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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 94, Revision 2 (FGE.94Rev2): Consideration of aliphatic amines and amides evaluated in an addendum to the group of aliphatic and aromatic amines and amides evaluated by the JECFA (68th meeting)[1]

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

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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 (the 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 12 aliphatic amines and amides evaluated by the JECFA at the 68th meeting in 2007. This revision is required owing to additional toxicity data on 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090]. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern and available data on metabolism and toxicity. The Panel agrees with JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the 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 been considered and for all 12 substances, the information is adequate.

KEY WORDS

JECFA 68th meeting, food safety, flavourings, aliphatic amines, aliphatic amides, FGE.94

[1] On request from the European Commission, Question No EFSA-Q-2013-00688, adopted on 25 March 2014.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft, Pia Lund and Karin Nørby and EFSA staff: 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 a scientific opinion 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 (the 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.

In Flavouring Group Evaluation 94, Revision 1 (FGE.94Rev1), the EFSA considered 12 aliphatic amines and amides evaluated by the JECFA at the 68th meeting. This revision 2 is made due to additional toxicity data received on 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] that were requested in the previous opinion.

The Panel concluded that no supporting Flavouring Group Evaluation was available for the substances in the present FGE.

Genotoxicity data from *in vitro* and *in vivo* studies were available for seven [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103 and 16.111] of the flavouring substances and the results did not indicate any concern for genotoxicity of the substances in this flavouring group.

In the previous version of FGE.94 (FGE.94Rev1), the Panel concluded that it could agree with the way the application of the Procedure has been performed by the JECFA for 11 substances. For one substance [FL-no: 16.090] no adequate NOAEL was available. A new 90-day study has now become available for [FL-no: 16.090] and a NOAEL to provide an adequate margin of safety can be derived.

For 10 of the 12 substances, use levels have been provided by the Industry. Based on the use levels, the mTAMDI figures calculated for nine substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103, 16.104, 16.111 and 17.035] are above the threshold of concern for their structural classes. For these nine substances more reliable data are needed. On the basis of such data the flavouring substances should be reconsidered using the Procedure. For the remaining two substances [FL-no: 16.100 and 16.101], use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment.

In order to determine whether the conclusion for the 12 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications are available for all 12 substances.

For all 12 substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035] the Panel agrees with the 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].

On 24 May 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids(CEF) adopted a scientific opinion on Flavouring Group Evaluation 94, Revision 1 (FGE.94Rev1): Consideration of aliphatic amines and amides evaluated in an addendum to the group of aliphatic and aromatic amines and amides evaluated by JECFA (68th meeting).

The Panel concluded that: "For one substance [FL-no: 16.090] additional toxicity data are still needed before the evaluation can be finalised. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been consiered and for one substance, [FL-no: 16.090], the composition of the stereoisomeric mixture has to be specified".

The requested data on 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] have now been submitted by the applicant.

The Commission requests EFSA to finalise its safety assessment on [FL-no: 16.090] within nine months from the receipt of this letter.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to finalise its safety assessment on [FL-no: 16.090] in accordance with Commission Regulation (EC) No 1565/2000.

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

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

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

ASSESSMENT

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 the 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 special 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, the 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 the 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 the 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 the 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, 2006).

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 the 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 the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

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

The JECFA uses the threshold of concern of 1.5 microgram (µg)/person/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 the 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.94, which contains a group of 12 aliphatic amines and amides, the Panel considered that additional toxicity data were needed for three substances [FL-no: 16.090, 16.095 and 16.111] before they could be evaluated as flavouring substances, as no adequate toxicity study from which a no observed adverse effect level (NOAEL) could be established was available, neither on the substances themselves nor on supporting substances.

In first revision of FGE.94 (FGE.94Rev1) new 90-day studies had been provided for [FL-no: 16.095 and 16.111] and NOAELs to provide adequate margins of safety could be derived. Furthermore, new metabolism and genotoxicity data had been provided for [FL-no: 16.111]. Finally, information on the stereoisomeric composition had been provided for three substances [FL-no: 16.090, 16.102 and 16.104] and ID tests and information on solubility in ethanol had been provided for two substances [FL-no: 16.100 and 16.101] (EFFA, 2010; Flavour Industry, 2012).

