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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 304 (FGE.304):

Five carboxamides from chemical group 30¹

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 five flavouring substances in the Flavouring Group Evaluation 304, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The substances were 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 the three substances [FL-no: 16.117, 16.123 and 16.125] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For the remaining two candidate substances [FL-no: 16.118 and 16.124], no appropriate NOAEL was available and additional data are required. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all five candidate substances.

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KEYWORDS

FGE.304, carboxamides, flavourings, food safety.

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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 asked to evaluate five flavouring substances in the Flavouring Group Evaluation 304, using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These five carboxamides [FL-no: 16.117, 16.118, 16.123, 16.124 and 16.125] (see Table 1) belong to chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The five flavouring substances possess chiral centres. All substances have been presented with specification of the stereoisomeric composition.

All candidate substances were assigned to structural class III, according to the decision tree approach presented by Cramer et al., 1978.

None of the candidate substances have been reported to occur naturally.

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.

Genotoxicity data are available for three substances. The Panel concluded that the data available do not give rise to safety concern with respect to genotoxicity for any of the candidate substances.

On the basis of the available data, the candidate substances are not expected to be hydrolysed. Owing to the lack of further data, the candidate substances cannot be anticipated to be metabolised to innocuous products.

According to the default MSDI approach, the four flavouring substances [FL-no: 16.118, 16.123, 16.124 and 16.125] in this group have intakes in Europe from 6.1 to 61 micrograms/*capita*/day, which are below the threshold of concern value for structural class III of 90 micrograms/person/day. For one substance [FL-no: 16.117], the intake of 120 microgram/*capita*/day is above the threshold of concern. However, an adequate NOAEL of 100 microgram/kg bw/day exists from a 90-day study with this candidate substance [FL-no: 16.117], which provides a margin of safety of 50000. This substance is structurally related to the two substances [FL-no: 16.123 and 16.125] for which a margin of safety of 3.3×10^5 , based on the combined estimated daily *per capita* intake, can be calculated. Therefore, the three substances [FL-no: 16.117, 16.123 and 16.125] are not anticipated to pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach. No NOAEL could be derived for the remaining two candidate substances [FL-no: 16.118 and 16.124] or

structurally related substances. Accordingly, further data are required for these two candidate substances.

When the estimated intakes were based on the mTAMDI approach, they ranged from 210 to 11000 microgram/person/day for the five flavouring substances belonging to structural class III. These intakes are above the threshold of concern of 90 microgram/person/day for structural class III substances. Therefore, for these five substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances 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 five flavouring substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all the flavouring substances. Thus, the final evaluation of the materials of commerce can be performed for all five substances.

In conclusion, for two substances [FL-no: 16.118 and 16.124], additional toxicity data are requested. For the remaining three substances [FL-no: 16.117, 16.123 and 16.125], the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

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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 2008/163/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 AS PROVIDED BY THE COMMISSION

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

The Commission requested EFSA to carry out an evaluation of the flavouring substances *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117], *N*-(2-(pyridine-2-yl)ethyl)-3-*p*-menthanecarboxamide [FL-no: 16.118], (1*R*,2*S*,5*R*)-*N*-(4-methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide [FL-no: 16.123], (1*R*,2*S*,5*R*)-*N*-cyclopropyl-5-methyl-2-isopropyl cyclohexanecarboxamide [FL-no: 16.124] and (2*S*,5*R*)-*N*-[4-(2-amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)cyclohexanecarboxamide [FL-no: 16.125], also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 304

1.1. Description

The present Flavouring Group Evaluation (FGE), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I), deals with five substances, *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117], *N*-(2-(pyridine-2-yl)ethyl)-3-*p*-menthanecarboxamide [FL-no: 16.118], (1*R*,2*S*,5*R*)-*N*-(4-methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide [FL-no: 16.123], (1*R*,2*S*,5*R*)-*N*-cyclopropyl-5-methyl-2-isopropyl cyclohexanecarboxamide [FL-no: 16.124] and (2*S*,5*R*)-*N*-[4-(2-amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)cyclohexanecarboxamide [FL-no: 16.125]. These flavouring substances (candidate substances) belong to chemical group 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). Three of the candidate substances [FL-no: 16.117, 16.123 and 16.125] are structurally related, the two others are not.

The five flavouring substances under consideration, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

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

The Panel is aware that there are three amides in the Register, (*N*-ethyl-2-isopropyl-5-methylcyclohexane carboxamide [FL-no: 16.013] (JECFA evaluated and considered in FGE.86), *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide [FL-no: 16.101] and *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111] (both JECFA evaluated and considered in FGE.94), showing partial structural similarity with the candidate substances in this FGE. However, these are not considered sufficiently structural similar and accordingly are not used as supporting substances for the candidate substances in the present FGE.

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 five candidate substances possess chiral centres. The substances have been presented with specification of the stereoisomeric composition. See Table 1.

1.3. Natural Occurrence in Food

None of the candidate substances have been reported to occur naturally (TNO, 2012).

2. Specifications

Purity criteria for the five substances have been provided by the Flavouring Industry (Flavour Industry, 2008d; Flavour Industry, 2008e; Flavour Industry, 2009u; Flavour Industry, 2010l) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the information is adequate for the candidate substances.

