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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 86, Revision 2 (FGE.86Rev2): Consideration of aliphatic and arylalkyl amines and amides evaluated by JECFA (65th meeting)[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 30 aliphatic and arylalkyl amines and amides evaluated by JECFA at the 65th meeting in 2005. This revision is required owing to additional available toxicity data on piperine [FL-no: 14.003] and deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091]. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological thresholds of concern and available data on metabolism and toxicity. The Panel agrees with JECFA's conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the Maximised Survey-derived Daily Intake (MSDI) approach for all substances considered in this FGE. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for all 30 substances, the information is adequate.

KEY WORDS

flavourings, safety, aliphatic amines, aliphatic amides, arylalkyl amines, JECFA, 65th meeting

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[1] On request from the European Commission, Question No EFSA-Q-2013-00865 and EFSA-Q-2014-00071, adopted on 19 December 2014.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Flavourings: Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby, and EFSA staff: Maria Carfí, Annamaria Rossi and Kim Rygaard Nielsen for the support provided to this scientific opinion.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver 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 CEF Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The previous version of this consideration, FGE.86Rev1, dealt with 34 aliphatic and arylalkyl amines and amides which are in the Register and which were evaluated by JECFA at its 65th meeting. The Panel concluded that no structurally related substances to support the evaluation in a corresponding Flavouring Group Evaluation (FGE) was available.

Since the publication of FGE.86Rev1, the Industry has informed that four of the substances, [FL-no: 11.014, 16.049, 16.093 and 16.094], are no longer used as flavouring substances in Europe and will therefore not be further considered in this FGE. The present consideration therefore only deals with 30 flavouring substances.

In FGE.86Rev1, the Panel agreed with the application of the Procedure as performed by JECFA for 27 of the 30 substances. For two substances, piperine [FL-no: 14.003] and deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091], the Panel did not agree with JECFA that appropriate studies are available for deriving NOAELs and concluded that additional toxicity data are required for these two substances.

The present revision of FGE.86Rev1 is due to the submission of the requested toxicity data for [FL-no: 14.003 and 16.091]. Based on the results of two 90-day oral toxicity studies received by EFSA the Panel now concludes that these two substances are not of safety concern when used as flavouring substances at the estimated levels of exposure based on the MSDI approach.

For N-isopentylidene isopentylamine [FL-no: 11.017] the Panel concluded that this substance can be metabolised to innocuous products and accordingly evaluated along the A-side of the Procedure (while JECFA evaluated [FL-no: 11.017] along the B-side). Like JECFA the Panel concluded that [FL-no: 11.017] is of no safety concern at estimated level of intake, based on the MSDI approach.

For 18 substances evaluated by JECFA through the Procedure use levels for the EU have been provided by the Industry [FL-no: 11.002, 11.004, 11.005, 11.007, 11.015, 11.016, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.080, 14.133, 14.141, 16.052, 16.091 and 16.092]. The mTAMDI figures calculated for the substances in structural class I are 340 µg/person per day, except for [FL-no: 16.092], for which the mTAMDI is 15000 µg/person/day, exceeding the threshold of 1800 µg/person/day for structural class I. The mTAMDI figures for the structural class II substances range from 200 to 340 µg/person/day, except for [FL-no: 14.141] for which the figure is 600 µg/person/day, exceeding the threshold of 540 µg/person/day for structural class II. For the two substances [FL-no: 16.052 and 16.091] in structural class III the mTAMDI is 200 and 1900 µg/person/day, respectively, and both exceed the threshold of concern of 90 µg/person/day for structural class III substances. Thus, for four substances [FL-no: 14.141, 16.052, 16.091 and 16.092] the intakes, estimated on the basis of the mTAMDI approach, exceed the threshold for their structural classes. Therefore more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be considered using the Procedure. Subsequently, additional data might become necessary.

For the remaining 12 substances [FL-no: 11.001, 11.003, 11.006, 11.009, 11.017, 14.003, 14.010, 14.064, 14.167, 16.006, 16.013 and 16.053] use levels are needed to calculate the mTAMDIs in order

to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 30 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity are available for all JECFA evaluated substances.

Thus, for all 30 JECFA evaluated aliphatic and arylalkyl amines and amides [FL-no: 11.001, 11.002, 11.003, 11.004, 11.005, 11.006, 11.007, 11.009, 11.015, 11.016, 11.017, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.003, 14.010, 14.064, 14.080, 14.133, 14.141, 14.167, 16.006, 16.013, 16.052, 16.053, 16.091 and 16.092] the Panel agreed with JECFA conclusion “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

EFSA has considered the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation of aliphatic and aromatic amines and amides in the Flavouring Group Evaluation 86, Revision 1 (FGE.86Rev1). The opinion was adopted on 25 November 2010. EFSA concluded in its opinion that for two substances [FL-no: 14.003 and 16.091] additional toxicity data are still needed before the evaluation can be finalised.

The requested data on piperine [FL-no: 14.003] and deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091] have now been submitted by the applicants.

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

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to finalise its safety assessment on piperine [FL-no: 14.003] and deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091] in accordance with Commission Regulation (EC) No 1565/2000.

INTERPRETATION OF TERMS OF REFERENCE

In the terms of reference as provided by the Commission substances are indicated as Aromatic Amines. This description is not appropriate and the Panel will use a description “Arylalkyl Amines” instead.

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

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

[6] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8-16.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000, hereafter named the "EFSA Procedure". This Procedure is based on the opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996; JECFA, 1997; JECFA, 1999), hereafter named the "JECFA Procedure". The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of particular importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour 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. It is noted that JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006a).

In the absence of more accurate 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.

As information on use levels for the flavouring substances has not been requested by JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram per Person per Day (Step B5) Used by JECFA

JECFA uses the threshold of concern of 1.5 µg/person per day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the

Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 µg per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 µg per day?")" (JECFA, 1999).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 µg per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

1. History of the Evaluation of the Substances in the Present FGE

In FGE.86, which considered 35 aliphatic and arylalkyl amines and amides, the Panel concluded that for five substances no applicable NOAEL was available for the substance itself or for a structurally related substance and accordingly further data are required.

The first Revision of Flavouring Group Evaluation 86 (FGE.86Rev1) included a re-consideration of three candidate substances [FL-no: 16.091, 16.093 and 16.094] as additional toxicity data had been submitted. Furthermore, EU production figures were provided for two substances [FL-no: 11.006 and 16.053] (EFFA, 2010). Furthermore, additional information on stereoisomeric composition [FL-no: 16.013], composition of mixture [FL-no: 11.017], specifications (data on solubility [FL-no: 14.064 and 14.168]) and missing ID-tests [FL-no: 11.017, 14.168 and 16.094] was received (EFFA, 2010) after publication of FGE.86 and included in Revision 1.

2-Propionyl pyrroline [FL-no: 14.168], which contains an α,β-unsaturated ketone structure has been withdrawn from FGE.86Rev1 and transferred to FGE.223 for evaluation with respect to a possible genotoxic potential.

FGE	Opinion adopted	Link	No. of substances
FGE.86	22 May 2008	http://www.efsa.europa.eu/en/efsajournal/pub/745.htm	35
FGE.86Rev1	25 November 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1926.htm	34
FGE.86Rev2			30

The present revision of FGE.86, FGE.86Rev2, includes evaluation of additional toxicity data provided for piperine [FL-no: 14.003] (EFFA, 2013; Bauter, 2013) and deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091] (Flavour Industry, 2013; Koetzner, 2013a; Koetzner, 2013b).

A search in the open literature did not reveal any pertinent new information.

Furthermore, the Industry has submitted the requested EU production figures for five substances [FL-no: 11.006, 14.167, 16.053, 16.091 and 16.092] (EFFA, 2004; Flavour Industry, 2004a, 2004b), and new information on the composition of the stereoisomeric mixture for [FL-no: 16.013] (EFFA, 2014). These new data have also been included in the present revision of FGE.86.

Finally, the industry has informed that four substances, *N*,*N*-dimethylphenethylamine [FL-no: 11.014], butyramide [FL-no: 16.049], *N*-cyclopropyl (2E,6Z)-nonadienamide [FL-no: 16.093] and *N*-ethyl (2E,6Z)-nonadienamide [FL-no: 16.094] are no longer supported for use as flavouring substances in Europe (DG SANCO, 2012) and the substances will therefore not be considered any further.

2. Presentation of the Substances in JECFA Flavouring Group

2.1. Description

2.1.1. JECFA Status

JECFA has evaluated a group of 37 flavouring substances consisting of aliphatic and arylalkyl amines and amides at the 65th meeting (JECFA, 2006b).

2.1.2. EFSA Considerations

Two of the 37 flavouring substances evaluated by JECFA in the group named “aliphatic and aromatic amines and amides” are not in the Register (1-amino-2-propanol and acetamide; JECFA-no: 1591 and 1592, respectively). A third substance evaluated by JECFA contains an α,β-unsaturated ketone moiety and has been considered with respect to genotoxicity in FGE.223, corresponding to subgroup 5.1 of FGE.19 (EFSA, 2008a), for which a final conclusion regarding its genotoxic properties could not be reached and additional data were requested. Since the publication of FGE.86Rev1, the industry has informed that four of the 34 substances are no longer supported for use as flavouring substances in EU (DG SANCO, 2012; DG SANCO, 2014). This consideration therefore only deals with 30 flavouring substances.

A summary of the specifications and structural formulas of the 30 substances is given in Table 1.

2.2. Isomers

2.2.1. Status

The following five substances [FL-no: 11.005, 11.020, 14.133, 16.013 and 16.092] in the group of JECFA evaluated aliphatic and arylalkyl amines and amides have one or more chiral centres. Two substances [FL-no: 14.003 and 16.091] can exist as geometrical isomers.

2.2.2. EFSA Considerations

Adequate information on stereoisomeric composition is available for all substances.

2.3. Specifications

2.3.1. Status

JECFA specifications are available for all 30 substances (JECFA, 2005). See Table 1

2.3.2. EFSA Considerations

The available specifications are considered adequate for all substances.

3. Intake Estimation

3.1. Status

For all 30 substances evaluated through JECFA Procedure intake data, based on the MSDI approach, are available for the EU. (See Table 5).

After publication of FGE.86, JECFA has re-evaluated flavouring substances for which updated tonnage (production) data were submitted to JECFA by Industry. These updated tonnage figures were included for the substances [FL-no: 11.002, 11.004, 11.005, 11.007, 11.015, 11.016, 11.017, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.080, 14.133, 14.141, 16.049 and 16.052] in FGE.86Rev1. Furthermore, the Industry has submitted production figures for the EU for five further substances [FL-no: 11.006, 14.167, 16.053, 16.091 and 16.092] (EFFA, 2004; Flavour Industry, 2004a, 2004b). These figures are included in the present revision of FGE.86.

3.2. EFSA Considerations

For 18 substances [FL-no: 11.002, 11.004, 11.005, 11.007, 11.015, 11.016, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.080, 14.133, 14.141, 16.052, 16.091 and 16.092], the Industry has submitted food categories[7] and use levels in these food categories, for normal and maximum use (EFFA, 2005; EFFA, 2007; Flavour Industry, 2004b) (see Table 6, Appendix A). Based on these normal use levels mTAMDI figures can be calculated (see Table 7, Appendix A), (EFSA, 2004).