FGE	Opinion adopted	Link	No. of substances
FGE.94	23 September 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1338.htm	12
FGE.94Rev1	24 May 2012	http://www.efsa.europa.eu/en/efsajournal/pub/2747.htm	12
FGE.94Rev2	26 March 2014		

The present revision of the FGE is due to additional toxicity data provided for the substance 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090]. The data provided are a 14-day range finding study and a 90-day dietary study (Bauter, 2013a; Bauter, 2013b). Furthermore, information on the composition of the stereoisomeric mixture has been provided for [FL-no: 16.090] (Flavour Industry, 2013).

New information were also provided on the stereoisomeric composition of [FL-no: 16.090] (EFFA, 2014).

2. Presentation of the Substances in the JECFA Flavouring Group

2.1. Description

2.1.1. JECFA Status

The JECFA has evaluated a group of 12 flavouring substances consisting of aliphatic and aromatic amines and amides at their 68th meeting (JECFA, 2007).

2.1.2. EFSA Considerations

All of the JECFA evaluated substances are in the Register and this consideration therefore deals with these 12 substances. The Panel concluded that there are no supporting substances from other FGEs for these 12 aliphatic amines and amides.

2.2. Isomers

2.2.1. Status

The following five substances [FL-no: 16.102, 16.103, 16.104, 16.105 and 16.111] have one or more chiral centres. Two substances can exist as geometrical isomers [FL-no: 16.090 and 16.095].

2.2.2. EFSA Considerations

Adequate information on the isomeric composition is available for all 12 substances. For the three stereoisomeric substances [FL-no: 16.095, 16.105 and 16.111], the CAS register number (CASrn) is considered to specify the stereoisomeric composition (Table 1).

2.3. Specifications

2.3.1. Status

The JECFA specifications are available for all substances (JECFA, 2008b).

2.3.2. EFSA Considerations

The European Flavour Industry has submitted specifications for all 12 substances commercially used in Europe (EFFA, 2006; Flavour Industry, 2004; Flavour Industry, 2006a; Flavour Industry, 2006b; Flavour Industry, 2007; Flavour Industry, 2008; Flavour Industry, 2006c). Although the JECFA specifications are available, the specifications used in this consideration are those submitted by the Industry. See Table 1. The specifications are considered adequate for all 12 substances.

3. Intake Estimation

3.1. Status

For all 12 substances evaluated through the JECFA Procedure intake data are available for the EU.

3.2. EFSA Considerations

For 10 JECFA evaluated substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035] normal and maximum use levels have been provided by the Flavour Industry in accordance with the Commission Regulation (EC) No 1565/2000 (EC, 2000; EFFA, 2006; Flavour Industry, 2004; Flavour Industry, 2006a; Flavour Industry, 2006b; Flavour Industry, 2006c; Flavour Industry, 2007; Flavour Industry, 2008) (Appendix A, Table A.1). Based on the normal use levels, mTAMDI figures (see Table 7) can be calculated (for calculation of mTAMDI figures, see e.g. FGE.03, Annex II (EFSA, 2004).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2008b)