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, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995a). 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 total annual volume of production of the five candidate substances from use as flavouring substances in Europe is approximately 1700 kg (Flavour Industry, 2008d; Flavour Industry, 2008e; Flavour Industry, 2009u; Flavour Industry, 2010l).

On the basis of the annual volumes of production reported for the five candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated. The estimated daily *per capita* intakes of the substances from use as a flavouring substance will be: 120 microgram/day for *N*-*p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117], 61 microgram/day for *N*-(2-(pyridine-2-yl)ethyl)-3-*p*-menthanecarboxamide [FL-no: 16.118] and below 12 microgram/day for the remaining substances (Table 2).

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

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

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.

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

For the five candidate substances, information on food categories and normal and maximum use levels⁵ were submitted by the Flavour Industry (Flavour Industry, 2008d; Flavour Industry, 2008e; Flavour Industry, 2009u; Flavour Industry, 2010l). The five candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances in Various Food Categories *

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	Only [FL-no: 16.123]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Only [FL-no: 16.123]
03.0	Edible ices, including sherbet and sorbet	Only [FL-no: 16.123]
04.1	Processed fruits	All except [FL-no; 16.117 and 16.118]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no; 16.124 and 16.125]
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Only [FL-no: 16.123]
07.0	Bakery wares	Only [FL-no: 16.123]
08.0	Meat and meat products, including poultry and game	None
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	None
10.0	Eggs and egg products	Only [FL-no: 16.123]
11.0	Sweeteners, including honey	Only [FL-no: 16.123]
12.0	Salts, spices, soups, sauces, salads, protein products etc.	Only [FL-no: 16.123]
13.0	Foodstuffs intended for particular nutritional uses	None
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	Only [FL-no: 16.123]
15.0	Ready-to-eat savouries	None
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	None

* All candidate substances are also used in chewing gum.

According to the Flavour Industry the normal use levels for the five candidate substances are in the range of 0.5 - 220 mg/kg food and the maximum use levels are in the range of 1 - 800 mg/kg (Flavour Industry, 2008d; Flavour Industry, 2008e; Flavour Industry, 2009u; Flavour Industry, 2010l).

All five candidate substances are also used in chewing gum, which is not covered by any of the above food categories. Normal/maximum use levels for chewing gum are 200/800 mg/kg for [FL-no: 16.117], 100/300 mg/kg for [FL-no: 16.118], 30/300 mg/kg for [FL-no: 16.123], 1200/4000 mg/kg for [FL-no: 16.124] and 400/800 mg/kg for [FL-no: 16.125].

For the substances [FL-no: 16.117 and 16.118], the Industry has informed that only 10 % of the amount added is released from the chewing gum (Sostmann, 2006). For [FL-no: 16.124 and 16.125] there is a release of 25.4 % and 10.5 %, respectively (Flavour Industry, 2009u). For the remaining substance [FL-no: 16.123] there is no information on % release. Taking these % releases and an intake estimate of 2 g chewing gum/day into consideration, the mTAMDI of the candidate substances is

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)

calculated based on the 16 food categories and the use of chewing gum. These figures are presented in Tables II.2.3 and 6.1.

The mTAMDI values for the five candidate substances from structural class III range from 210 to 11000 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

The hydrolysis of [¹⁴C]-*N-p*-benzenenitrile-menthanecarboxamide [FL-no: 16.117] was studied in rat and human hepatic microsomes (Sipes and Kong, 2012). As a positive control the hydrolysis of isoeugenol acetate, a known substrate of carboxyl esterase, was used. The results show that metabolically active male rat or human microsomes did not hydrolyse *N-p*-benzenenitrile-menthanecarboxamide [FL-no: 16.117].

The possible release of cyanide from the candidate substance *N-p*-benzenenitrile-menthanecarboxamide [FL-no: 16.117] during metabolism was studied in rat and human hepatocytes. Incubations of up to 250 µM of the candidate substance with human or rat hepatocytes for up to 4 hours only resulted in release of low amounts, if any, of cyanide. Proper positive control incubations with benzyl nitrile and sodium cyanide were included in the study (Wolff and Skibbe, 2007).

Specific information regarding absorption, distribution, metabolism and excretion is not available for the remaining four candidate substances. The candidate aromatic amides are anticipated to being absorbed from the gastrointestinal tract like other aromatic amides. Aromatic amides are expected to be metabolised to polar metabolites which are eliminated in the urine or bile (James, 1974; Schwen, 1982). No absorption data are available for the non-aromatic amide (1*R*,2*S*,5*R*)-*N*-Cyclopropyl-5-methyl-2-isopropyl cyclohexanecarboxamide [FL-no: 16.124] or structurally substances.

The hydrolysis of a substance with partial structure similarity to [FL-no: 16.117] from FGE.94Rev1, *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111], was studied in artificial pancreatic juice and rat liver homogenate (Poet et al., 2005). Based on the disappearance of the employed substrate, [FL-no: 16.111] was hydrolysed in artificial pancreatic juice with a half-life of 43 ± 14.7 min. and a first order rate constant (*K*) of 1.06 ± 0.426 hr⁻¹. In 20 fold-diluted liver homogenate the disappearance of [FL-no: 16.111] was considerably faster (half-life: 0.802 ± 0.191 min.). However, the potential hydrolysis products, *p*-menthane-3-carboxylic acid, glycine ethylester and glycine, were only detected at trace levels. This indicates that the disappearance of [FL-no: 16.111] under the employed *in vitro*-conditions is due to the hydrolysis of the ethyl ester bond rather than the hydrolysis of the amide bond.