For the remaining 12 substances [FL-no: 11.001, 11.003, 11.006, 11.009, 11.017, 14.003, 14.010, 14.064, 14.167, 16.006, 16.013 and 16.053] use levels are needed to calculate the mTAMDIs.

[7] Annex III, Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8-16.

SUMMARY OF SPECIFICATION DATA

Table 1: Summary of Specification Data (JECFA, 2005)

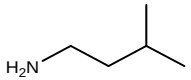
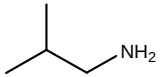
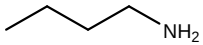
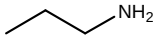
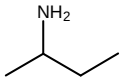
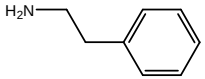
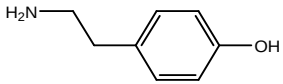
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments/ Reference for specifications
11.001 1587	3-Methylbutylamine		3219 512 107-85-7	Liquid $C_5H_{13}N$ 87.16	Soluble Soluble	95 - 97 - NMR 98 %	1.405 - 1.411 0.747 - 0.753	
11.002 1583	Isobutylamine		4239 513 78-81-9	Liquid $C_4H_{11}N$ 73.14	Soluble Soluble	68 - MS 95 %	1.391 - 1.397 0.731 - 0.737	
11.003 1582	Butylamine		3130 524 109-73-9	Liquid $C_4H_{11}N$ 73.14	Soluble Soluble	78 - NMR 99 %	1.398 - 1.404 0.732 - 0.740	
11.004 1580	Propylamine		4237 601 107-10-8	Liquid C_3H_9N 59.11	Soluble Soluble	48 - MS 95 %	1.384 - 1.390 0.714 - 0.720	
11.005 1584	*sec*-Butylamine		4240 707 13952-84-6	Liquid $C_4H_{11}N$ 73.14	Soluble Soluble	63 - MS 95 %	1.387 - 1.393 0.715 - 0.721	Racemate.
11.006 1589	Phenethylamine		3220 708 64-04-0	Liquid $C_8H_{11}N$ 121.18	Soluble Soluble	194 - 195 - NMR 95 %	1.526 - 1.532 (25°) 0.961 - 0.967	
11.007 1590	2-(4-Hydroxy-phenyl)ethylamine		4215 709 51-67-2	Solid $C_8H_{11}NO$ 137.18	Soluble Soluble	- 165 MS 95 %	n.a. n.a.	
11.009 1610	Trimethylamine		3241 10497 75-50-3	Gas C_3H_9N 59.11	Soluble Soluble	3 - 4 - NMR 98 %	n.a. 0.667 - 0.675 (4°)	

Table 1: Summary of Specification Data (JECFA, 2005)

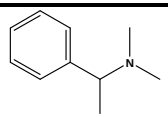
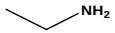
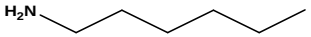
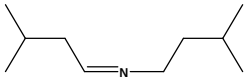
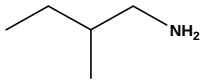
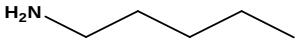
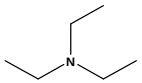
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments/ Reference for specifications
11.014 1613	*N,N*-Dimethylphenethylamine		4248 19342-01-9	Liquid $C_{10}H_{15}N$ 149.24	Soluble Soluble	183 - MS 95 %	1.500 - 1.506 0.898 - 0.904	No longer supported by Industry (DG SANCO, 2012).
11.015 1579	Ethylamine		4236 10477 75-04-7	Gas C_2H_7N 45.08	Soluble Soluble	17 -81 MS 95 %	n.a. 0.682 - 0.686 (10°)	
11.016 1588	Hexylamine		4243 10478 111-26-2	Liquid $C_6H_{15}N$ 101.19	Soluble Soluble	130 - MS 95 %	1.415 - 1.421 0.761 - 0.767	
11.017 1606	*N*-Isopentylidene isopentylamine		3990 35448-31-8	Liquid $C_{10}H_{21}N$ 155.29	Insoluble Soluble	145 - 148 - MS 98 %	1.422 - 1.428 0.768 - 0.774	
11.018 1581	Isopropylamine		4238 10480 75-31-0	Liquid C_3H_9N 59.11	Soluble Soluble	34 - MS 95 %	1.367 - 1.373 0.687 - 0.693	
11.020 1586	2-Methylbutylamine		4241 10484 96-15-1	Liquid $C_5H_{13}N$ 87.16	Soluble Soluble	96 - MS 95 %	1.417 - 1.423 0.777 - 0.779	Racemate.
11.021 1585	Pentylamine		4242 11734 110-58-7	Liquid $C_5H_{13}N$ 87.16	Soluble Soluble	103 - MS 95 %	1.418 - 1.424 0.750 - 0.759	
11.023 1611	Triethylamine		4246 10496 121-44-8	Liquid $C_6H_{15}N$ 101.19	Soluble Soluble	88 - MS 95 %	1.395 - 1.401 0.724 - 0.730	

Table 1: Summary of Specification Data (JECFA, 2005)

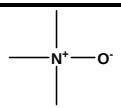
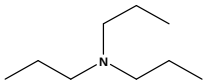
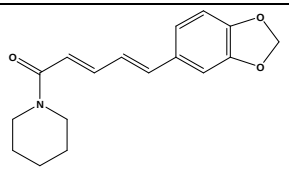
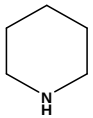
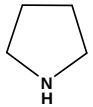
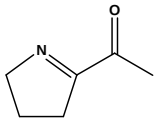
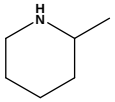
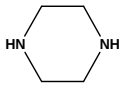
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments/ Reference for specifications
11.025 1614	Trimethylamine oxide		4245 10494 1184-78-7	Solid C_3H_9NO 75.11	Soluble Soluble	- 213 MS 95 %	n.a. n.a.	
11.026 1612	Tripropylamine		4247 10495 102-69-2	Liquid $C_9H_{21}N$ 143.27	Soluble Soluble	156 - MS 95 %	1.411 - 1.417 0.754 - 0.760	
14.003 1600	Piperine		2909 492 94-62-2	Solid $C_{17}H_{19}O_3N$ 285.34	Very slightly soluble Soluble	- 128 - 130 NMR 97 %	n.a. n.a.	Register name to be changed to (E,E)-piperine.
14.010 1607	Piperidine		2908 675 110-89-4	Liquid $C_5H_{11}N$ 85.15	soluble Soluble	106 - IR 98 %	1.450 - 1.454 0.858 - 0.862	
14.064 1609	Pyrrolidine		3523 10491 123-75-1	Liquid C_4H_9N 71.12	Soluble Freely soluble	87 - 89 - IR NMR 95 %	1.440 - 1.446 0.847 - 0.853	
14.080 1604	2-Acetyl-1-pyrroline		4249 99583-29-6	Solid C_6H_9NO 111.14	Soluble Soluble	- 19 MS 95 %	n.a. n.a.	
14.133 1608	2-Methylpiperidine		4244 109-05-7	Liquid $C_6H_{13}N$ 99.18	Soluble Soluble	118 - MS 95 %	1.442 - 1.448 0.838 - 0.844	Racemate.
14.141 1615	Piperazine		4250 110-85-0	Solid $C_4H_{10}N_2$ 86.14	Soluble soluble	- 109 MS	n.a. n.a.	

Table 1: Summary of Specification Data (JECFA, 2005)

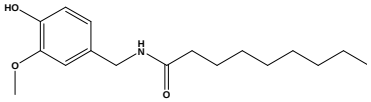
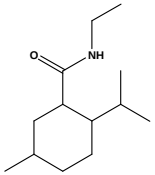
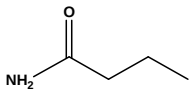
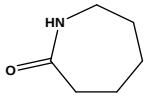
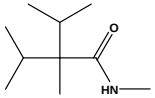
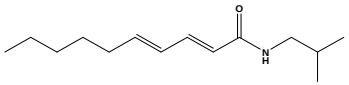
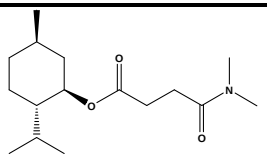
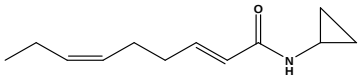
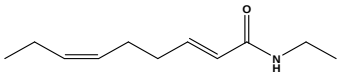
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments/ Reference for specifications
						95 %		
14.167 1603	1-Pyrroline		3898 5724-81-2	Liquid C_4H_7N 69.10	Soluble Soluble	87 - 89 - NMR 99 %	1.440 - 1.446 0.849 - 0.855	
16.006 1599	*N*-Nonanoyl 4-hydroxy-3-methoxybenzylamide		2787 590 2444-46-4	Solid $C_{17}H_{27}O_3N$ 293.41	Slightly soluble Soluble	- 124 - 128 NMR 96 %	n.a. n.a.	
16.013 1601	*N*-Ethyl-2-isopropyl-5-methylcyclohexane carboxamide		3455 2298 39711-79-0	Solid $C_{13}H_{25}ON$ 211.35	Insoluble Soluble	- 91 - 93 NMR 98 %	n.a. n.a.	60 - 90 % (1R, 2S, 5R) or L- and 5 - 40 % (1S,2R,5S) or D- with minor amounts of other diastereomers (up to 10 %) (EFFA, 2014).
16.049 1593	Butyramide		4252 541-35-5	Solid C_4H_9NO 87.12	Soluble Soluble	- 115 NMR MS 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
16.052 1594	1,6-Hexalactam		4235 105-60-2	Solid $C_6H_{11}NO$ 113.16	Soluble Soluble	- 70 NMR MS 95 %	n.a. n.a.	
16.053 1595	2-Isopropyl- N,2,3-trimethylbutanamide		3804 10459 51115-67-4	Solid $C_{10}H_{21}ON$ 171.28	insoluble Soluble	- 56 - 64 NMR 99 %	n.a. n.a.	
16.091 1598	Deca-(2*E*,4*E*)-dienoic acid isobutyl-amide		4148 18836-52-7	Solid $C_{14}H_{25}NO$ 223.36	Insoluble Soluble	- 82 - 90 IR NMR MS 95 %	n.a. n.a.	