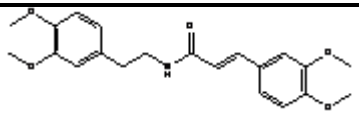
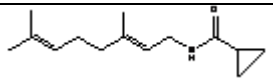
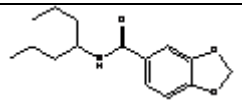
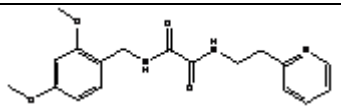
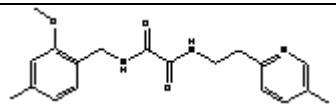
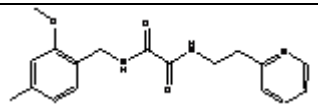
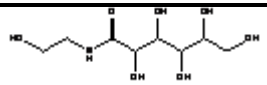
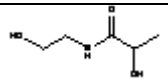
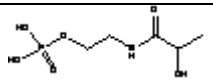
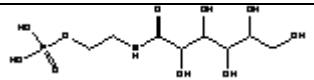
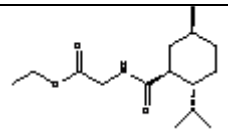
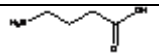
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments / Reference for specifications
16.090 1777	3-(3,4-Dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide		4310 69444-90-2	Solid $C_{21}H_{25}NO_5$ 371.43	Practically insoluble or insoluble Slightly soluble	127.9 IR NMR MS 99 %	n.a. n.a.	(Flavour Industry, 2006b). 95 % (E)-isomer (EFFA, 2014).
16.095 1779	Cyclopropanecarboxamide, *N*-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]-		4267 744251-93-2	Solid $C_{14}H_{23}NO$ 221.00	Insoluble Soluble	146 (at 1 hPa) 53 IR NMR 98 %	n.a. n.a.	(Flavour Industry, 2004).
16.098 1767	*N*-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide		4232 745047-51-2	Solid $C_{15}H_{21}NO_3$ 263.34	Insoluble Sparingly soluble	116 IR NMR MS 99 %	n.a. n.a.	(Flavour Industry, 2006c).
16.099 1768	*N*-(2,4-Dimethoxy-benzyl)-*N'*-(2-pyridin-2-yl-ethyl)-oxalamide		4233 745047-53-4	Solid $C_{18}H_{21}N_3O_4$ 343.38	Insoluble Sparingly soluble	123 IR NMR MS 99 %	n.a. n.a.	(Flavour Industry, 2006c). [FL-no: 16.099, 16.100 and 16.101] should be named by the same nomenclature principles.
16.100 1769	*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide		4234 745047-94-3	Solid $C_{19}H_{23}N_3O_3$ 341.41	Insoluble Sparingly soluble	- 132 - 133 IR NMR MS 99 %	n.a. n.a.	(Flavour Industry, 2008). [FL-no: 16.099, 16.100 and 16.101] should be named by the same nomenclature principles.
16.101 1770	*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide		4231 745047-97-6	Solid $C_{18}H_{21}N_3O_3$ 327.38	Insoluble Sparingly soluble	- 128 - 129 IR NMR 99 %	n.a. n.a.	(Flavour Industry, 2008). [FL-no: 16.099, 16.100 and 16.101] should be named by the same nomenclature principles.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2008b)

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
16.102 1772	2,3,4,5,6-Pentahydroxy-*N*-(2-hydroxyethyl)-hexanamide		4254 686298-93-1	Solid $C_8H_7NO_7$ 239.22	Soluble Soluble	 99 - 100 IR NMR MS 99 %	n.a. 1.562	(Flavour Industry, 2008). Only one diastereomer (2R,3S,4S,5R) (Flavour Industry, 2012). Register name to be changed to (2R,3S,4S,5R)-2,3,4,5,6-Pentahydroxy-*N*-(2-hydroxyethyl)hexanamide.
16.103 1774	Propanamide, 2-hydroxy-*N*-(2-hydroxyethyl)-		4256 5422-34-4	Liquid $C_5H_{11}NO_3$ 133.15	Soluble Moderate	380 IR NMR MS 99 %	1.481-1.491 1.185-1.196	(Flavour Industry, 2008).
16.104 1775	2-[(2-Hydroxypropanoyl)amino] ethyl dihydrogen phosphate		 782498-03-7	Solid $C_5H_{12}NO_6$ 213.13	Soluble Moderate	 200 IR NMR MS 95 %	n.a. 1.521	(EFFA, 2006; Flavour Industry, 2008). Racemate (EFFA, 2010).
16.105 1773	(2R,3S,4S,5R)-2-[(2,3,4,5,6-Pentahydroxyhexanoyl)amino]ethyl dihydrogen phosphate		4255 791807-20-0	Solid $C_8H_{18}NO_{10}P$ 319.21	Soluble Moderate	 130 IR NMR MS 95 %	n.a. 1.76	(EFFA, 2006; Flavour Industry, 2008).
16.111 1776	Glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester.		4309 68489-14-5	Solid $C_{15}H_{27}NO_3$ 269.38	Practically insoluble Soluble	151 (2.7 hPa) 80-82 IR NMR MS 99 %	n.a. n.a.	(Flavour Industry, 2007).
17.035 1771	4-Amino-butyric acid		 56-12-2	Solid $C_4H_9NO_2$ 103.12	Slightly soluble Practically insoluble	 200 IR NMR MS 100 %	n.a n.a.	(Flavour Industry, 2006a).

(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[7] from the JECFA (JECFA, 2008a)

In vitro and *in vivo* genotoxicity testing has been performed on six flavouring substances [FL-no: 16.090, 16.098, 16.099, 16.102, 16.103 and 16.111] in this group. The results of these studies are summarised in Table 2 and described below.