Data on the candidate substance [FL-no: 16.117] and another carboxamide [FL-no: 16.111] demonstrate that there is no hydrolysis of the amide bond under the *in vitro* conditions. Owing to the lack of further data, the candidate substances cannot be anticipated to be metabolised to innocuous products.

For more detailed information, see Annex III.

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 five candidate substances from chemical group 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2.

Step 1

All five candidate substances are classified in structural class III according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

Step 2 requires consideration of the metabolism of the candidate substances. The five candidate substances [FL-no: 16.117, 16.118, 16.123, 16.124 and 16.125] 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 five candidate substances are allocated to structural class III. Four of the candidate substances [FL-no: 16.118, 16.123, 16.124 and 16.125] have estimated European daily *per capita* intakes (MSDI) ranging from 6.1 to 61 microgram (Table 2). These intakes are below the threshold of concern of 90 microgram/person/day for structural class III. Accordingly, they proceed to step B4 of the Procedure.

One candidate substance *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117] has an estimated European daily *per capita* intake (MSDI) of 120 microgram (Table 2a), which is above the threshold of concern of 90 microgram/person/day for structural class III. Therefore, data must be available on the substance or closely related substances to perform a safety evaluation. On the basis of a 90-day study in rats exposed to *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117] in the diet, a No-Observed Adverse-Effect-Level (NOAEL) of 100 mg/kg bw/day was identified. The MSDI value of 120 microgram/capita/day is equivalent to 2 microgram/kg bw/day, at a body weight of 60 kg. Thus, the margin of safety is 50000.

Based on results of the safety evaluation through the Procedure, *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117] is not anticipated to pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

Step B4

A NOAEL of 100 mg/kg bw/day was reported for *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117]. This substance is structurally related to the two substances [FL-no: 16.123 and 16.125]. The combined estimated daily *per capita* intake of 18 microgram for the two candidate substances corresponds to 0.3 microgram/kg bw/day, at a body weight of 60 kg. Thus, a margin of safety of 3.3×10^5 can be calculated. Therefore, the two substances [FL-no: 16.123 and 16.125] are not anticipated to pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

No NOAEL could be derived for the two candidate substances [FL-no: 16.118 and 16.124] or structurally related substances from studies with a sufficient duration of exposure. Accordingly, further data are required for these two candidate substances.

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

The mTAMDI intakes for the five candidate substances in structural class III range from 210 to 11000 microgram/person/day, which all are above the threshold of concern of 90 microgram/person/day.

Accordingly, further information is required for all candidate substances. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the intake estimates based on the MSDI and mTAMDI approaches, see Table 6.1.

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

FL-no	EU Register name	MSDI ($\mu\text{g}/\text{capita}/\text{day}$)	mTAMDI ($\mu\text{g}/\text{person}/\text{day}$)	Structural class	Threshold of concern ($\mu\text{g}/\text{person}/\text{day}$)
16.117	N-p-Benzeneacetonitrile-menthanecarboxamide	120	4400	Class III	90
16.118	N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide	61	2500	Class III	90
16.123	(1R,2S,5R)-N-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide	12	210	Class III	90
16.124	(1R,2S,5R)-N-Cyclopropyl-5-methyl-2-isopropyl cyclohexanecarboxamide	6.1	11000	Class III	90
16.125	(2S,5R)-N-[4-(2-Amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)cyclohexanecarboxamide	6.1	7900	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

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

On the basis of the reported annual production volumes in Europe (Flavour Industry, 2008d; Flavour Industry, 2008e; Flavour Industry, 2009u; Flavour Industry, 2010l), the combined estimated daily *per capita* intake as flavourings of the three structurally similar candidate substances [FL-no: 16.117, 16.123 and 16.125] assigned to class III is 138 microgram, which exceeds the threshold of concern for a substance belonging to structural class III of 90 microgram/person/day.

The combined estimated intake of 138 microgram/*capita*/day corresponds to 2.3 microgram/kg bw/day, which is more than 40000 fold lower than the NOAEL of 100 mg/kg bw/day for *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117] (See Section 8.2).

The combined intake for the remaining two substances in class III [FL-no: 16.118 and 16.124] is 67 microgram, *capita*/day, which do not exceeds the threshold of concern for substances belonging to structural class III of 90 microgram/person/day.

8. Toxicity

8.1. Acute Toxicity

Data are available for three candidate substances [FL-no: 16.117, 16.118 and 16.123]. Oral LD₅₀ values are in the range of 300 to more than 2000 mg/kg bw in rats (Mallory, 2004; Groom, 2007; Bradshaw, 2008).

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

A 90-day oral dosing study in rats is available for the candidate substance [FL-no: 16.117] and two 28-day oral dosing studies in rats are available for the candidate substance [FL-no: 16.118].