Table 1: Summary of Specification Data (JECFA, 2005)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [(a)] Solubility in ethanol [(b)]	Boiling point, °C [(c)] Melting point, °C ID test Assay minimum	Refrac. Index [(d)] Spec.gravity [(e)]	EFSA comments/ Reference for specifications
16.092 1602	*N,N*-Dimethyl menthyl succinamide		4230 544714-08-1	Liquid $C_{16}H_{30}O_2N_2$ 282.43	Slightly soluble Soluble	380 - IR NMR 95 %	1.522 - 1.530 0.965 - 0.975	Register name to be changed to (1R,2S,5R) N,N-Dimethyl menthyl succinamide.
16.093 1597	*N*-Cyclopropyl (2*E*,6Z)-nonadienamide		4087 608514-55-2	Solid $C_{12}H_{19}NO$ 193.29	Sparingly soluble Soluble	- 33 - 37 IR NMR 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
16.094 1596	*N*-Ethyl (2*E*,6*Z*)-nonadienamide		4113 608514-56-3	Liquid $C_{11}H_{19}NO$ 181.28	Sparingly soluble Soluble	120 (0.8 hPa) - IR NMR MS 96 %	1.484 - 1.493 0.910 - 0.920	No longer supported by Industry (DG SANCO, 2014).

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

4. Genotoxicity Data

4.1. Genotoxicity Studies – Text Taken[8] from JECFA (JECFA, 2006b)

In vitro

No mutagenicity was found in the standard Ames assay when various strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, TA1535, TA1537, TA1538, TA1530, TA1531, TA1532 and TA1964) were incubated with up to 10000 µg/plate of ethylamine [FL-no: 11.015], isopropylamine [FL-no: 11.018], butylamine [FL-no: 11.003], isobutylamine [FL-no: 11.002], pentylamine [FL-no: 11.021], acetamide (No. 1592, not in Register), 2-isopropyl-N,2,3-trimethylbutyramide [FL-no: 16.053], deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091], piperine [FL-no: 14.003], piperidine [FL-no: 14.010], pyrrolidine [FL-no: 14.064], trimethylamine [FL-no: 11.009], triethylamine [FL-no: 11.023] or piperazine [FL-no: 14.141] with or without metabolic activation (Green and Savage, 1978; Haworth et al., 1978; Andrews et al., 1980; Florin et al., 1980; Haworth et al., 1983; Mortelmans et al., 1986; Zeiger et al., 1987; Karekar et al., 1996; King, 2003).

In a host-mediated assay in which *S. typhimurium* strain TA1950, TA1951, TA1952 or TA1964 was injected intraperitoneally into mice followed by an intramuscular injection of 800 mg/kg bw of piperidine or pyrrolidine, no mutagenicity was observed (Green and Savage, 1978).

There was no evidence of DNA damage when *Escherichia coli* 343/591 uvrB-/recA-/lac+ or uvrB+/recA+/lac+ was incubated with up to 1080 mM (63793 µg/ml) of acetamide (No. 1592, not in Register) or up to 33.7 mM (2870 µg/ml) of piperidine [FL-no: 14.010] (Hellmér and Bolcsfoldi, 1992). In the SOS Chromotest with *E. coli* PQ37, the N-nitroso derivative of tyramine [FL-no: 11.007] gave positive results (Ohshima et al., 1989).

Assays in mammalian cell lines have been performed with tyramine [FL-no: 11.007], acetamide (No. 1592, not in Register), 2-isopropyl-N,2,3-trimethylbutyramide [FL-no: 16.053], and piperidine [FL-no: 14.010]. Unscheduled DNA synthesis was not increased when WI-38 human cells were incubated with 125 - 2000 µg/ml of 2-isopropyl-N,2,3-trimethylbutyramide (Skinner, 1978). No single-strand DNA breaks were reported when 0.03 - 1000 mM (2 - 59068 µg/ml) of acetamide or 0.03 - 3 mM (2.6 to 255 µg/ml) of piperidine were incubated with rat hepatocytes (Sina et al., 1983). Mixed results have been reported with tyramine and piperidine in the mouse lymphoma forward mutation assay: positive results were reported for both compounds when tested at up to 823 and 688 µg/ml, respectively, in L5178Y mouse lymphoma cells with and without metabolic activation;, but only at cytotoxic doses (Wangenheim and Bolcsfoldi, 1988). No mutagenic effects were reported when tyramine and 2-isopropyl-N,2,3-trimethylbutyramide were tested at concentrations of up to 3500 and 1000 µg/ml, respectively, in L5178Y mouse lymphoma cells (Kirby et al., 1978; McGregor et al., 1988). No mutagenic effects were observed when piperidine was tested at concentrations of up to 512 µg/ml without metabolic activation in L5178Y mouse lymphoma cells; however, equivocal results were noted when metabolic activation was added (Garberg et al., 1988).

In vivo

In male and female C57BL/6, male CBA, male CD1 and male BDF_1 mice, a single dose of acetamide (No. 1592, not in Register) of up to 5000 mg/kg bw did not induce micronuclei in the bone marrow or peripheral blood when administered by gavage or intraperitoneal injection (Mirkova, 1996; Morita et al., 1997). Micronuclei were found in the bone marrow of female C57BL/6 mice given 3.39 mmol/kg bw (approximately 200 mg/kg bw) of acetamide by gavage 30 and 6 h before termination; however, no dose-response relation was seen, as only a single dose was used (Chieli et al., 1987).

[8] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

Piperine [FL-no: 14.003] did not induce micronuclei in the bone marrow of male Swiss mice given a single dose of 10 or 20 mg/kg bw by gavage (Karekar et al., 1996) or two intraperitoneal doses (at 0 and 24 h) for a total dose of up to 4 mg/kg bw (Muralidhara and Narasimhamurthy, 1990).

Male and female 1C3F1 mice were given a single dose of 1000 mg/kg bw of 1,6-hexalactam [FL-no: 16.052] by gavage, and bone marrow was sampled from groups of 10 animals after 24, 30 and 48 h. Colchicine was administered to the mice 1 h before sacrifice. No chromosomal aberrations were seen (Adler and Ingwersen, 1989). The Comet assay was used to quantify DNA damage in cells from organs of male ddy mice given either acetamide (No. 1592, not in Register) or 1,6-hexalactam [FL-no: 16.052]. No DNA damage was reported in mice given a single dose of 2000 mg/kg bw 1,6-hexalactam by gavage; however, DNA damage was reported in the stomach, colon, lungs and bone marrow of male mice given a single intraperitoneal injection of acetamide at 2,000 mg/kg bw (Sasaki et al., 2000).

1,6-Hexalactam [FL-no: 16.052] did not induce replicative DNA synthesis in rat or mouse hepatocytes after treatment *in vivo* or *in vitro* at a dose of 350 or 700 mg/kg bw or 250 or 500 mg/kg bw, respectively (Uno et al., 1994; Miyagawa et al., 1995). In the mouse spot test, a single [route not stated but assumed to be intraperitoneal] injection of 1,6-hexalactam at a dose of up to 500 mg/kg bw significantly increased the frequency of spots over those in controls (Neuhäuser-Klaus and Lehmacher, 1989); however, statistically significant effects were observed in only one of three or four trials. It has been suggested that the colour spots observed were indicative of mitotic recombination and not mutation (Fahrig, 1989). Moreover, administration of 700 mg/kg bw in one trial did not significantly increase the frequency of spots over that in controls (Neuhäuser-Klaus and Lehmacher, 1989).

Female *Drosophila melanogaster* larvae fed up to 20 mmol/l (2263 µg/ml) of 1,6-hexalactam [FL-no: 16.052] showed sex-linked recessive lethal mutations and somatic mutation-mitotic recombination, whereas male larvae fed up to 5 mmol/l (566 µg/ml) did not have sex-linked recessive lethal mutations (Vogel, 1989).

Piperidine [FL-no: 14.010] and pyrrolidine [FL-no: 14.064] were tested for promoting activity in male Wistar rats given a single dose of 100 mg/kg bw of the test substance by gavage in dimethyl sulphoxide or 1 % Tylose. The number of mitoses in the adrenal cortex was examined 36 h after dosing. Only administration of pyrrolidine in dimethyl sulphoxide caused a statistically significant increase (approximately two-fold) in the number of mitoses over that in controls (Danz and Urban, 1979).

Piperine [FL-no: 14.003] and piperidine [FL-no: 14.010] did not cause mutations in male germ cells, as assessed by sperm shape abnormality and tests for dominant lethal mutations in mice and hamsters. Mice given piperine at doses of up to 75 mg/kg bw/day by gavage or up to 4 mg/kg bw per day by intraperitoneal injection for 5 days showed no sperm shape abnormalities or dominant lethal mutations (Muralidhara and Narasimhamurthy, 1990; Karekar et al., 1996; Daware et al., 2000). In another study, an oral dose of 400 mg/kg bw/day of piperidine for 40-100 days did not induce sperm shape abnormalities in mice or hamsters (Bempong and Scully, 1983).

Conclusion on genotoxicity

Negative results were reported in bacterial assays for reverse mutation with 15 aliphatic and aromatic amine and amide derivatives: ethylamine, isopropylamine, butylamine, isobutylamine, sec-butylamine, pentylamine, acetamide, 2-isopropyl-N,2,3-trimethylbutyramide, Deca-(2E,4E)-dienoic acid isobutyl-amide, piperine, piperidine, pyrrolidine, trimethylamine, triethylamine and piperazine.

Two substances, tyramine and piperidine, gave both positive and negative results in the mouse lymphoma assay, particularly at cytotoxic concentrations, while nitrosated tyramine gave positive results in the SOS Chromotest with *E. coli*.

Piperine and piperidine consistently gave negative results in a variety of studies *in vivo*, whereas acetamide, 1,6-hexalactam and pyrrolidine gave mainly negative results with some positive findings.

For a summary of *in vitro / in vivo* genotoxicity data considered by JECFA, see Table 2.

4.2. EFSA Considerations

The only valid positive *in vivo* genotoxicity studies cited by JECFA are related to acetamide, which JECFA considered inappropriate to be used as a flavouring substance due to its reported carcinogenicity in both rats and mice, and consequently it was not evaluated using the Procedure.

N-nonanoyl 4-hydroxy-3-methoxybenzylamide [FL-no: 16.006] (Nonivamide, pelargonyl vanillylamide (PAVA)) is structurally related to capsaicin. Capsaicin has been evaluated by SCF in 2002 (SCF, 2002) and concluded to have shown genotoxic effects *in vitro* and *in vivo* and accordingly deleted from the Register. However, *N*-nonanoyl 4-hydroxy-3-methoxybenzylamide has more recently been evaluated by the UK Committee on Toxicity of Chemicals in Food in 2004 (COT, 2004) and concluded not to be an *in vivo* mutagen. The Panel agrees with this conclusion made by COT in 2004, based on the negative results of a bone marrow cytogenetic assay (COT, 2002) and an *in vivo* liver unscheduled DNA synthesis assay (Clay, 2003). Accordingly *N*-nonanoyl 4-hydroxy-3-methoxybenzylamide [FL-no: 16.006] can be evaluated through the Procedure along the B-side, which is also done by JECFA.

For 1,6-hexalactam [FL-no: 16.052] the Panel noted an increased frequency of spots in only one of three or four trials in the mouse spot tests. In addition, sex-linked recessive lethal mutations and somatic mutations were reported in female *Drosophila* larvae fed 1,6-hexalactam. However, 1,6-hexalactam did not show carcinogenic effects in male and female mice and rats following daily administration for two years of up to 2250 mg/kg bw (mice) or up to 350 mg/kg bw (rats).