In vitro

N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide ([FL-no: 16.098], 2,3,4,5,6-pentahydroxy-*N*-(2-hydroxyethyl)-hexanamide ([FL-no: 16.102], propanamide, 2-hydroxy-*N*-(2-hydroxyethyl)- ([FL-no: 16.103] and 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] were tested in *Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 and *Escherichia coli* WP2uvrA at concentrations up to 5000 µg/plate, with and without S9 activation. There was no evidence of an increase in revertants (Uhde, 2004; Verspeek-Rip, 2004a; Verspeek-Rip, 2004b; Zhang, 2004a).

N-(2,4-dimethoxy-benzyl)-*N*′-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] induced an increase in the number of revertants in *S. typhimurium* TA1535 in the absence (but not in the presence) of metabolic activation compared with control values; however, no dose–response was observed, and the mean number of revertants was reported to be below historical spontaneous reversion or negative control values. When tested under the conditions of the pre-incubation assay at concentrations of up to 5000 µg/plate, *N*-(2,4-dimethoxy-benzyl)-*N*′-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] induced an increase in the number of revertants in *S. typhimurium* TA100 in the presence of metabolic activation, but only at a concentration of 62 µg/plate; no dose–response pattern was observed, and no significant increases in the number of revertants were reported in the absence of metabolic activation at concentrations of up to 5000 µg/plate. Moreover, *N*-(2,4-dimethoxy-benzyl)-*N*′-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] consistently tested negative in several other strains of *S. typhimurium* (TA98 and TA1537) and in *E. coli* WP2uvrA in both the absence and presence of metabolic activation, in both plate incorporation and pre-incubation assays, at concentrations of up to 5000 µg/plate. Given the lack of a dose-dependent response, non-reproducibility of results and the fact that the number of revertants was below historical control values, it was concluded that *N*-(2,4-dimethoxy-benzyl)-*N*′-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] was non-mutagenic (Zhang, 2005a).

Glycine, *N*-[[(1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] induced a slight increase in the number of revertants in *S. typhimurium* TA100 and TA1535 in the absence of metabolic activation compared with control values; however, the increase was not statistically significant. In a set of confirmatory experiments, *S. typhimurium* TA100 and TA1535 were re-tested at concentrations of 0, 2000, 3000, 4000 or 5000 µg/plate without metabolic activation (Table 2). The study reported an increase in revertant colonies in strain TA1535 that was reproducible and, at the highest concentration tested, was significantly above in-house historical controls. The report concluded that *N*-[[(1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] was weakly mutagenic to TA1535 under the test conditions. In contrast, increases observed in the revertant colonies in *S. typhimurium* TA100, although statistically significant, were small and did not follow a dose–response pattern. Moreover, glycine, *N*-[[(1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] consistently tested negative in several other strains of *S. typhimurium* (TA98 and TA1537) and in *E. coli* WP2uvrA in both the absence and presence of metabolic activation, at concentrations of up to 5000 µg/plate (Thompson, 2005).

N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide [FL-no: 16.098] and *N*-(2,4-dimethoxy-benzyl)-*N*′-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] produced no evidence of genotoxicity at 0, 21,

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

62, 190, 560, 1670 or 5000 µg/ml in standard chromosomal aberration assays in Chinese hamster ovary cells cultured with and without S9 metabolic activation (Zhang, 2004b; Zhang, 2005b).

In vivo

In a standard mouse micronucleus bone marrow assay, groups of 21 male Swiss albino (CD-1) mice per dose were injected intraperitoneally with 0, 175, 350 or 700 mg *N*-(1-propylbutyl)-1,3-benzodioxole-5-carboxamide [FL-no: 16.098]/kg bw. At 24, 36 and 48 hours following dose administration, seven mice from each group were killed, and their femoral bone marrow was harvested, fixed and stained. No statistically significant differences were observed in the number of polychromatic erythrocytes with micronuclei between the test groups and the negative control (Pucaj, 2004a).

In a standard mouse micronucleus bone marrow assay using the same protocol as described above, groups of 21 male Swiss albino (CD-1) mice per dose were injected intraperitoneally with 0, 200, 400 or 800 mg *N*-(2,4-dimethoxy-benzyl)-*N'*-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099]/kg bw. At 24, 36 and 48 hours following dose administration, seven mice from each group were killed, and their femoral bone marrow was harvested, fixed and stained. No statistically significant differences were observed in the number of polychromatic erythrocytes with micronuclei between the test groups and the negative control (Pucaj, 2004b).