90-day oral toxicity study with N-p-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117]

Groups of male and female rats (N = 10 or 15, control and highest dose of which 5 were in recovery groups for additional 28 days) were administered *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117] in the diet at concentration corresponding to doses of 0, 100, 300 and 1000 mg/kg bw/day for 90 days. The study was conducted in accordance with OECD test Guidelines no. 408 (Eapen, 2007). Significant effects ascribed to the exposure were a slight increase in methemoglobin in females dosed 1000 mg/kg bw/day, increased cholesterol and potassium in males dosed 300 and 1000 mg/kg bw/day, which were normalised after the recovery period. Females dosed with 100, 300 and 1000 mg/kg bw/day had reduced alanin transaminase (ALAT), females dosed with 100 and 1000 mg/kg bw had reduced aspartate transaminase (ASAT). However, decrease in ASAT and ALAT is of no toxicological relevance. Females dosed with 300 and 1000 mg/kg bw/day had reduced triglyceride. These effects were normalised after the recovery period. No dose related effects were detected on urine analysis or on macroscopic examination. Significant increased liver weight relative to body weight was observed in both males and females dosed 300 and 1000 mg/kg bw/day. There were no histological substance-related changes in any tissue, including the thyroids in the rats examined after the primary and the recovery or recovery periods (see studies with [FL no: 16.118] described below (Eapen, 2007). A NOAEL of 100 mg/kg bw/day could be derived.

28-day oral toxicity studies with N-(2-(pyridine-2-yl)ethyl)-3-p-menthanecarboxamide [FL-no: 16.118]

Groups of male and female Sprague-Dawley rats (N = 5) were administered *N*-(2-(pyridine-2-yl)ethyl)-3-*p*-menthanecarboxamide [FL-no: 16.118] in the diet at concentration corresponding to doses of 0, 100, 300 and 1000 mg/kg bw/day for 28 days (Chase, 2008). Treatment related changes were detected in the liver and thyroid at all dose levels and due to the presence of fatty vacuolation in the liver, a NOAEL could not be established.

In another study, groups of male and female Sprague-Dawley rats (N = 8) were administered *N*-(2-(pyridine-2-yl)ethyl)-3-*p*-menthanecarboxamide [FL-no: 16.118] in the diet at concentrations corresponding to doses of 0, 10, 50 and 300 mg/kg bw/day for 28 days (Eapen, 2008). Significant effects ascribed to the exposure were higher albumin and globin, lower triglyceride in males in the 300 mg/kg bw/day group, higher cholesterol in males and females in the 300 mg/kg bw/day group, higher T₃ in males and females in 300 mg/kg group and in females in the 50 mg/kg bw/day group, and increased absolute and relative liver weight in both males and females was found in the 300 mg/kg bw/day group. Follicular cell hypertrophy of the thyroid gland were observed in 4 females (300 mg/kg bw/day), 7 males (300 mg/kg bw/day), 2 males (50 mg/kg bw/day), 1 male (10 mg/kg bw/day) and 1 male (0 mg/kg bw/day); the significance of these findings was not reported (Eapen, 2008). A NOAEL of 10 mg/kg bw/day could be derived, but owing to the short duration of this study, this NOAEL cannot be used for safety assessment of this and structurally related substances.

The toxicity data are summarised in Annex IV, Table IV.2

8.3. Developmental / Reproductive Toxicity Studies

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

8.4. Genotoxicity Studies

With three candidate substances, *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117], *N*-(2-(pyridine-2-yl)ethyl)-3-*p*-menthanecarboxamide [FL-no: 16.118] and (1*R*,2*S*,5*R*)-*N*-(4-methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide [FL-no: 16.123], valid bacterial mutagenicity studies have been performed in absence and in presence of metabolic activation up to sufficiently high concentrations. These studies did not provide indications for genotoxic activity (Sokolowski, 2004b; May, 2007; Bowles, 2008).

With the candidate substance *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117] also a chromosomal aberration test in human lymphocytes has been carried out, which provided no indication of clastogenicity (Bowen, 2007), but this test was of limited validity as the negative result in presence of metabolic activation was not confirmed in a second test. However, in an additional study with this substance, again a negative result in presence of metabolic activation was obtained (Woods, 2008), so that overall the conclusion that [FL-no: 16.117] did not show clastogenic potential *in vitro* could be drawn. An *in vivo* bone marrow micronucleus assay in the mouse did not indicate a clastogenic potential for [FL-no: 16.117] either, but that result was of limited relevance due to absence of target organ toxicity (Pritchard, 2007).

With substance [FL-no: 16.118] at concentrations ranging from 100 to 300 µg/ml, a negative result was obtained in a human lymphocyte test for chromosomal aberrations after 3 hours of exposure in presence of metabolic activation. A repeat assay to confirm this negative result was not carried out. A negative result was also obtained with this substance at concentrations ranging from 260 to 300 µg/ml after 3 hours of exposure in absence of metabolic activation, but in the repeat assay to confirm this negative result with the substance at concentrations ranging from 25 - 160 µg/ml, an equivocal result (4.5 % cells with chromatid breaks at the highest level tested (160 µg/ml) vs. 1 % in the non-exposed cells) was obtained. This increased incidence was outside the historical control range, but it was not statistically significant in comparison with the concurrent control (Mason, 2007). Additional scoring of hundred extra metaphases from the Mason (2007) study was performed by Pritchard (Pritchard, 2011), to provide more robust data from this study. The result from additional scoring showed no increase in the percentage cells with aberrations excluding gaps. Furthermore, the aberration frequencies fell within the historical control range.

Conclusion on genotoxicity:

The data available do not give rise to safety concern with respect to genotoxicity for the candidate substances.