The Panel agreed with JECFA that the available studies on genotoxicity did not preclude the evaluation of the aliphatic and arylalkyl amines and amides by using the Procedure.

For *N*-cyclopropyl (2E,6Z)-nonadienamide [former candidate substance FL-no: 16.093, not used as flavour in EU any more] additional genotoxicity data have been submitted by EFFA (Bowles, 2003). The substance was tested in a bacterial reverse mutation test using *S. typhimurium* strains TA98, TA100, TA1535, 1537 and *E. coli* strain WP2uvrA with and without metabolic activation (see Table 3). It was concluded to be negative regarding the induction of mutagenicity.

5. New Toxicity Data Considered by the Panel in FGE.86Rev2

5.1. 90-day Dietary Toxicity Study in Crl:CD (SD) Rats with Piperine [FL-no: 14.003]

A 90-day study was performed with piperine [FL-no: 14.003] (Bauter, 2013). The study was performed according to OECD Guideline (TG 408). Four groups of adult Crl: Sprague-Dawley® CD® IGS rats (10/sex/group) were maintained on diets, calculated to provide piperine intake levels of 4.8, 14.5 and 47.8 mg/kg bw/day in males and 4.8, 14.6 and 48.4 mg/kg bw/day in females, giving an average daily intake of 0 (vehicle), 5, 15 or 50 mg/kg bw/day for males and females for at least 90 days.

Homogeneity, stability, and concentration analyses of the test diets indicate that piperine was homogeneously distributed, stable and was considered to have met target concentrations in the diet for all intake levels.

Prior to study initiation and again on day 86, the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. The animals were observed for viability, signs of gross toxicity, and behavioral changes, occurring at least once daily during the study, and weekly for a battery of detailed clinical parameters. Body weights were recorded twice during acclimation, including prior to test

initiation (day 0), and together with food consumption, approximately weekly thereafter, and prior to sacrifice. Urine and blood samples were collected on day 85 from all study animals for urinalysis, hematology, and clinical chemistry determinations. Coagulation assessments were performed on day 92 or 93, prior to necropsy. Gross necropsies and histological evaluation of selected organs and tissues were performed on all study animals.

There were no mortalities, clinical or ophthalmological changes, attributable to piperine administration. Decreased male body weight gain (20 % reduction) and male (15 % reduction) and female (12 % reduction) food consumption at 50 mg/kg bw/day target dietary intake were considered the result of decreased food intake related to administration of high dietary concentrations of piperine since there was no effect on food efficiency. No effect was observed on the final body weights.

There were no gross and microscopic changes or clinical pathology or organ weight changes attributed to the administration of piperine. Some statistically significant changes in hematology, coagulation, and clinical chemistry parameters were not dose-dependent, small in magnitude and within the range of historical values. A statistical significant and dose-dependent increase in cholesterol in males was observed at 15 and 50 mg/kg bw/day, with approximately 30 % and 55 %, respectively. No effect on cholesterol was observed in females. Similarly, no changes in organ weight or relative organ weight were observed in males or females, except for a reduction in relative epididymides weight at 5 and 50 mg/kg bw/day in males. This change was small and not dose-dependent, and therefore of limited toxicological relevance.

Based on the dose dependent increased in plasma cholesterol levels in males at the mid and high dose, the Panel decided that the lowest dose level of 5 mg/kg bw/day should be considered as the NOAEL.

5.2. 14- and 90-Day Dietary Study in Rats with Deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091]

14-day study

A 14-day dose-range finding GLP study was performed with the candidate substance deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no:16.091] (Koetzner, 2013b). Five groups of adult Crl: Sprague-Dawley® CD® IGS rats (5/sex/group) were placed into two control and three test groups. The test compound was added to the diet in a 10 % solution of the vehicle (55 % 1,2-propylene glycol, 45 % diethyl malonate). The control groups were a basal diet control (no vehicle) as well as a control which received the vehicle. The diets were prepared such that the food consumption (based on food intake and body weight) resulted in an intake of the candidate substance of 33, 170 and 330 mg/kg bw/day for males, and 35, 185 and 380 mg/kg bw/day for females during 14 days, respectively. Based on reductions in food consumption and decreases in body weight and body weight gain in the high dose group and the absence of such changes in the middle dose group indicates that male and female rats can be expected to tolerate a dose level of approximately 170 (males) or 185 (females) mg/kg bw/day of deca-(2E,4E)-dienoic acid isobutyl-amide in a study of longer duration.

90-day study

A 90-day study was performed with deca-(2E,6Z)-dienoic acid isobutyl-amide [FL-no: 16.091] (Koetzner, 2013a). The study was performed according to OECD guideline (TG 408). Five groups of adult Crl: Sprague-Dawley® CD® IGS rats (10/sex/group) were placed into two control and three test groups. The diets were prepared such that the food consumption (based on food intake and body weight) resulted in an intake of the vehicle (55 % 1,2-propylene glycol, 45 % diethyl malonate), of 0 or 1000 mg/kg bw/day and of the test diets (containing 10 % of the test compound in the vehicle) of 100, 400 or 1000 mg/kg bw/day, resulting in intake of respectively 10, 40 and 100 mg/kg bw/day of the test article, deca-(2E,4E)-dienoic acid isobutyl-amide for males and females during 90 days, based on body weight and feed consumption data.

Homogeneity, stability, and concentration analyses of the test diets indicate that deca-(2E,4E)-dienoic acid isobutyl-amide was homogeneously distributed, stable and was considered to meet target concentrations in the diet for all intake levels.

The animals were examined by focal illumination and indirect ophthalmoscopy prior to initiation and again at the end of the study (day 85), observed for viability, signs of gross toxicity and behavioral changes at least once daily during the study and weekly for a battery of detailed clinical observations. Urine and blood samples were collected on day 90 from all study animals for urinalysis, hematology and clinical chemistry determinations and additional blood samples were collected for coagulation assessments on Day 95/96, prior to necropsy. Gross necropsies and histological evaluation of selected organs and tissues were performed on all study animals.

There were no mortalities, clinical observations, ophthalmological, clinical pathology, body weight, body weight gain, food consumption (males) or food efficiency changes attributable to deca-(2E,4E)-dienoic acid isobutyl-amide administration. A statistically significant reduction in food consumption was observed at the highest dose level, 100 mg/kg bw/day, in females, but not in males. There were no macroscopic findings or organ weight changes attributable to the administration of deca-(2E,4E)-dienoic acid isobutyl-amide. Microscopically, hypertrophy of the acinar cells in the submandibular salivary gland was observed in males at 40 mg/kg bw/day (4/10) and 100 mg/kg bw/day (10/10) and in females only at 100 mg/kg bw/day (9/10) at 100 mg/kg bw/day. Hypertrophy was characterised microscopically by diffuse enlargement of acinar cells with slightly basophilic, stippled cytoplasm. The severity was predominantly slight in males at 40 mg/kg bw/day and moderate at 100 mg/kg bw/day, indicating a dose-dependent effect. Since the changes in the submandibular salivary glands were not observed in the naïve and vehicle control groups in male and female, this effect was attributed to the test substance.

Based on the toxicological endpoints evaluated, the no-adverse-effect level (NOAEL) for administration of deca-(2E,4E)-dienoic acid isobutyl-amide in the diet (as a 10 % mixture in vehicle) was determined to be 10 mg/kg bw/day for males as indicated by the histological changes in the submandibular salivary glands at 40 and 100 mg kg bw day.

A summary of the toxicity studies is given in Table 4.

6. Application of the Procedure

6.1. Application of the Procedure to Aliphatic and Arylalkyl Amines and Amides by JECFA (JECFA, 2006b)

After publication of FGE.86, JECFA has re-evaluated flavouring substances for which estimated intake was originally based on anticipated poundage data (JECFA, 2009). New annual production volumes were submitted to JECFA by the Flavour Industry for [FL-no: 11.002, 11.004, 11.005, 11.007, 11.015, 11.016, 11.017, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.080, 14.133, 14.141 and 16.052]. JECFA concluded that there was “no safety concern” for these substances.

No new monograph was prepared, so all text about anticipated poundage in the below text should not be taken into account.

Step 1.

In applying the Procedure for the safety evaluation to these flavouring substances, the Committee assigned 15 substances [FL-no: 11.001, 11.002, 11.003, 11.004, 11.005, 11.009, 11.015, 11.016, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026 and 16.092] to structural class I, eight flavouring substances [FL-no: 11.006, 11.007, 14.010, 14.064, 14.080, 14.133, 14.141 and 14.167] to structural class II and the remaining seven flavouring substances [FL-no: 11.017, 14.003, 16.006, 16.013, 16.052, 16.053 and 16.091] to structural class III.

Step 2.

Twenty-three flavouring substances in this group, namely all those in structural classes I and II [FL-no: 11.001, 11.002, 11.003, 11.004, 11.005, 11.006, 11.007, 11.009, 11.015, 11.016, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.010, 14.064, 14.080, 14.133, 14.141, 14.167 and 16.092] are predicted to be metabolised to innocuous products. The evaluation of these substances therefore proceeded via the A-side of the Procedure.

For the seven flavouring substances in structural class III, namely the medium chain saturated and unsaturated aliphatic and alicyclic amides [FL-no: 11.017, 14.003, 16.006, 16.013, 16.052, 16.053, 16.091,] limited metabolic data were available, and evaluation of these substances therefore proceeded via the B-side of the Procedure.

Step A3.

The estimated daily *per capita* exposures to all 15 flavouring substances in structural class I are below the threshold of concern (1800 µg/person/day for class I). Three of these 15 substances [FL-no: 11.001, 11.003 and 11.009] are reported to be currently used as flavouring substances, and, according to the Procedure, the current use and exposure levels of these three substances raise no safety concern. The other 12 substances [FL-no: 11.002, 11.004, 11.005, 11.015, 11.016, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026 and 16.092] are proposed for use as flavouring substances. Although, according to the Procedure, the use of these 12 substances raises no safety concern at the exposure estimated from anticipated volumes of production, less uncertain estimates are needed. The estimated daily per capita exposure to all nine flavouring substances in structural class II is below the threshold of concern (540 µg/day). Three of these nine substances [FL-no: 11.006, 14.010 and 14.064] are reported to be used as flavouring substances, and, according to the Procedure, their use raises no safety concern at current estimated level of exposure. The other five substances [FL-no: 11.007, 14.080, 14.133, 14.141 and 14.167] are proposed for use as flavouring substances. Although, according to the Procedure, use of these six substances raises no safety concern at the exposure levels estimated from anticipated volumes of production, less uncertain exposure estimates are needed.

Step B3.

The estimated *per capita* exposures to five of the flavouring substances in structural class III [FL-no: 11.017, 14.003, 16.006, 16.052 and 16.091] are below the threshold of concern (90 µg/person/day). One of these substances [FL-no: 14.003] is reported to be used as a flavouring substance in Europe and the USA, one [FL-no: 16.006] is reported to be used in Europe and to be proposed for use in the USA, and three [FL-no: 11.017, 16.052 and 16.091] are proposed for use in both regions. For those five substances proposed for use in flavours in one or more region [FL-no: 11.017, 14.003, 16.006, 16.052 and 16.091] less uncertain exposure estimates are needed. In accordance with the Procedure, evaluation of these eight flavouring substances proceeded to Step B4.

