine (about 15.5% of the original
9 dose) within 96 hours (Bhat & Chandrasekhara, 1987)".

10 "In rats given a single oral dose (not specified) of *N*-ethyl-para-menthane-[3-¹⁴C]-carboxamide (*N*-ethyl-2-
11 isopropyl-5-methylcyclohexanecarboxamide), 64.2% and 28.7% of the dose was excreted in urine and
12 faeces, respectively, over 5 days. Almost 50% of the radioactivity was secreted into the bile within 2 days,
13 most within 24 hours, indicating enterohepatic circulation of the parent compound or its metabolites. The
14 peak plasma concentration (0.3% of the total dose) was reached within 1 hour. Subsequently, the compound
15 was eliminated with a half-life of 11 hours. Whole-body autoradiography showed that most of the
16 radioactivity was in the liver, kidneys and gastrointestinal tract. The results indicate that the substance was
17 rapidly and extensively converted into more polar metabolites of unknown structure (James, 1974)".

18 The following text on metabolism, including hydrolysis, of amides is taken from JECFA (JECFA, 2006a):

19 "The metabolic fate of *N*-ethyl-para-menthane-[3-¹⁴C]-carboxamide was examined in one male and one
20 female dog given a single dose oral dose of 10 mg/kg bw. The substance was readily absorbed and rapidly
21 eliminated in the urine (72% of the dose within the first 24 hours) and faeces (11% of the dose within 5
22 days). No parent compound was detected in the urine. The main urinary metabolites were glucuronide or
23 sulphate conjugates, whereas the faeces contained mainly unchanged compound. Radioactivity was detected
24 (detection limit= 0.05 ppm) in the liver, adrenal glands (male only), testes and kidney (female only) 5 days
25 after treatment. Peak plasma levels were reached within 4 hours. Subsequently the compound was eliminated
26 with a half-life of about 70 minutes. Plasma radioactivity was determined to consist mostly (> 90%) of
27 metabolites of the test substance. About 70% was bound to plasma protein *in vitro*, but < 10% of the
28 radioactivity was protein-bound *in vivo*. The author noted that rats metabolised the test substance to polar
29 unconjugated metabolites, while dogs metabolised it to conjugates; however, both species metabolised it
30 extensively and eliminated it rapidly (James, 1974)".

31 "These studies indicate that the amides in the group of flavouring agents are quickly absorbed, metabolised
32 and excreted, mainly in urine but also partly in the faeces".

33 "Aliphatic amides have been reported to undergo limited hydrolysis. Extensive hydrolysis of aliphatic
34 amides of various lengths was observed after incubation with rabbit liver extracts; however, hydrolysis was
35 significantly slower for aliphatic amides with fewer than five or more than 10 carbons (Bray et al., 1949)".

36 "After administration of 1.5-5.0 g of acetamide or butyramide to rabbits, 62% of the dose of acetamide was
37 recovered unchanged in the urine within 24 hours, while only 13% of the butyramide dose was recovered
38 unchanged".

39 "Studies in which rats were given an oral dose (170 mg/kg bw) of piperine or dogs were given an oral dose
40 (10 mg/kg bw) of *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide indicated that amide hydrolysis
41 products are not major metabolites of these compounds (James, 1974; Bhat & Chandrasekhara, 1986a)".

42 "The metabolism of piperine was studied in groups of male albino Wistar rats given a dose of 170 mg/kg bw
43 by gavage or 85 mg/kg bw by intraperitoneal injection. Urine and faeces were collected every 24 hours for
44 12 days, while control urine and faeces samples were collected for 3 days from rats fed a control diet before

1 dosing. No unchanged piperine was detected in urine after exposure by either route; however, there was
 2 increased excretion of conjugated glucuronides, sulphates and phenols, with maximum excretion of all three
 3 on days 1-4. Demethylation of piperine was suggested by an increase in conjugated phenols. Over 8 days,
 4 about 36% of the gavage dose was excreted in urine as conjugated phenols and 62% as
 5 methylenedioxyphenyl metabolites. About 19% of the intraperitoneal dose was excreted as phenolics and
 6 about 72% as methylenedioxyphenyl derivatives (Bhat & Chandrasekhara, 1986a). The proposed pathways
 7 for the metabolism of piperine in rats involved in addition of amide hydrolysis to piperic acid, metabolic
 8 oxidative cleavage of the benzylic alkene function results in a series of vanilloyl and piperonyl derivatives,
 9 which are excreted free of in conjugated form, mainly in the urine (Bhat & Chandrasekhara, 1987)".