Genotoxicity data are summarised in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The five candidate substances, *N-p*-benzeneacetonitrile-menthanecarboxamide [FL-no: 16.117], *N*-(2-(pyridine-2-yl)ethyl)-3-*p*-menthanecarboxamide [FL-no: 16.118], (1*R*,2*S*,5*R*)-*N*-(4-methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide [FL-no: 16.123], (1*R*,2*S*,5*R*)-*N*-cyclopropyl-5-methyl-2-isopropyl cyclohexanecarboxamide [FL-no: 16.124] and (2*S*,5*R*)-*N*-[4-(2-amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)cyclohexanecarboxamide [FL-no: 16.125], are carboxamides from chemical group 30.

The five flavouring substances possess chiral centres. All substances have been presented with specification of the stereoisomeric composition.

All candidate substances were assigned to structural class III, according to the decision tree approach presented by Cramer et al., 1978.

None of the candidate substances have been reported to occur naturally.

Genotoxicity data are available for three of the substances. The Panel concluded that the data available do not give rise to safety concern with respect to genotoxicity for any of the candidate substances.

On the basis of the available data, the candidate substances are not expected to be hydrolysed. Owing to the lack of further data, the candidate substances cannot be anticipated to be metabolised to innocuous products.

According to the default MSDI approach, the four flavouring substances [FL-no: 16.118, 16.123, 16.124 and 16.125] in this group have intakes in Europe from 6.1 to 61 micrograms/*capita*/day, which are below the threshold of concern value for structural class III of 90 micrograms/person/day. For one substance [FL-no: 16.117], the intake of 120 microgram/*capita*/day is above the threshold of concern. However, an adequate NOAEL of 100 microgram/kg bw/day exists from a 90-day study with this candidate substance [FL-no: 16.117], which provides a margin of safety of 50000. This substance is structurally related to the two substances [FL-no: 16.123 and 16.125] for which a margin of safety of 3.3×10^5 , based on the combined estimated daily *per capita* intake, can be calculated. Therefore, the three substances [FL-no: 16.117, 16.123 and 16.125] are not anticipated to pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach. No NOAEL could be derived for the remaining two candidate substances [FL-no: 16.118 and 16.124] or structurally related substances. Accordingly, further data are required for these two candidate substances.

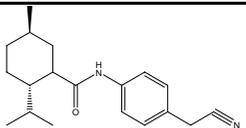
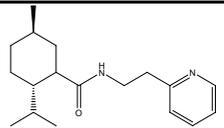
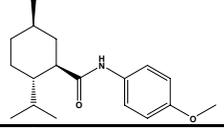
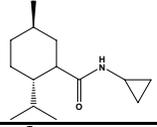
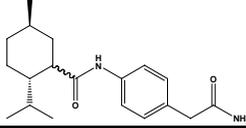
When the estimated intakes were based on the mTAMDI approach, they ranged from 210 to 11000 microgram/person/day for the five candidate substances from structural class III, which are above the threshold of concern for structural class III of 90 microgram/person/day. Therefore more reliable exposure data are required for these five substances [FL-no: 16.117, 16.118, 16.123, 16.124 and 16.125]. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for all the flavouring substances. Thus, the final evaluation of the materials of commerce can be performed for all five substances.

In conclusion, for two candidate substances [FL-no: 16.118 and 16.124] the Panel considered that additional toxicity data are needed. For the remaining three flavouring substance [FL-no: 16.117, 16.123 and 16.125] evaluated using the Procedure, the Panel considered that they would present no safety concern at the estimated levels of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 304

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 304

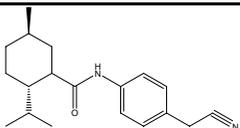
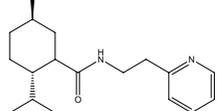
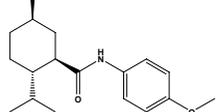
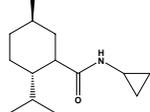
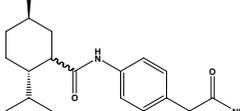
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
16.117	N-p-Benzeneacetonitrile-menthanecarboxamide		4496 852379-28-3	Solid C ₁₉ H ₂₆ N ₂ O 298.43	Insoluble Insoluble	147-151.3 IR NMR MS 99 %	n.a. n.a.	In accordance with CASrn, Register name to be changed to (1R, 3R, 4S)-N-p-Benzeneacetonitrile-menthanecarboxamide. Min assay 99 % (sum of isomers: Min. 94 % (1R, 3R, 4S)-N-p-Benzeneacetonitrile-menthanecarboxamide and 0 - 5 % (1R, 3S, 4S)-N-p-Benzeneacetonitrile-menthanecarboxamide. (Flavour Industry, 2012e).
16.118	N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide		4549 847565-09-7	Solid C ₁₈ H ₂₈ N ₂ O 288.43	Soluble Soluble	83 IR NMR MS 99 %	n.a. n.a.	In accordance with CASrn, Register name to be changed to (1R, 2S, 5R)-N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide. (Flavour Industry, 2012e).
16.123	(1R,2S,5R)-N-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl)-cyclohexanecarboxamide		4681 68489-09-8	Solid C ₁₈ H ₂₇ NO ₂ 289.42	Insoluble Soluble	177.7 IR NMR MS 95 %	n.a. n.a.	
16.124	(1R,2S,5R)-N-Cyclopropyl-5-methyl-2-isopropylcyclohexanecarboxamide		4693 73435-61-7	Solid C ₁₄ H ₂₅ NO 223.19	Soluble Soluble	125 IR NMR MS 95 %	n.a. 0.23	
16.125	(2S,5R)-N-[4-(2-Amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)cyclohexanecarboxamide		4684 1119711-29-3	Solid C ₁₉ H ₂₈ N ₂ O ₂ 316.2	Sparingly soluble Soluble	186-188 IR NMR MS 95 %	n.a. 0.4	