The *per capita* exposures in the USA of the two remaining flavouring substances in structural class III, 2-isopropyl-N-2,3-trimethylbutyramide ([FL-no: 16.053]; exposure, 1054 µg/day) and N-ethyl-2-isopropyl-5-methylcyclohexane carboxamide ([FL-no: 16.013]; exposure, 127 µg/day), exceed the threshold of concern for their structural class (90 µg/person/day). In accordance with the Procedure, data must be available on these substances or closely related substances for a safety evaluation. For [FL-no: 16.053], which is proposed for use as a flavouring substance, a less uncertain exposure estimate is needed.

Step B4.

The No Observed Effect Level (NOEL) of 750 mg/kg bw/day for 1,6-hexalactam [FL-no: 16.052] in a 90-day feeding study in rats (NTP, 1982) is at least 2.5×10^{10} times higher than the estimated

exposure from its proposed use as a flavouring substance in Europe (0.00002 µg/kg bw/day) and in the USA (0.00003 µg/kg bw/day).

The NOEL of 572 mg/kg bw/day for the structurally related substance, N-isobutyl-2,6,8-decatrienamide [FL-no: 16.121] (Moore, 2002), is applicable to N-ethyl (2E,6Z)-nonadienamide [FL-no: 16.094]] and deca-(2E,4E)-dienoic acid isobutylamide [FL-no: 16.091], as they follow similar pathways of metabolism. This NOEL is 600000 times the estimated exposure to N-ethyl (2E,6Z)-nonadienamide [FL-no: 16.094] from its proposed use as a flavouring substance in the USA (1 µg/kg bw/day) and at least 600000 times the estimated exposure to deca-(2E,4E)-dienoic acid isobutylamide [FL-no: 16.091] from its proposed use as flavouring substance in Europe and in the USA (both 1 µg/kg bw/day).

The NOEL of 8.4 mg/kg bw/day for N-nonanoyl 4-hydroxy-3-methoxybenzylamide [FL-no: 16.006] (Posternak et al., 1969) is more than 70000 times the estimated exposure from its proposed use as a flavouring substance in Europe (0.1 µg/kg bw/day) and 8.4×10^6 times that in the USA (0.001 µg/kg bw/day).

The NOEL of 20 mg/kg bw/day for piperine [FL-no: 14.003] (Bhat and Chandrasekhara, 1986) is 50000 times the estimated exposure to piperine from its reported use as a flavouring substance in Europe (0.4 µg/kg bw/day) and 2×10^7 times that in the USA (0.001 µg/kg bw/day).

The NOEL of 115 mg/kg bw/day for the structurally related substance sec-butylamine [FL-no: 11.005] (Gage, 1970) is applicable to N-isopentylidene isopentylamine [FL-no: 11.017] and is at least 5.75×10^8 times the estimated intake to N-isopentylidene isopentylamine from its proposed use as flavouring substance in Europe (0.0001 µg/kg bw/day) and in the USA (0.0002 µg/kg bw/day).

Consideration of flavour substances with high exposure, evaluated via the B-side of the Procedure

In accordance with the Procedure, more data on toxicity were considered to evaluate the safety of 2-isopropyl-*N*-2,3-trimethylbutyramide [FL-no: 16.053] and *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide [FL-no: 16.013], as the estimated exposure levels from proposed use [FL-no: 16.053] and reported use [FL-no: 16.013] as flavouring substances were determined to exceed the threshold of concern for structural class III (90 µg per person per day).

The results of three studies in Sprague-Dawley (CD®) rats treated by gavage were available on 2-isopropyl-*N*-2,3-trimethylbutanamide [FL-no: 16.053]: a 14-day study in groups of six rats of each sex at a dose of 0, 5, 25 or 50 mg/kg bw in corn oil twice daily (Nixon and Alden, 1978); a 14-week study in groups of 30 rats of each sex at a dose of 0, 10, 50 or 100 mg/kg bw in corn oil once daily (Pence, 1980a); and a 14-week study in groups of 30 rats of each sex at a dose of 0, 1, 2, 5, 10 or 50 mg/kg bw in corn oil once daily (Cheng, 1982). The studies showed treatment-related hepatic and renal toxicity at doses of 10 mg/kg bw and higher. The NOEL was 5 mg/kg bw/day, on the basis of histopathological lesions in the kidneys of male rats in the 14-week study (Cheng, 1982). A study of reproductive and teratogenic toxicity in rats at a dose of 0, 10, 50 or 100 mg/kg bw showed no reproductive effects or foetal abnormalities (Pence, 1980b). The NOEL of 5 mg/kg bw/day is 280 times the estimated daily exposure to 2-isopropyl-*N*-2,3-trimethylbutyramide [FL-no: 16.053] when used as a flavouring substance in the USA (18 µg/kg bw/day).

Two studies were conducted on N-ethyl-2-isopropyl-5-methylcyclohexane carboxamide [FL-no: 16.013] in rat treated by gavage: a 28-day study in groups of six Crj:CD(SD) rats of each sex at a dose of 0, 8, 40, 200 or 1000 mg/kg bw/day (Miyata, 1995) and a 22-week study in groups of 15 Sprague-Dawley (CFY) rats of each sex at a dose of 0, 100, 300 or 725 mg/kg bw/day. Mild toxicity in the liver and kidneys was observed at doses of 40 mg/kg bw and above. Two further studies were conducted in beagle dogs given gelatine capsules: a 28-day study in groups of one male and one female given a dose of 0, 600, 1000 or 1500 mg/kg bw/day and a 52-week study in groups of three animals of each sex given a dose of 0, 100, 300 or 1000 mg/kg bw/day (James, 1974). These studies showed mild toxic

effects in the liver at all doses. The NOEL of 8 mg/kg bw/day in these studies is 1000000 times the estimated daily exposure to N-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide when used as a flavouring substance in Europe (0.008 µg/kg bw/day) and 4000 times that in the USA (2 µg/kg bw/day).

The additional toxicity data indicate that 2-isopropyl-*N*-2,3-trimethylbutanamide [FL-no: 16.053] and N-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide [FL-no: 16.013] would not be expected to raise safety concerns at their estimated levels of exposure when used as flavouring substances. For one of these substances [FL-no: 16.053], however, less uncertain exposure estimates are needed, as the existing estimate was based on anticipated poundage.

In conclusion, JECFA evaluated all 30 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the aliphatic and arylalkyl amines and amides are summarised in Table 6: Summary of Safety Evaluation by JECFA (JECFA, 2006b).

6.2. EFSA Considerations

After the publication of FGE.86 Industry has submitted additional data (toxicity data and mutagenicity data) on substance [FL-no: 16.095] (evaluated in FGE.94 (EFSA CEF Panel, 2010)) to support the evaluation of [FL-no: 16.091]. However, the Panel consider the substance [FL no: 16.095] not to be sufficiently structurally related to the candidate substance [FL-no: 16.091] owing to no cyclopropyl group in [FL-no: 16.091]. Due to the structural difference, routes of metabolism will also be different and also difference in toxicity must be anticipated.

The Panel agrees with the application of the Procedure as performed by JECFA for 27 of the 30 substances in the group.

N-isopentylidene isopentylamine [FL-no: 11.017] is anticipated to be completely hydrolysed to isopentylamine and isopentylaldehyde, which are further metabolised to innocuous products. Accordingly [FL-no: 11.017] can be anticipated to be metabolised to innocuous products and evaluated along the A-side of the Procedure. JECFA evaluated [FL-no: 11.017] along the B-side. As the estimated European *per capita* intake of 0.0073 µg is below the threshold of concern for structural class III substances of 90 µg/person/day, the Panel concluded (as did JECFA) that there was no safety concern of the estimated level of intake of [FL-no: 11.017] based on the MSDI approach.

For piperine [FL-no: 14.003], JECFA derives a NOAEL of 20 mg/kg bw/day from a 56-day feeding study, in which groups of six rats were given different doses of black pepper or oleoresin corresponding to up to approximately 20 mg/kg bw/day or 100 mg piperine/kg feed corresponding to up to approximately 10 mg/kg bw/day. No histopathology was performed. The Panel did not agree with JECFA that the study is appropriate for deriving a NOAEL to be used at step B4 of the Procedure for piperine [FL-no: 14.003], and accordingly additional data were required. In response to this data request the Flavour Industry submitted a 90-day oral toxicity study in rats (summarised in Section 5.1) with piperine [FL-no: 14.003]. Based on this new study the Panel could derive a NOAEL of 5 mg/kg bw/day. When the exposure estimate for [FL-no: 14.003], based on MSDI approach, of 6.2 µg *per capita* per day is compared to the NOAEL for [FL-no: 14.003], an adequate margin of safety of more than 4.8×10^4 can be calculated for piperine.

For deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091] JECFA makes use of a NOAEL derived from a structurally related substance. A NOAEL of 572 mg/kg bw/day for N-isobutyl-2,6,8-decatrienamide [FL-no: 16.121] has been derived from a 28-day feeding study with groups of 10 rats given different amounts of an extract of unknown purity from gold root (*Halopsis longiper*) with an estimated concentration of 50 % of N-isobutyl-2,6,8-decatrienamide (Moore, 2002). This study is also considered in FGE.303, in which N-isobutyl-2,6,8-decatrienamide [FL-no: 16.121] is the candidate substance. The Panel did not agree with JECFA that the study is appropriate for deriving a NOAEL to

be used at step B4 of the Procedure for the substance [FL-no: 16.091], and accordingly additional data are required. In response to this request expressed in FGE.86, the Flavour Industry has now submitted a palatability and range-finding 14-day study and a 90-day oral toxicity study in rats (the 90-day toxicity study is summarised in Section 5.2) with substance [FL-no: 16.091]. Based on this new study the Panel could derive a NOAEL of 10 mg/kg bw/day. When the exposure estimate for [FL-no: 16.091], based on MSDI approach, of 11 µg *per capita* per day is compared to the NOAEL for [FL-no: 16.091], an adequate margin of safety of more than 5.5×10^4 can be calculated for deca-(2E,4E)-dienoic acid isobutyl-amide.

The Panel considered further the possible consequences of nitrosation after ingestion of the secondary and tertiary amine and secondary amide candidate substances according to the approach described in the Annex to the minutes of the 30th AFC Panel meeting, May 2008 (EFSA, 2008b). From these considerations, the Panel concluded that extremely large margins of exposure could be calculated ($>> 10^9$) for nitrosated products possibly formed from amines used as flavouring substances in foods. Such large margins of exposure indicate that a risk of carcinogenicity resulting from such possible nitrosation products is virtually absent.

The Panel also noted that this conclusion is not applicable for foods preserved with nitrites, because for such foods the conditions for nitrosation, either in the foods themselves or after consumption in the stomach may differ substantially from the worst-case conditions on which the calculations in the above mentioned Annex were based.