In a similar standard mouse micronucleus bone marrow assay, male NMRI BR mice (five per group) were administered aqueous 2,3,4,5,6-pentahydroxy-*N*-(2-hydroxyethyl)-hexanamide [FL-no: 16.102] at 0 (negative or positive control) or 2000 mg/kg bw via gavage. Femoral bone marrow was isolated at 24 or 48 hours post-administration. Treatment and control mice showed no difference in the ratio of polychromatic to normochromatic erythrocytes. 2,3,4,5,6-Pentahydroxy-*N*-(2-hydroxyethyl)-hexanamide [FL-no: 16.102] showed no mutagenic potential in the mouse micronucleus assay (Buskens, 2004).

Employing the same standard mouse micronucleus bone marrow assay as used above, male NMRI BR mice (five per group) were administered aqueous propanamide, 2-hydroxy-*N*-(2-hydroxyethyl)- [FL-no: 16.103] at 0 (negative or positive control) or 2000 mg/kg bw via gavage. Femoral bone marrow was isolated 24 or 48 hours after administration. Treated and control mice showed no difference in the ratio of polychromatic to normochromatic erythrocytes. Propanamide, 2-hydroxy-*N*-(2-hydroxyethyl)- [FL-no: 16.103] showed no mutagenic potential in the mouse micronucleus assay (Buskens, 2004).

Conclusion on genotoxicity

On the weight of evidence, negative results were obtained with the flavouring agents of this group when tested in *in vitro* mutation assays in *S. typhimurium* and *E. coli*, as well as in mammalian cells. Negative results were also obtained in *in vivo* micronucleus assays.

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

4.2. Genotoxicity Study on [FL-no: 16.111]

A Mouse Lymphoma Assay for glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl) cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] (Flanders, 2006) was submitted after the publication of FGE.94.

The study was conducted according to an adequate design to assess the potential mutagenicity of the test material on the thymidine kinase, TK +/-, locus of the L5178Y mouse lymphoma cell line (Flanders, 2006). L5178Y TK +/- 3.7.2c mouse lymphoma cells (heterozygous at the thymidine kinase locus) were treated with the test material at eight dose levels, in duplicate, together with vehicle (solvent) and positive controls. The entire experiment was repeated to confirm the result of the first experiment. Four hours exposures were used both with and without activation in Experiment 1. In

Experiment 2, the exposure time without activation was increased to 24 hours. The dose range of test material, plated for expression of mutant colonies, was selected following the results of a preliminary cytotoxicity test and was 42.03 to 672.5 µg/ml in the absence of metabolic activation and 84.06 to 1008.75 µg/ml in the presence of metabolic activation for the first experiment. For the second experiment the dose range plated for expression of mutant colonies was 10.51 to 504.38 µg/ml without metabolic activation and 42.03 to 672.5 µg/ml with metabolic activation.

The maximum dose level used was limited by test material induced cytotoxicity. A precipitate of test material was observed at 1345 µg/ml during the course of the study. The vehicle (solvent) controls had mutant frequency values that were considered acceptable for the L5178Y cell line at the TK +/- locus. The positive control materials induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system.

The test material did not induce any statistically significant or concentration-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in either the first or the second experiment incorporating dose levels that exhibited optimum levels of cytotoxicity.

Study results are presented in Table 3.

4.3. EFSA Considerations

Genotoxicity data from *in vitro* studies are available for seven substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103 and 16.111] and *in vivo* studies were available for four substances [FL-no: 16.098, 16.099, 16.102 and 16.103] of the 12 flavouring substances evaluated by the JECFA.

The Panel noted that conflicting positive results were obtained for *N*-(2,4-dimethoxy-benzyl)-*N'*-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] in the one study by (Zhang, 2005a) when tested in the *S. typhimurim* TA1535 and TA100. However, no concentration–related response was observed, the positive results with TA1535 and TA100 were not reproducible and concomitantly, the tests with TA98 and TA1537 were consistently negative (JECFA, 2008a). The Panel concluded that the available data did not raise concern about genotoxicity in the Ames test.

For the consideration in FGE.94 on cyclopropanecarboxamide, *N*-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]- [FL-no: 16.095] an additional genotoxicity study was provided by the Industry (Next Century Incorporated, 2004) after the JECFA evaluated the substance at the 68th meeting (JECFA, 2007). In this study, the substance was tested in a bacterial reverse mutation test using *S. typhimurium* strains TA97a, TA98, TA100, TA1535, and *E. coli* strain WP2uvra with and without metabolic activation. It was concluded to be negative for the induction of mutagenicity (see Table 4).

Glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] did not induce gene mutations at the thymidine kinase locus in L5178Y cells in the Mouse Lymphoma Assay (Flanders, 2006).

Overall, the Panel considered that the available data did not raise concern with respect to genotoxicity.

5. *In Vitro* Hydrolysis Study on [FL-no: 16.111]

The hydrolysis of glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] was studied in artificial pancreatic juice and rat liver homogenate (Poet et al., 2005). Based on the disappearance of the employed substrate, [FL-no: 16.111] was hydrolysed in artificial pancreatic juice with a half-life of 43 ± 14.7 min and a first order loss rate (K) of 1.06 ± 0.426 hour^{-1}. In 20 fold-diluted liver homogenate the disappearance of [FL-no: 16.111] was considerably faster (half-life: 0.802 ± 0.191 min). However, the potential hydrolysis products p-menthane-3-carboxylic acid, glycine ethylester and glycine were only detected at trace levels. This indicates that the disappearance of [FL-no: 16.111], under the employed *in vitro* conditions, is due to the hydrolysis of the ethyl ester bond rather than the hydrolysis of the amide bond.

This stability of the amide bond is in agreement with data provided for *N*-benzonitrile-*p*-menthan-3-carboxamide, [FL-no: 16.117]. This structurally related substance was not hydrolysed when incubated with pooled hepatic microsomes from male rats or male humans under conditions in which hydrolytic enzymes were shown to be active (Sipes and Kong, 2012).

In conclusion, the Panel considers that the candidate substance [FL-no: 16.111] cannot be expected to be metabolised to innocuous products.

6. 90-Day Studies on [FL-no: 16.090, 16.095 and 16.111]

Three 90-day studies requested in first version of this FGE have been submitted for [FL-no: 16.090, 16.095 and 16.111] by the Industry.

6.1. 90-Day Dietary Toxicity Study in Crl:CD (SD) Rats on Cyclopropanecarboxamide, *N*-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]- [FL-no: 16.095]

A 90-day dietary toxicity study followed by a 28-day recovery period was performed with cyclopropanecarboxamide, *N*-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]- [FL-no: 16.095] in rats (Bauter, 2011). The study was performed according to OECD Guideline 408 under GLP. The dose levels tested were 0, 11, 110 and 1100 mg/kg diet, equal to mean daily exposures of 0, 0.7, 7.3, and 73.3 mg/kg bw/day in the male rats and of 0, 0.8, 8.1, and 80.1 mg/kg bw/day in the female rats. Each test group consisted of 10 animals per sex. Recovery groups were included for the control and high dose groups as well. Clinical observations, functional observation battery and motor activity were recorded. The feed homogeneity was checked by dietary chemical analysis. Data on body weight and individual food consumption were collected throughout the in-life phase of the study. Blood samples were taken for complete haematological, clinical chemical and serological analyses. At study termination, body weight, organ and tissue weights were recorded after macroscopical examination and complete histopathology was performed on the animals of the control and highest dose groups. No substance-related effects were found implying that the highest dose group is the NOAEL of 1100 mg/kg diet which is equal to approximately 73 mg *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide/kg bw/day in male rats.

6.2. 90-Day Oral (by Gavage) Toxicity Study in Crl:CD (SD) Rats on Glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111]

A 90-day oral toxicity study by gavage in Crl:CD (SD) rats (males and females) followed by 14-day recovery period was performed with glycine, *N*-[[(1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] (Kirkpatrick, 2011). The study was performed according to OECD Guideline 408 under GLP. The substance was administered at doses of 0, 25, 75, 225 and 675 mg/kg bw/day to 10 animals/sex/dose group via gavage. Recovery groups were included for the control and high dose groups. The following parameters were evaluated: daily clinical observation, weekly recording of individual body weight and food consumption while clinical chemistry, hematology, blood clotting parameters and urinalysis were done at study termination and at the end of the recovery period. A modified Irwin test, performed before start and on week 12 of the study, was conducted in order to evaluate any potential effect on the central nervous system. Ophtalmology examination was performed on week 1 and week 12 of the study. Complete necropsies were conducted on all animals and selected organs were weighted. Selected tissues were examined microscopically from all animals in the control and the 675 mg/kg bw/day groups at the primary necropsy. Kidney, liver, heart and gross lesions were examined microscopically from all animals at the scheduled necropsies. Sections of kidneys were also evaluated for presence of α_{2u}-globulin by immunohistochemistry. Spermatogenic endpoints (motility, morphology and numbers) were evaluated for all males at the scheduled necropsies.