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

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
16.117	N-p-Benzeneacetonitrile-menthanecarboxamide		120	Class III B3: Intake above threshold Data available Adequate NOAEL exists		6)	
16.118	N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide		61	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
16.123	(1R,2S,5R)-N-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide		12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
16.124	(1R,2S,5R)-N-Cyclopropyl-5-methyl-2-isopropylcyclohexanecarboxamide		6.1	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
16.125	(2S,5R)-N-[4-(2-Amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)cyclohexanecarboxamide		6.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

- 1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
- 2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
- 3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
- 4) No safety concern based on intake calculated by the MSDI approach of the named compound.
- 5) Data must be available on the substance or closely related substances to perform a safety evaluation.

- 6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).*
- 7) *Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.*
- 8) *No conclusion can be drawn due to lack of information on the purity of the material of commerce.*

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 products⁶ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous⁷ (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.

⁶ "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

⁷ "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

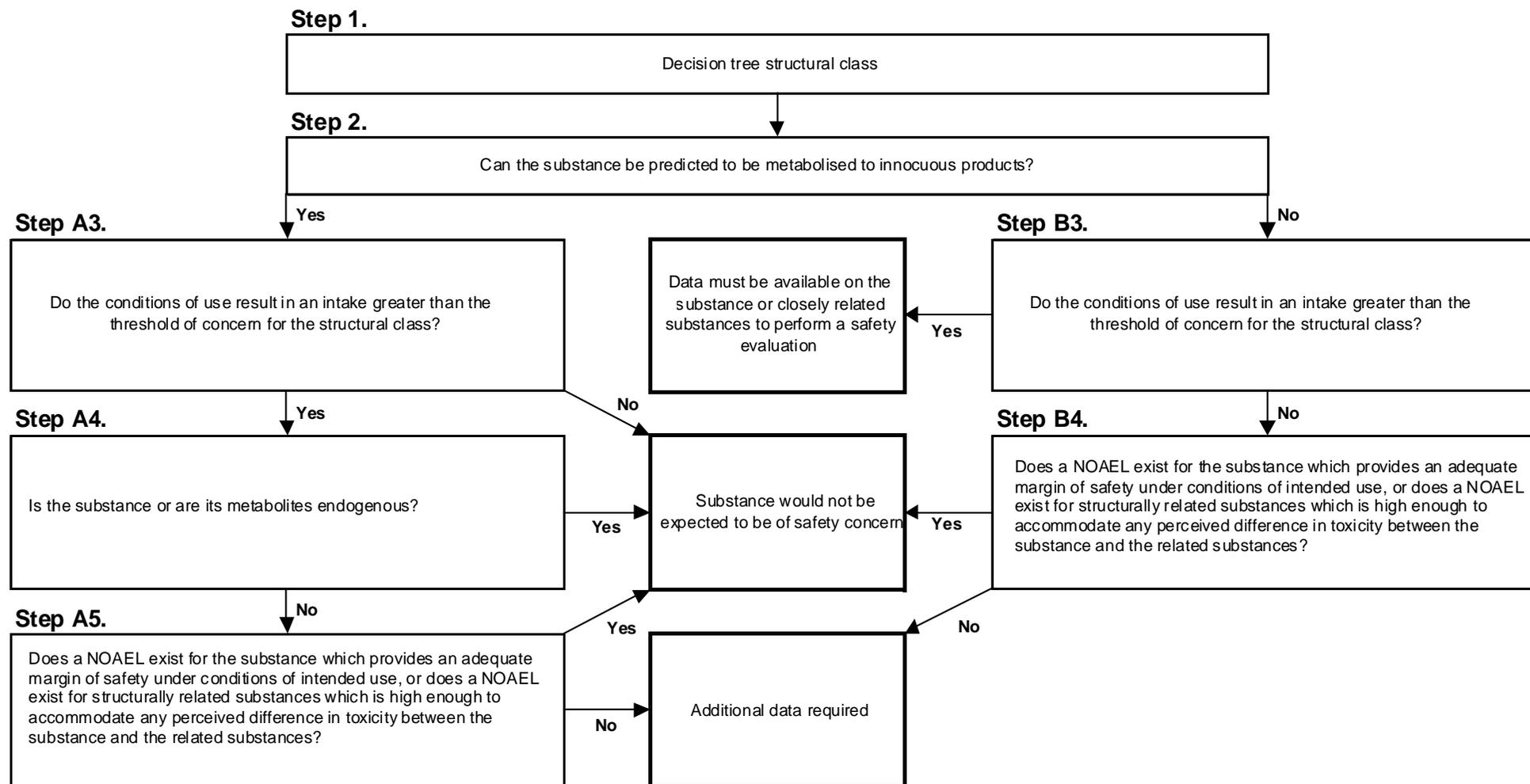


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

ANNEX II: USE LEVELS / mTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

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

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.304* (Flavour Industry, 2008d; Flavour Industry, 2008e; Flavour Industry, 2009u; Flavour Industry, 2010l).