CONCLUSION

The Panel has considered 30 out of the 37 substances in JECFA flavouring group of aliphatic and arylalkyl amines and amides.

Two of the 37 substances, (1-amino-2-propanol and acetamide; JECFA-no: 1591 and 1592, respectively), evaluated by JECFA in this group are not used as flavouring substances in EU and are therefore not included in the Register and accordingly not in the Union List. A third substance evaluated by JECFA is an α,β-unsaturated ketone [FL-no: 14.168] considered with respect to genotoxicity in FGE.223, corresponding to subgroup 5.1 of FGE.19, for which a final conclusion regarding its genotoxic properties could not be reached and additional data were requested. Since the publication of FGE.86Rev1, the Industry has informed that four substances are no longer used as flavouring substances in the EU. This consideration therefore only deals with 30 flavouring substances.

This revision of FGE.86 is made because additional toxicity data, which were requested in previous opinions, have now been provided for piperine [FL-no: 14.003] and deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091].

In previous versions of FGE.86 the Panel agreed with the application of the Procedure as performed by JECFA for 27 of the 30 substances. For N-isopentylidene isopentylamine [FL-no: 11.017] the Panel concluded that this substance can be metabolised to innocuous products and accordingly evaluated along the A-side of the Procedure to be of no safety concern at step A3 (while JECFA evaluated [FL-no: 11.017] along the B-side). For two substances [FL-no: 14.003 and 16.091] the Panel concluded that no appropriate studies are available for deriving NOAELs to provide an adequate margin of safety. Therefore, the Panel concluded that in FGE.86 additional toxicity data are required for these two substances.

Since the publication of FGE.86Rev1, two 90-day oral toxicity studies have become available for piperine [FL-no: 14.003] and deca-(2E,4E)-dienoic acid isobutyl-amide [FL-no: 16.091]. From these studies the Panel could derive NOAELs which can provide adequate margins of safety for these two substances at the estimated levels of exposure based on the MSDI approach..

For 18 substances [FL-no: 11.002, 11.004, 11.005, 11.007, 11.015, 11.016, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.080, 14.133, 14.141, 16.052, 16.091 and 16.092], food categories and use levels in these has been provided by the Industry. Based on these use levels, mTAMDI figures could be calculated. For one substance ([FL-no: 16.092] from structural class I the mTAMDI of 15000 µg/person/day exceeds the threshold for the structural class of 1800 µg/person/day. Also for one substance ([FL-no: 14.141] in structural class II the mTAMDI of 600 µg/person/day exceeds the threshold for the structural class of 540 µg/person/day and finally two substances [FL-no: 16.052 and 16.091] in structural class III exceed the threshold of the structural class of 90 µg/person/day with mTAMDI figures of 200 and 770 µg/person/day, respectively. Thus, for these four substances, [FL-no: 14.141, 16.052, 16.091 and 16.092], more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be considered using the Procedure. Subsequently, additional data might become necessary.

For the remaining 12 substances [FL-no: 11.001, 11.003, 11.006, 11.009, 11.017, 14.003, 14.010, 14.064, 14.167, 16.006, 16.013 and 16.053] use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity are available for all JECFA evaluated substances.

In conclusion, for all JECFA evaluated aliphatic and arylalkyl amines and amides [FL-no: 11.001, 11.002, 11.003, 11.004, 11.005, 11.006, 11.007, 11.009, 11.015, 11.016, 11.017, 11.018, 11.020, 11.021, 11.023, 11.025, 11.026, 14.003, 14.010, 14.064, 14.080, 14.133, 14.141, 14.167, 16.006, 16.013, 16.052, 16.053, 16.091 and 16.092] the Panel agrees with JECFA conclusion “no safety concern at estimated levels of intake based on the MSDI approach”.

SUMMARY OF GENOTOXICITY DATA

Table 2: Genotoxicity Data (*In Vitro / In Vivo*) evaluated by JECFA (JECFA, 2006b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
In Vitro						
11.015 1579	Ethylamine	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	100 to 10000 µg/plate	Negative[1]	Mortelmans et al., 1986
11.018 1581	Isopropylamine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	10 to 10000 µg/plate	Negative[1]	Zeiger et al., 1987
11.003 1582	Butylamine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	3.3 to 3333 µg/plate	Negative[1]	Zeiger et al., 1987
		Reverse Mutation	*S.typhimurium* TA100	3 µmol/plate (219 µg/plate)[2]	Negative[1]	Florin et al., 1980
11.002 1583	Isobutylamine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	33 to 10000 µg/plate	Negative[1]	Mortelmans et al., 1986
11.005 1584	sec-Butylamine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	10 to 3333 µg/plate	Negative[1]	Zeiger et al., 1987
11.021 1585	Pentylamine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	33 to 3333 µg/plate	Negative[1]	Mortelmans et al., 1986
11.007 1590	2-(4-Hydroxy-phenyl)ethylamine	Forward Mutation	Mouse lymphoma L5178Y cells	500 to 3500 µg/ml	Negative	McGregor et al., 1988
		Forward Mutation	Mouse lymphoma L5178Y cells	0.08, 0.80, 2.0, 4.0 or 6.0 mM (11, 109, 274, 548 and 823 µg/ml)[3,4]	Positive [5,6]	Wangenheim and Bolcsfoldi, 1988
		Forward Mutation	Mouse lymphoma L5178Y cells	0.40, 0.80, 1.60, 2.39 or 3.20 mM (55, 109, 220, 327 and 439 µg/ml)[3,4]	Positive [6,7]	Wangenheim and Bolcsfoldi, 1988
- 1592	Acetamide (not in Register)	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	100 to 10000 µg/plate	Negative[1]	Haworth et al., 1983
		DNA Repair	*Escherichia coli* 343/591 *uvr*B$^-$/*rec*A$^-$/*lac*$^+$ and *uvr*B$^+$/*rec*A$^+$/*lac*$^+$	Up to 1,080 mM (63793 µg/ml)[8]	Negative[1]	Hellmér and Bolcsfoldi, 1992
		Single Strand DNA Breaks	Rat hepatocytes	0.03, 0.3, 3, 10, 30, 100, 300 or 1000 mM (2, 18, 177, 591, 1772, 5907, 17720 or 59068 µg/ml)[8]	Negative	Sina et al., 1983
16.053	2-Isopropyl- N,2,3-	Reverse Mutation	*S.typhimurium* TA98,	200, 1000, 5000, 10000, or 20000	Negative[1]	Haworth et al., 1978

Table 2: Genotoxicity Data (*In Vitro / In Vivo*) evaluated by JECFA (JECFA, 2006b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
1595	trimethylbutanamide		TA100, TA1535, TA1537, TA1538	µg/plate		
		Forward Mutation	Mouse lymphoma L5178Y cells	0.01 to 1000 µg/ml	Negative[1]	Kirby et al., 1978
		Unscheduled DNA Synthesis	WI-38 cells (human)	125 to 2000 µg/ml[9]	Negative[1]	Skinner, 1978
16.091 1598	Deca-(2*E*,4*E*)-dienoic acid isobutyl-amide	Reverse Mutation	*S.typhimurium* TA98, TA100, TA102, TA1535, TA1537	5 to 1500 µg/plate[10]	Negative[5]	King, 2003
		Reverse Mutation	*S.typhimurium* TA98, TA100, TA102, TA1535, TA1537	5 to 5000 µg/plate[11]	Negative[7]	King, 2003
14.003 1600	Piperine	Reverse Mutation	*S.typhimurium* TA97a, TA98, TA100, TA102	0.01, 0.5, or 10 µmol/plate (3, 143 and 2853 µg/plate)[12]	Negative[1]	Karekar et al., 1996
		Reverse Mutation (pre-incubation)	*S.typhimurium* TA97a, TA98, TA100, TA102	0.005, 0.05, 0.5 or 5 µmol/plate (1, 14, 143 and 1427 µg/plate)[12,13]	Negative[1]	Karekar et al., 1996
		Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1,000 µg	Negative[1]	Andrews et al., 1980
14.010 1607	Piperidine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (255 µg/plate)[14]	Negative[1]	Florin et al., 1980
		Reverse Mutation	*S.typhimurium* TA1530, TA1531, TA1532, TA1964	1 to 5 mg/plate (1,000 to 5,000 µg/plate)[14]	Negative	Green and Savage, 1978
		Reverse Mutation (microsomal assay)	*S.typhimurium* TA1530, TA1531, TA1532, TA1964	0.15 M (12772 µg/ml)[14,15]	Negative	Green and Savage, 1978
		Reverse Mutation (host-mediated, mice)	*S.typhimurium* TA1950, TA1951, TA1952, TA1964	800 mg/kg bw[16]	Negative	Green and Savage, 1978
		Forward Mutation	Mouse lymphoma L5178Y cells	3.03, 4.04, 5.05, 6.06 or 7.07 mM (258, 344, 430, 516 and 602 µg/ml)[14]	Positive [5,6]	Wangenheim and Bolcsfoldi, 1988
		Forward Mutation	Mouse lymphoma L5178Y cells	4.04, 5.05, 6.06, 7.07 or 8.08 mM (344, 430, 516, 602 or 688 µg/ml)[14]	Negative[7]	Wangenheim and Bolcsfoldi, 1988
		Forward Mutation	Mouse lymphoma L5178Y	2.0, 4.01 or 6.01 mM	Negative[5]	Garberg et al., 1988

Table 2: Genotoxicity Data (*In Vitro / In Vivo*) evaluated by JECFA (JECFA, 2006b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
			cells	(170, 341 or 512 µg/ml)[14]		
		Forward Mutation	Mouse lymphoma L5178Y cells	2.0, 4.01, 6.01 or 8.02 mM (170, 341, 512 or 683 µg/ml)[14]	Equivocal [7,17]	Garberg et al., 1988
		DNA Repair	*Escherichia coli* 343/591 *uvr*B$^-$/*rec*A$^-$/*lac*$^+$ and *uvr*B$^+$/*rec*A$^+$/*lac*$^+$	33.7 mM (2870 µg/ml)[14,18]	Negative[5]	Hellmér and Bolcsfoldi, 1992
		DNA Repair	*Escherichia coli* 343/591 *uvr*B$^-$/*rec*A$^-$/*lac*$^+$ and *uvr*B$^+$/*rec*A$^+$/*lac*$^+$	101 mM (8600 µg/ml)[14,18]	Negative[5]	Hellmér and Bolcsfoldi, 1992
		Single Strand DNA Breaks	Rat hepatocytes	0.03, 0.3 or 3 mM (2.6, 26 and 255 µg/ml)[14]	Negative	Sina et al., 1983
14.064 1609	Pyrrolidine	Reverse Mutation	*S.typhimurium* TA100	Up to 3 µmol/plate (213 µg/plate)[19]	Negative[1]	Florin et al., 1980
		Reverse Mutation	*S.typhimurium* TA1530, TA1531, TA1532, TA1964	1000 to 5000 µg/plate[3]	Negative	Green and Savage, 1978
		Reverse Mutation (microsomal assay)	*S.typhimurium* TA1530, TA1531, TA1532, TA1964	0.5 M (35561 µg/ml)[19]	Negative	Green and Savage, 1978
		Reverse Mutation (host-mediated, mice)	*S.typhimurium* TA1950, TA1951, TA1952, TA1964	800 mg/kg bw[16]	Negative	Green and Savage, 1978
11.009 1610	Trimethylamine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	10 to 1000 µg/plate	Negative[1]	Mortelmans et al., 1986
11.023 1611	Triethylamine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	10 to 10000 µg/plate	Negative[1]	Zeiger et al., 1987
14.141 1615	Piperazine	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	33 to 3167 µg/plate	Negative[1]	Haworth et al., 1983
In Vivo						
- 1592	Acetamide (not in Register)	DNA Damage (Comet assay)	Male ddy mice	2000 mg/kg bw[20]	Positive [21]	Sasaki et al., 2000
		Micronuclei (bone marrow)	C57B1/6 mice	2500 or 5000 mg/kg bw[22]	Negative	Mirkova, 1996
		Micronuclei (bone	Male CBA mice	5000 mg/kg bw[22]	Negative	Mirkova, 1996