Increase in the number ofmonocytes (in males), neutrophils , and white blood cells in both genders were observed in the high-dose treated group. These increases declined after the recovery period.

Lower hematocrit values were seen at 225 and 675 mg/kg bw/day in the males but not in the females. However, lower hematocrit, accompanied by decrease in hemoglobin, red blood cell count, mean corpuscular volume and increase in red cell distribution width were observed after the recovery period in both sexes at 675 mg/kg/ bw/day.

At 675 mg/kg bw/day, in both genders it was observed an increase in serum creatinine, urea nitrogen, triglycerides, total urine volume and a reduction in serum albumin/globulin ratios, chloride levels and specific gravity. An increase in calcium level was observed only in males.

These changes were not present after the recovery period.

At gross macroscopy, enlarged kidneys with rough surface were observed in one male and pale kidney was reported for one female rat at 675 mg/kg bw/day, which correlated with the histopathological observation of renal tubular degeneration. An increase in liver and kidney weight was observed in male and female rats at 675 mg/kg bw/day, though this finding was no longer present after the recovery period.

Test substance-related microscopic findings were noted in the kidney (tubular degeneration and dilatation, interstitial fibrosis and tubular epithelium vacuolation) and in the liver (periportal hepatocellular vacuolation and centrilobular hepatocellular hypertrophy) in both male and female rats and in the heart of females (increase in incidence of cardiomyopathy) at 675 mg/kg bw/day. Furthermore, tubular hyaline droplets were observed in male kidney at all doses. However, this finding was not dose-related and considered to correlate with the increase in male rat specific α-2μ-globulin observed at immunohistohemical investigation. Microscopic changes in the kidney, liver and heart were also present after the recovery period, but they were less severe.

Renal changes observed in both genders and cardiomyopathy observed only in female rats at 675 mg/kg bw/day were considered adverse effects. Haematological changes observed at 225 and 675 mg/kg bw/day were also considered adverse as they were dose- related. Red cells changes were also present after a recovery period of 14 days at the dose of 675 mg/kg bw/day. Therefore, the Panel established a NOAEL of 75 mg/kg bw/day.

6.3. 14-Day and 90-Day Dietary Study in Rats on 3-(3,4 Dimethoxyphenyl)-*N*-[2-(3,4 dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090]

In a 14-day dietary palatability and general toxicity study, groups of male and female Hsd:SD® rats (5/sex/dietary intake level) were fed a diet that contained 0 (dietary control), 3000, 6000 and 12 000 mg 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] per kg feed (Bauter, 2013a). These dietary levels were calculated to correspond to an actual intake of 275, 542 and 1075 mg/kg bw/day for males and 276, 553 and 902 mg/kg bw/day for females. Clinical observations were recorded daily and body weights and food consumption observations were made on day 0, 3, 7, 10 and 14. No mortality was observed throughout the course of the study and the general condition of the rats was unremarkable. Body weight, weight gain and food efficiency was reduced for females fed diets with 6000 and 12 000 mg test substance/kg feed, and for males fed 12 000 mg/kg feed when compared to controls (Bauter, 2013a).

A 90-day dietary study was performed with 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] (Bauter, 2013b). The study was performed according to OECD Guideline 408 and the requirements of US FDA GLP. Four groups (10/sex/dietary intake level) of male and female CRL Sprague-Dawley CD®IGS rats were fed a diet that contained 0 (dietary control), 350, 1050 and 4200 mg of 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide per kg feed. The levels corresponded to a calculated daily intake of 0, 23, 69 and 279 mg/kg bw for males and 0, 26, 82 and 340 mg/kg bw for females. Ophthalmologic examinations were conducted prior to the initiation of the study and on day 88. Clinical observations of toxicity were performed on day 0 and weekly until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Near the end of the study period (day 78 - 81), functional observations of

sensory reactivity to different stimuli, grip strength and motor activity were assessed. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed during week 12 after overnight fast. Urine was collected during the 15 hours prior to the blood draw. At termination of the study, all survivors were sacrificed and subject to full necropsy.