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
16.117	-	-	-	-	-	150	-	-	-	-	-	-	-	-	1	0	-	-
	-	-	-	-	-	250	-	-	-	-	-	-	-	-	5	0	-	-
16.118	-	-	-	-	-	80	-	-	-	-	-	-	-	-	1	0	-	-
	-	-	-	-	-	150	-	-	-	-	-	-	-	-	5	0	-	-
16.123	0,5	0,5	0,5	0,5	-	1	0,5	0,5	-	-	0,5	1	1	-	0,1	0,2	-	-
	5	10	10	5	-	20	5	5	-	-	10	20	20	-	1	1	-	-
16.124	-	-	-	10	10	220	-	-	-	-	-	-	-	-	10	0	-	-
	-	-	-	30	30	800	-	-	-	-	-	-	-	-	40	0	-	-
16.125	-	-	-	20	20	70	-	-	-	-	-	-	-	-	10	0	-	-
	-	-	-	100	100	300	-	-	-	-	-	-	-	-	50	0	-	-

*All five candidate substances are also used in chewing gum, which is not covered by any of the above food categories. Normal/maximum use levels for chewing gum is 200/800 mg/kg for [FL-no: 16.117], 100/300 mg/kg for [FL-no: 16.118], 30/300 mg/kg for [FL-no: 16.123], 1200/4000 mg/kg for [FL-no: 16.124] and 400/800 mg/kg for [FL-no: 16.125].

For the two substances [FL-no: 16.117 and 16.118] the Industry has informed that only 10 % of the amount added is released from the chewing gum (Sostmann, 2006). For [FL-no: 16.124 and 16.125] there is a release of 25.4 % and 10.5 %, respectively (Flavour Industry, 2009u). For the remaining substance [FL-no: 16.123] there is no information on % release. Taking these % releases and an intake estimate of 2 g chewing gum/day into consideration, the mTAMDI of the candidate substances based on the 16 food categories and the use of chewing gum is calculated. These figures is presented in tables II.2.3 and 6.1.

II.2 mTAMDI Calculations

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

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

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

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

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

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

Food categories according to Commission Regulation 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		

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	
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food	
10.0	Eggs and egg products	Food	
11.0	Sweeteners, including honey		Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.		Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food	
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts		Exception c
15.0	Ready-to-eat savouries		Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food	

The mTAMDI values (see Table II.2.3) are presented for each of the five flavouring substances in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (Flavour Industry, 2008d; Flavour Industry, 2008e; Flavour Industry, 2009u; Flavour Industry, 2010l). The mTAMDI values are only given for the highest reported normal use 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)
16.117	N-p-Benzeneacetonitrile-menthanecarboxamide	4400	Class III	90
16.118	N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide	2500	Class III	90
16.123	(1R,2S,5R)-N-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide	210	Class III	90
16.124	(1R,2S,5R)-N-Cyclopropyl-5-methyl-2-isopropylcyclohexanecarboxamide	11000	Class III	90
16.125	(2S,5R)-N-[4-(2-Amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)cyclohexanecarboxamide	7900	Class III	90

The calculation of mTAMDI for the candidate substances takes into account the information Industry has provided on release from the chewing gum matrix (Flavour Industry, 2009u; Flavour Industry, 2010l; Sostmann, 2006).

ANNEX III: METABOLISM

The hydrolysis of [¹⁴C]-*N-p*-benzenenitrile-menthancarboxamide (BMC) [FL-no: 16.117] was studied in rat and human hepatic microsomes (Sipes and Kong, 2012). As a positive control the hydrolysis of isoeugenol acetate, a known substrate of carboxyl esterase, was used. The radiochemical purity of [¹⁴C]-BMC [FL-no: 16.117] were > 99 %.

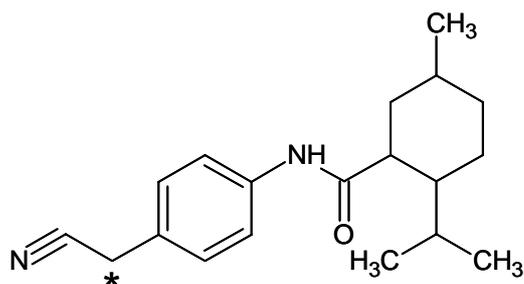


Figure 1. The structure of [¹⁴C]-*N-p*-benzenenitrile-menthancarboxamide [FL-no: 16.117], with location of the [¹⁴C] label (*).

The hydrolytic assay was carried out in a total volume of 0.4 ml of 0.1 M potassium phosphate buffer with pH 7.4 containing [¹⁴C]-BMC (100 μM or 20 μM) or isoeugenol acetate (500 μM) and pooled hepatic microsomes from male F-344 rats or male humans. At each time point (5, 10, 30 and 60 min.) an aliquot of the reaction mixture was removed from the incubation and mixed with ice cold ethanol to terminate the reaction. The [¹⁴C]-BMC and its metabolites were analysed with a reversed phase HPLC-radiometric analysis. Control incubations were conducted with heat denatured microsomes. No hydrolysis was detected at any time point when either active or heat-inactivated hepatic microsomes were used. Both hepatic microsomes from rat and humans hydrolysed isoeugenol acetate to isoeugenol.

The results show that metabolically active male rat or human microsomes did not hydrolyse *N-p*-benzenenitrile-menthancarboxamide [FL-no: 16.117].