10 The panel, in addition to the studies identified by the JECFA, also retrieved the following study by Kawada
 11 and Iwai (1985) in which the metabolism in rats of dihydrocapsaicin was investigated *in vivo* and *in vitro*:
 12 Within 48 hours after oral administration of dihydrocapsaicin (20 mg/kg bw) to male adult rats, unchanged
 13 dihydrocapsaicin and eight of its metabolites were identified in urine; i.e. dihydrocapsaicin (8.7 % of total
 14 dose), vanillylamine (4.7 %), vanillin (4.6 %), vanillyl alcohol (37.6 %) and vanillic acid (19.2 %) as free
 15 forms and/or their glucuronides. The proportions of free and and glucuronide metabolites in urine 14.5 %
 16 and 60.5 % of the total dose. Cell-free extracts of rat liver catalysed the hydrolysis of dihydrocapsaicin to
 17 vanillylamine and 8-methyl nonanoic acid. The former compound was further transformed to vanillin *in situ*.
 18 Dihydrocapsaicin-hydrolyzing enzyme activity was found in various organs of rats. The activity was located
 19 mainly in the liver (Kawada & Iwai, 1985).

20 The Panel also had a further look at the study performed by Bray et al. (1949) and concluded that simple
 21 aliphatic amides, such as formamide, acetamide, propionamide, n-butyramide and n-valeramide were
 22 reported to undergo hydrolysis in rabbits after oral administration. The extent of hydrolysis increased with
 23 increasing chain-length and ranged from 28 to 97 % of the dose. Complete hydrolysis was reported for
 24 phenylacetamide in rabbits. For the aliphatic amides, increased hydrolysis was seen with increased chain-
 25 lengths following incubation with rabbit liver extracts and liver slices (Bray et al., 1949).

26 III.3. Summary and Conclusions

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

29 Simple aliphatic amides, such as formamide, acetamide, propionamide, n-butyramide and n-valeramide were
 30 reported to undergo hydrolysis in rabbits after oral administration. The extent of hydrolysis increased with
 31 increasing chain-length and ranged from 28 to 97 % of the dose. Complete hydrolysis was reported for
 32 phenylacetamide in rabbits. For the aliphatic amides, increased hydrolysis was seen with increased chain-
 33 lengths following incubation with rabbit liver extracts and liver slices (Bray et al., 1949).

34 Aliphatic and aromatic amides are expected to be readily absorbed and partly metabolised to polar
 35 metabolites, which are eliminated in the urine or bile (James, 1974; Schwen, 1982). Hydrolysis of the amide
 36 bond has been reported as a metabolic pathway for the amides dihydrocapsaicin and piperine *in vivo* in rats
 37 (Kawada & Iwai, 1985; Bhat & Chandrasekhara, 1987).

38 In summary, like other aliphatic and aromatic amides, the candidate substance is anticipated to be absorbed
 39 from the gastrointestinal tract and at least partly hydrolysed. However, due to the lack of specific information
 40 on hydrolysis and metabolism and given the limited knowledge on hydrolysis of amides in general, it cannot
 41 be anticipated that the candidate substance is metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for the candidate substance and the supporting substance of the present Flavouring Group Evaluation.