Table 2: Genotoxicity Data (*In Vitro / In Vivo*) evaluated by JECFA (JECFA, 2006b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
		marrow)				
		Micronuclei (bone marrow and peripheral blood)	Male CD1 mice	500 to 5000 mg/kg bw[23]	Negative	Morita et al., 1997
		Micronuclei (bone marrow and peripheral blood)	Male BDF1 mice	1250 to 5000 mg/kg bw[23]	Negative	Morita et al., 1997
		Micronuclei (bone marrow)	Female C57B1/6 mice	3.39 mmol/kg bw (200 mg/kg bw)[24,25]	Positive	Chieli et al., 1987
16.052 1594	1,6-Hexalactam	DNA Damage (Comet assay)	Male ddy mice	2000 mg/kg bw[22]	Negative	Sasaki et al., 2000
		Replicative DNA Synthesis	Male F344 rats	350 or 700 mg/kg bw[26]	Negative	Uno et al., 1994
		Replicative DNA Synthesis	Male B6C3F_1 mice	250 or 500 mg/kg bw[22]	Negative	Miyagawa et al., 1995
		Mammalian Spot	(C57B1xT)F_1 mouse embryos	400 or 500 mg/kg bw[22]	Positive [27]	Fahrig, 1989
		Mammalian Spot	(TxHT)F_1 mouse embryos	500 mg/kg bw[28]	Positive [29]	Neuhäuser-Klaus and Lehmacher, 1989
		Mammalian Spot	(TxHT)F_1 mouse embryos	700 mg/kg bw[28]	Negative	Neuhäuser-Klaus and Lehmacher, 1989
		Sex-Linked Recessive Lethals	Male *Drosophila melanogaster* larvae	5.0 mM[30] (566 µg/ml)[31]	Negative	Vogel, 1989
		Sex-Linked Recessive Lethals	Female *Drosophila melanogaster* larvae	5.0 or 20.0 mM[30] (566 or 2263 µg/ml)[31]	Positive	Vogel, 1989
		Somatic Mutation/Mitotic Recombination	Female *Drosophila melanogaster* larvae	2.5, 5.0, 10.0 or 20.0 mM[40] (283, 566, 1132 and 2263 µg/ml)[31]	Positive	Vogel, 1989
		Chromosomal Aberrations (bone marrow)	Male and female 1C3F_1 mice	1000 mg/kg bw[22]	Negative	Adler and Ingwersen, 1989
14.003 1600	Piperine	Micronuclei (bone marrow)	Male Swiss mice	10 or 20 mg/kg bw[22]	Negative	Karekar et al., 1996
		Micronuclei (bone marrow)	Male Swiss mice	1, 2 or 4 mg/kg bw[32]	Negative	Muralidhara and Narasimhamurthy, 1990

Table 2: Genotoxicity Data (*In Vitro / In Vivo*) evaluated by JECFA (JECFA, 2006b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
		Sperm Morphology	Male Swiss mice	10 or 50 mg/kg bw/day[33]	Negative	Karekar et al., 1996
		Sperm Morphology	Male Swiss mice	35, 50 or 75 mg/kg bw/day[34]	Negative	Daware et al., 2000
		Sperm Morphology	Male Swiss mice	1, 2 or 4 mg/kg bw/day[35]	Negative	Muralidhara and Narasimhamurthy, 1990
		Dominant Lethal Mutations	Male and Female Swiss mice	10 or 50 mg/kg bw[22]	Negative	Karekar et al., 1996
		Dominant Lethal Mutations	Male Swiss mice	4 mg/kg bw/day[35]	Negative	Muralidhara and Narasimhamurthy, 1990
14.010 1607	Piperidine	Mitosis in Adrenocortical Cells	Male Wistar rats	100 mg/kg bw in DMSO[22]	Negative	Danz and Urban, 1979
		Mitosis in Adrenocortical Cells	Male Wistar rats	100 mg/kg bw in 1 % Tylose[22]	Negative	Danz and Urban, 1979
		Sperm Morphology	Male hybrid mice	400 mg/kg bw/day[36]	Negative	Bempong and Scully, 1983
		Sperm Morphology	Male golden Syrian hamsters	400 mg/kg bw/day[36]	Negative	Bempong and Scully, 1983
14.064 1609	Pyrrolidine	Mitosis in Adrenocortical Cells	Male Wistar rats	100 mg/kg bw in 1 % Tylose[22]	Negative	Danz and Urban, 1979
		Mitosis in Adrenocortical Cells	Male Wistar rats	100 mg/kg bw in DMSO[22]	Positive	Danz and Urban, 1979

[1] With and without S9.
[2] Calculated using the molecular weight of butrylamine (73.14 g/mol).
[3] Calculated using the molecular weight of tyramine (137.18 g/mol).
[4] Actual compound used in this study was tyramine hydrochloride at concentrations of 0.101 to 7.59 mM (18 to 1318 µg/ml) without metabolic activation, and 0.506 to 4.05 mM (88 to 703 µg/ml) with metabolic activation.
[5] Without metabolic activation.
[6] Significant increases in mutation frequency were observed only at cytotoxic doses.
[7] With metabolic activation.
[8] Calculated using the molecular weight of acetamide (59.07 g/mol).
[9] Cytotoxic at 2000 µg/ml.
[10] Toxic and precipitates at 1,500 µg/plate.
[11] Toxic and precipitates at 5,000 µg/plate.
[12] Calculated using the molecular weight of piperine (285.34 g/mol).

[13] Toxic at 5 µmol/plate without metabolic activation.
[14] Calculated using the molecular weight of piperidine (85.15 g/mol).
[15] Highest non-cytotoxic concentration.
[16] Intraperitoneal injection of *S. typhimurium* strain with intramuscular injection of test material.
[17] Results observed did not meet the criteria for positive or negative classification.
[18] Concentration at which a significant reduction in the number of colonies of each strain was observed; however, the highest concentration of piperidine tested was 1,010 mM.
[19] Calculated using the molecular weight of pyrrolidine (71.12 g/mol).
[20] Administered via a single intraperitoneal injection.
[21] Increase in DNA damage was observed in the stomach, colon, lungs and bone marrow of mice.
[22] Administered via a single gavage dose.
[23] Single, double, or quadruple intraperitoneal injections, separated by 24 hours, were administered.
[24] Administered by gavage at 30 and 6 hours prior to sacrifice.
[25] Calculated using the molecular weight of acetamide (59.07 g/mol).
[26] Administered via a single subcutaneous injection.
[27] Frequency of spots of genetic relevance was significantly increased relative to controls only in 1 out of 3 trials, and only at the highest dose (500 mg/kg bw).
[28] Administered at a single dose (route not specified).
[29] Significant increase in spots of genetic relevance was observed only in 1 out of 4 groups receiving 500 mg/kg body weight.
[30] Administered in the diet.
[31] Calculated using the molecular weight of 1,6-hexalactam (113.16 g/mol).
[32] Intraperitoneal injection in 2 instalments at 0 and 24 hours.
[33] Administered via gavage for 5 days.
[34] Administered orally for 5 consecutive days.
[35] Administered intraperitoneally for 5 days, followed by a 35-day maintenance period.
[36] Piperidine was administered orally to mice for 100 days. However, on day 40 and every subsequent 5 days, 3 mice were killed for examination of sperm morphology.

Table 3: Additional *In Vitro* Genotoxicity Data Considered by the Panel in FGE.86Rev1

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference	Comments
16.093 1597	*N*-Cyclopropyl (2*E*,6*Z*)-nonadienamide	Reverse Mutation	*S.typhimurium* TA98, TA100, TA1535, TA1537	Up to 5000 µg/plate	Negative[1]	Bowles, 2003	
		Reverse Mutation	*E.coli WP2 uvrA*$^-$	Up to 5000 µg/plate	Negative[1]		
16.095 1779	*N*-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide	Reverse Mutation	*S.typhimurium* TA97a, TA1535	Up to 2000 µg/plate	Negative	Next Century Incorporated, 2004	Cytotoxic at different concentration with and without S9
		Reverse Mutation	*S.typhimurium* TA98, TA100	Up to 5000 µg/plate	Negative		
		Reverse Mutation	*Escherichia coli WP2 uvrA (328)*	Up to 2000 µg/plate	Negative		

[1] With and without S9.

SUMMARY OF TOXICITY DATA

Table 4: Toxicity Data Considered by the Panel in FGE.86Rev2

Chemical Name [FL-no]	Species; Sex No/group	Route	Doses (mg/kg bw/day)	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
Deca-(2*E*,4*E*)-dienoic acid isobutyl-amide [16.091]	Rat; M, F 5	Diet	0, 333 (350), 1686 (1858) and 3271 (3782)	14	Range-finding	Koetzner, 2013b	
	Rat; M, F 10	Diet	0, 10, 40 and 100	90	10	Koetzner, 2013a	
Piperine [14.003]	Rat; M, F 10	Diet	0, 5, 15 and 50	90	5	Bauter, 2013	

SUMMARY OF SAFETY EVALUATIONS

Table 5: Summary of Safety Evaluation by JECFA (JECFA, 2006b)

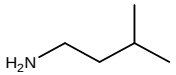
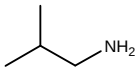
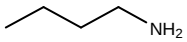
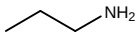
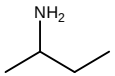
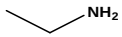
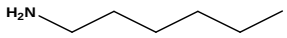
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (µg/*capita*/day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d),(e)]	EFSA conclusion on the named compound[(f)]	EFSA conclusion on the material of commerce
11.001 1587	3-Methylbutylamine		24 0.07	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.002 1583	Isobutylamine		0.012 0.09	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.003 1582	Butylamine		89 0.01	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.004 1580	Propylamine		0.012 0.02	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.005 1584	sec-Butylamine		0.012 2	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	Racemate. No safety concern at the estimated level of intake based on the MSDI approach.
11.009 1610	Trimethylamine		130 70	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.015 1579	Ethylamine		0.012 0.2	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.016	Hexylamine		0.024	Class I	(d)	No safety concern at	No safety concern at