The hydrolysis of a substance with partial structure similarity to [FL-no: 16.117] from FGE.94Rev1, *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111], was studied in artificial pancreatic juice and rat liver homogenate (Poet et al., 2005). Based on the disappearance of the employed substrate, [FL-no: 16.111] was hydrolysed in artificial pancreatic juice with a half-life of 43 ± 14.7 min. and a first order rate constant (K) of 1.06 ± 0.426 hr⁻¹. In 20 fold-diluted liver homogenate the disappearance of [FL-no: 16.111] was considerably faster (half-life: 0.802 ± 0.191 min.). However, the potential hydrolysis products *p*-menthane-3-carboxylic acid, glycine ethylester and glycine were only detected at trace levels. This indicates that the disappearance of [FL-no: 16.111] under the employed *in vitro*-conditions is due to the hydrolysis of the ethyl ester bond rather than the hydrolysis of the amide bond.

The possible release of cyanide from the candidate substance *N-p*-benzenenitrile-menthancarboxamide [FL-no: 16.117] during metabolism was studied in rat and human hepatocytes. Incubations of up to 250 μM of the candidate substance with human or rat hepatocytes for up to 4 hours only resulted in release of low amounts, if any, of cyanide. Proper positive control incubations with benzyl nitrile and sodium cyanide were included in the study (Wolff and Skibbe, 2007).

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

The candidate aromatic amides are anticipated to being absorbed from the gastrointestinal tract like other aromatic amides. Aromatic amides are expected to be metabolised to polar metabolites which are eliminated in the urine or bile (James, 1974; Schwen, 1982). No absorption data are available for the non-aromatic amide (1*R*,2*S*,5*R*)-*N*-cyclopropyl-5-methyl-2-isopropyl cyclohexanecarboxamide [FL-no: 16.124] or supporting substances.

Data on the candidate substance [FL-no: 16.117) and the carboxamide [FL-no: 16.111] demonstrate that there is no hydrolysis of the amide bond under the *in vitro* conditions. Owing to the lack of further data the candidate substances cannot be anticipated to be metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for the two candidate substances 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
N-p-benzeneacetonitrile-menthanecarboxamide [16.117]	Rat	M,F	Oral	>2000	(Mallory, 2004)	
N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide [16.118]	Rat	F	Oral	Between 300 and 2000	(Groom, 2007)	
(1R,2S,5R)-N-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide [16.123]	Rat	F	Oral	>2000	(Bradshaw, 2008)	

Subacute / subchronic / chronic / carcinogenic toxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 30.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
N-p-benzeneacetonitrile-menthanecarboxamide [16.117]	Rat; M,F 10	Diet	100, 300, 1000	90 days	100	(Eapen, 2007)	
N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide [16.118]	Rat; M,F 5	Diet	100, 300, 1000	28 days		(Chase, 2008)	
	Rat; M,F 8	Diet	10, 50, 300	28 days	10	(Eapen, 2008)	

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental and reproductive toxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 30.

GENOTOXICITY

In vitro mutagenicity/genotoxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 30.

TABLE IV.4: GENOTOXICITY (IN VITRO)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
N-p-benzeneacetone trile-menthanecarboxamide [16.117]	Reverse mutation	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537 and <i>E. coli</i> WP2 <i>uvrA</i>	3 - 5000 microg/plate	Negative ¹	(Sokolowski, 2004b)	Valid study.
	Chromosomal aberration	Human lymphocytes	373 - 2984 microg/ml	Negative	(Bowen, 2007)	Valid together with the study by Woods, 2008.
	Chromosomal aberration	Human lymphocytes	367 - 2938 microg/ml	Negative ²	(Woods, 2008)	Confirmatory test for Bowen, 2007.
N-(2-(Pyridine-2-yl)ethyl)-3-p-menthanecarboxamide [16.118]	Reverse mutation	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537 and <i>E. coli</i> WP2 <i>uvrA</i>	5 - 5000 microg/plate	Negative ¹	(May, 2007)	Valid study.
	Chromosomal aberration	Human lymphocytes	25 - 300 microg/ml	Equivocal ³ Negative ³	(Mason, 2007) (Pritchard, 2011)	Valid study.
	Chromosomal aberration	Human lymphocytes	100 - 300 microg/ml	Negative ²	(Mason, 2007)	Limited relevance (no repeat study).
(1R,2S,5R)-N-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide [16.123]	Reverse mutation	<i>S. typhimurium</i> , TA98, TA100, TA102, TA1535 and TA1537	50 - 5000 microg/plate	Negative ¹	(Bowles, 2008)	Valid study.

¹ With and without metabolic activation.

² With metabolic activation.

³ Without metabolic activation

In vivo mutagenicity/genotoxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 30.

TABLE IV.5: GENOTOXICITY (IN VIVO)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
N-p-benzeneacetone trile-menthanecarboxamide [16.117]	Micronucleus induction	Mice	Gavage	500 - 2000 mg/kg bw/day	Negative	(Pritchard, 2007)	Of limited relevance due to absence of target tissue toxicity.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
ALAT	Alanin Transaminase
ASAT	Aspartate Transaminase
BMC	<i>N-p</i> -benzenenitrile-menthanecarboxamide
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)
HPLC	High Performance Liquid Chromatography
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
OECD	Organisation for Economic Co-operation and Development
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