TABLE IV.1: ACUTE TOXICITY

| Chemical Name [FL-no] | Species | Sex | Route | LD ₅₀ (mg/kg bw) | Reference | Comments |
|---|---------|-----|--------|--------------------------------|----------------|----------|
| Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no: 16.115] | Rat | F | Gavage | > 2000 | (Vaeth, 2007) | |
| (N-3,7-Dimethyl-2,6- Octadienylcyclopropylcarboxamide [FL-no: 16.095]) | Rat | | | > 2000 | (Merkel, 2004) | |

M = Male; F = Female; NR = Not reported.

Subacute / subchronic / chronic / carcinogenic toxicity data are not available for the candidate substance but one study is available for the supporting substance (JECFA, 2008b).

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

| Chemical Name [FL-no] | Species; Sex ² No./Group ³ | Route | Dose levels | Duration (days) | NOAEL (mg/kg bw/day) | Reference | Comments |
|--|---|-------|---|--|-------------------------|-----------------|----------|
| (N-3,7-Dimethyl-2,6- Octadienylcyclopropylcarboxamide [FL-no: 16.095]) | M; F 5/the two mid doses 10/control and high dose | diet | 0, 0.92, 9 and 92 (M) 0, 0.98, 10 and 97 (F) | 28 (unsupplemented feed for add. 14 days) | 92 | (Merkel, 2005b) | |

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No Developmental and reproductive toxicity data are available neither for the candidate substance nor for structurally related substances of the present Flavouring Group Evaluation.

In vitro mutagenicity/genotoxicity data are available for the candidate substance of the present Flavouring Group Evaluation and for the one supporting substance.

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

| Chemical Name [FL-no] | Test System | Test Object | Concentration | Result | Reference | Comments |
|---|-------------|--|---------------------|-----------------------|-----------------------------------|----------|
| Cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide [FL-no: 16.115] | Ames test | <i>S. typhimurium</i> TA98; TA100; TA102; TA1535; TA1537 | 1000 µg/plate | Negative ¹ | (August, 2007) | |
| (N-3,7-Dimethyl-2,6-octadienylcyclopropylcarboxamide [FL-no: 16.095]) | Ames test | <i>S. typhimurium</i> TA97a, TA98; TA100; TA1535 <i>E. Coli</i> WP2uvra | Up to 5000 µg/plate | Negative ¹ | (Next Century Incorporated, 2004) | |

¹ With and without metabolic activation.

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

No *In vivo* mutagenicity/genotoxicity data are available neither for the candidate substance nor for structurally related substances of the present Flavouring Group Evaluation.

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ABBREVIATIONS

| | |
|------------------|--|
| ADI | Acceptable Daily Intake |
| ADME | Absorption, Distribution, Metabolism and Excretion |
| BW | Body weight |
| CAS | Chemical Abstract Service |
| CEF | Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service |
| CHO | Chinese hamster ovary (cells) |
| CoE | Council of Europe |
| DNA | Deoxyribonucleic acid |
| EC | European Commission |
| EFSA | The European Food Safety Authority |
| EU | European Union |
| FAO | Food and Agriculture Organization of the United Nations |
| FEMA | Flavor and Extract Manufacturers Association |
| FGE | Flavouring Group Evaluation |
| FLAVIS (FL) | Flavour Information System (database) |
| ID | Identity |
| IOFI | International Organization of the Flavour Industry |
| IR | Infrared spectroscopy |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| LD ₅₀ | Lethal Dose, 50%; Median lethal dose |
| MS | Mass spectrometry |
| MSDI | Maximised Survey-derived Daily Intake |
| mTAMDI | Modified Theoretical Added Maximum Daily Intake |
| NAD | Nicotinamide Adenine Dinucleotide |
| NADP | Nicotinamide Adenine Dinucleotide Phosphate |
| No | Number |
| NOAEL | No Observed Adverse Effect Level |
| NOEL | No Observed Effect Level |
| NTP | National Toxicology Program |
| SCE | Sister Chromatid Exchange |
| SCF | Scientific Committee on Food |
| SMART | Somatic Mutation and Recombination Test |
| TAMDI | Theoretical Added Maximum Daily Intake |
| UDS | Unscheduled DNA Synthesis |
| WHO | World Health Organisation |