Table 5: Summary of Safety Evaluation by JECFA (JECFA, 2006b)

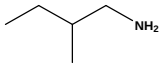
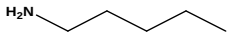
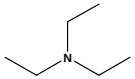
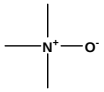
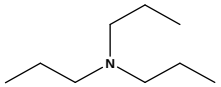
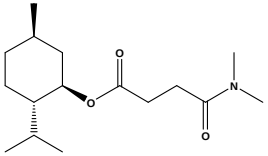
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (µg/*capita*/day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d),(e)]	EFSA conclusion on the named compound[(f)]	EFSA conclusion on the material of commerce
1588			0.007	A3: Intake below threshold		the estimated level of intake based on the MSDI approach.	the estimated level of intake based on the MSDI approach.
11.018 1581	Isopropylamine		0.012 0.02	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.020 1586	2-Methylbutylamine		0.012 0.02	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	Racemate. No safety concern at the estimated level of intake based on the MSDI approach.
11.021 1585	Pentylamine		0.037 0.2	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.023 1611	Triethylamine		0.073 0.9	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.025 1614	Trimethylamine oxide		2.3 0.09	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.026 1612	Tripropylamine		0.012 0.02	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.092 1602	*N*,*N*-Dimethyl menthyl succinamide		61 88	Class I A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. Register name to to

Table 5: Summary of Safety Evaluation by JECFA (JECFA, 2006b)

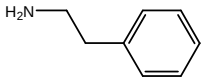
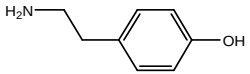
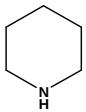
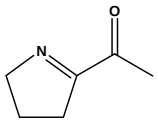
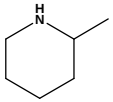
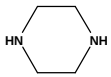
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (µg/*capita*/day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d),(e)]	EFSA conclusion on the named compound[(f)]	EFSA conclusion on the material of commerce
							be changed to (1R,2S,5R)- N,N-Dimethyl menthyl succinamide.
11.006 1589	Phenethylamine		0.075 0.05	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
11.007 1590	2-(4-Hydroxy-phenyl)ethylamine		0.012 0.02	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.010 1607	Piperidine		88 96	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.064 1609	Pyrrolidine		0.12 2	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.080 1604	2-Acetyl-1-pyrroline		0.012 0.1	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.133 1608	2-Methylpiperidine		0.012 0.002	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	Racemate. No safety concern at the estimated level of intake based on the MSDI approach.
14.141 1615	Piperazine		0.012 0.002	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 5: Summary of Safety Evaluation by JECFA (JECFA, 2006b)

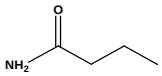
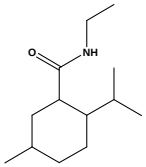
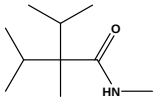
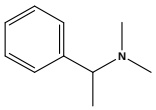
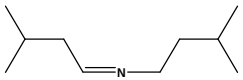
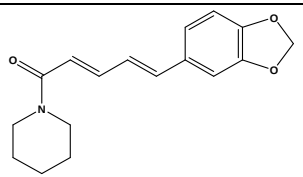
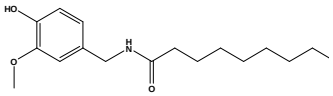
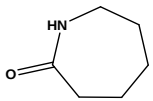
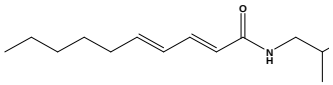
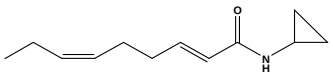
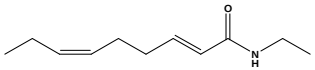
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (µg/*capita*/day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d),(e)]	EFSA conclusion on the named compound[(f)]	EFSA conclusion on the material of commerce
14.167 1603	1-Pyrroline		0.012 0.4	Class II A3: Intake below threshold	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.049 1593	Butyramide		0.012 0.002	Class II A3: Intake below threshold	(d)	The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity/carcinogenicity.	No longer supported by Industry (DG SANCO, 2012).
16.013 1601	*N*-Ethyl-2-isopropyl-5-methylcyclohexane carboxamide		0.4 127	Class III B3: Intake above threshold	Data must be available (e)	No safety concern at the estimated level of intake based on the MSDI approach. Toxicity data available to establish an adequate NOAEL.	No safety concern at the estimated level of intake based on the MSDI approach.
16.053 1595	2-Isopropyl- N,2,3-trimethylbutanamide		24 1054	Class III B3: Intake above threshold	Data must be available (e)	No safety concern at the estimated level of intake based on the MSDI approach. Toxicity data available to establish an adequate NOAEL.	No safety concern at the estimated level of intake based on the MSDI approach.
11.014 1613	*N,N*-Dimethyl-phenethylamine		0.012 0.09	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	Toxicity data required.	No longer supported by Industry (DG SANCO, 2012).
11.017 1606	*N*-Isopentylidene isopentylamine		0.012 0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	EFSA concluded at step A3: No safety concern at the estimated level of	No safety concern at the estimated level of intake based on the MSDI approach.

Table 5: Summary of Safety Evaluation by JECFA (JECFA, 2006b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (µg/*capita*/day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d),(e)]	EFSA conclusion on the named compound[(f)]	EFSA conclusion on the material of commerce
						intake based on the MSDI approach.	
14.003 1600	Piperine		6.2 0.07	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	No safety concern at the estimated level of intake based on the MSDI approach. Toxicity data available to establish an adequate NOAEL.	No safety concern at the estimated level of intake based on the MSDI approach. CASrn in the Register refers to the trans,trans isomer.
16.006 1599	*N*-Nonanoyl 4-hydroxy-3-methoxybenzylamide		6.0 0.07	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.052 1594	1,6-Hexalactam		0.012 0.002	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.091 1598	Deca-(2*E*,4*E*)-dienoic acid isobutyl-amide		11 83	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	No safety concern at the estimated level of intake based on the MSDI approach. Toxicity data available to establish an adequate NOAEL.	No safety concern at the estimated level of intake based on the MSDI approach.
16.093 1597	*N*-Cyclopropyl (2*E*,6*Z*)-nonadienamide		61 40	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	Toxicity data required.	No longer supported by Industry (DG SANCO, 2012).
16.094 1596	*N*-Ethyl (2*E*,6*Z*)-nonadienamide		61 88	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)	Toxicity data required.	No longer supported by Industry (DG SANCO, 2014).

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

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4. EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

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

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APPENDIX A

Table 6: Normal and Maximum use levels (mg/kg) available for JECFA evaluated Substances

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**
11.002	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.004	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.005	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.007	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	1 5	- -	1 5	1 5	0.2 1
11.015	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.016	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.018	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.020	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.021	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.023	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.025	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
11.026	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
14.080	0.4 2	0.1 0.5	0.4 2	0.4 2	- -	1 5	0.2 1	2 10	0.2 1	0.2 1	- -	- -	0.1 0.5	0.2 1	- -	1 5	1 5	0.2 1
14.133	0.4	0.1	0.4	0.4	-	1	0.2	2	0.2	0.2	-	-	0.1	0.2	-	1	1	0.2

Table 6: Normal and Maximum use levels (mg/kg) available for JECFA evaluated Substances

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**
	2	0.5	2	2	-	5	1	10	1	1	-	-	0.5	1	-	5	5	1
14.141	4	0.1	0.4	0.4	-	1	0.2	2	0.2	0.2	-	-	0.1	0.2	-	1	1	0.2
	2	0.5	2	2	-	5	1	10	1	1	-	-	0.5	1	-	5	5	1
16.006	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	300	-	-	-	-	-	-	-	-	-	-	-	-	-
16.052	0.4	0.1	0.4	0.4	-	1	0.2	1	0.1	0.1	-	-	0.1	0.2	-	1	1	0.2
	2	0.5	2	2	-	5	1	5	0.4	0.4	-	-	0.5	1	-	5	5	1
16.091	0.02	0.02	0.2	-	-	2	-	0.2	0.02	0.02	-	-	0.02	-	2	2	0.02	0.02
	1	1	10	-	-	100	-	10	1	1	-	-	1	-	100	100	1	1
16.092	0.4	0.4	0.4	-	-	40	-	4	0.4	0.4	-	-	0.4	-	40	40	0.4	0.4
	4	4	4	-	-	400	-	40	4	4	-	-	4	-	400	400	4	4

Table 7: Estimated intakes based on the MSDI- and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
11.001	3-Methylbutylamine	24	0.07		Class I	1800
11.002	Isobutylamine	0.012	0.09	340	Class I	1800
11.003	Butylamine	89	0.01		Class I	1800
11.004	Propylamine	0.012	0.02	340	Class I	1800
11.005	sec-Butylamine	0.012	2	340	Class I	1800
11.009	Trimethylamine	130	70		Class I	1800
11.015	Ethylamine	0.012	0.2	340	Class I	1800
11.016	Hexylamine	0.024	0.007	340	Class I	1800
11.018	Isopropylamine	0.012	0.02	340	Class I	1800
11.020	2-Methylbutylamine	0.012	0.02	340	Class I	1800
11.021	Pentylamine	0.037	0.2	340	Class I	1800
11.023	Triethylamine	0.073	0.9	340	Class I	1800
11.025	Trimethylamine oxide	2.3	0.09	340	Class I	1800
11.026	Tripropylamine	0.012	0.02	340	Class I	1800
16.092	N,N-Dimethyl menthyl succinamide	61	88	15000	Class I	1800
11.006	Phenethylamine	0.075	0.05		Class II	540
11.007	2-(4-Hydroxyphenyl)ethylamine	0.012	0.02	340	Class II	540
14.010	Piperidine	88	96		Class II	540
14.064	Pyrrolidine	0.12	2		Class II	540
14.080	2-Acetyl-1-pyrroline	0.012	0.1	340	Class II	540
14.133	2-Methylpiperidine	0.012	0.002	340	Class II	540
14.141	Piperazine	0.012	0.002	600	Class II	540

Table 7: Estimated intakes based on the MSDI- and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
14.167	1-Pyrroline	0.012	0.4		Class II	540
16.013	N-Ethyl-2-isopropyl-5-methylcyclohexane carboxamide	0.4	127		Class III	90
16.053	2-Isopropyl- N,2,3-trimethylbutanamide	24	1054		Class III	90
11.017	N-Isopentylidene isopentylamine	0.012	0.01		Class III	90
14.003	Piperine	6.2	0.07		Class III	90
16.006	N-Nonanoyl 4-hydroxy-3-methoxybenzylamide	6.0	0.07	1300	Class III	90
16.052	1,6-Hexalactam	0.012	0.002	200	Class III	90
16.091	Deca-(2E,4E)-dienoic acid isobutyl-amide	11	83	770	Class III	90

ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DNA	Deoxyribonucleic acid
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)
GLP	Good laboratory practice
ID	Identity
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
SCF	Scientific Committee on Food
WHO	World Health Organisation