Four animals died during the course of the study. On day 64, a single male rat of the 350 mg/kg feed group was found dead for indeterminate cause (no clinical signs). On day 14, 35 and 75, three female rats in the 4200 mg/kg feed group, respectively, were found dead. The clinical observations for the three dead female rats included intermittent slight tremors, vocalisation, prone posture and moderate to extreme ataxia, clonic convulsion, palor, tremors, twitches, hyperactivity and irregular respiration in the female that died on day 14. The female deaths are attributable to the very high concentration of the test material in the diet. All other clinical signs were regarded by the CEF Panel as incidental and not related to 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide in the diet.

All surviving animals included in the study were normal upon ophthalmic examination on day 88, therefore the test substance was not considered an ocular toxicant. Significant and dose-dependent reductions in body weight and body weight gain were reported for the 4200 mg/kg feed female group throughout the study. The male rats and the 1050 and 350 mg/kg feed female rats showed body weights and body weight gains that were overall comparable to the concurrent controls. Food consumption and efficiency were comparable among groups receiving the test material and controls with minor variations that were incidental. Functional observational battery results were comparable between test and control groups as were motor activity measurements. Notable differences in haematology parameters were reported for the 1050 and 4200 mg/kg feed female group. Statistically significant decreases in haemoglobin and haematocrit levels and increased red cell width were reported and correlated to increases in haematopoiesis and erythroid hyperplasia in the spleen and bone marrow. Additionally, for the 1050 and 4200 mg/kg feed groups significant dose-dependent changes in haematological parameters were observed, including increased absolute reticulocyte counts, decreased red blood cell counts, increased mean corpuscular volume (for females) and increased mean corpuscular haemoglobin (only in the 4200 mg/kg female group). The study director associated these findings with regenerative anemia in response to 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide in the diet. Coagulation parameters were comparable between test and control groups for both sexes. There were no statistically significant alterations in clinical chemistry parameters measured when test groups were compared to both concurrent and historical controls.

Organ weight and ratio measurements showed statistically significant differences in adrenal, spleen, liver and kidney of male and female rats in the 4200 mg/kg group when compared to controls.

Relevant microscopic changes were reported for the 4200 mg/kg feed male and female groups. Tan/brown, granular cytoplasmic proximal tubular epithelial pigment of the kidneys was observed in 7/10 males and 6/7 females. Minimal globular pigment was noted in the cytoplasm of the liver Kupffer cells in 8/10 males and 5/7 females and a slight increase in hematopoiesis with brown/tan, globular, pigment in the cytoplasm of fixed macrophages of the splenic red pulp in all males and females of the 4200 mg/kg feed group. There was also a slight increase in the cytoplasmic cortical vacuolisation of the adrenals in 7/10 males and 7/7 females of the 4200 mg/kg group. For 7/7 females of the same dose group it was observed a slight-moderate brain mineralisation and vacuolisation of the neutrophil grey area of the forebrain and a slight erythroid hyperplasia of the bone marrow (Bauter, 2013b).

Based on the toxicological endpoints described above, and with special consideration of the dose-dependent effects on hematological parameters, the Panel decided that the no-adverse-effect level (NOAEL) for 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide in the diet is 350 mg/kg feed, which corresponds to an estimated daily intake of 23 and 26 mg/kg bw/day for males and females, respectively.

7. Application of the Procedure

7.1. Application of the Procedure to Aliphatic Amines and Amides Substances by the JECFA (JECFA, 2008a)

According to the JECFA five of the substances belong to structural class I and seven to structural class III using the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

The JECFA concluded five aliphatic amines and amides substances [FL-no: 16.102, 16.103, 16.104, 16.105 and 17.035] at step A3 in the JECFA Procedure – meaning that the substances are expected to be metabolised to innocuous products (step 2) and concluded that the intakes for all substances are below the thresholds for their structural class I (step A3).

The remaining seven flavouring substances in this group cannot be predicted to be metabolised to innocuous products. The estimated daily per capita intakes of these flavouring substances are below the threshold of concern (i.e. 90 µg/person per day) for structural class III, and a No Observed Adverse Effect Level (NOAEL) exists to provide an adequate margin of safety to the estimated intake as flavouring substances (step B4).

Step B4.

For *N*-(1-propylbutyl)-1,3-benzodioxole-5-carboxamide [FL-no: 16.098] (*N*-(heptan-4-yl)benzo[*d*][1,3]dioxole-5-carboxamide), the no-observed-effect level (NOEL) of 20 mg/kg bw per day from a 93-day study in rats (Kot, 2005a) provides an adequate margin of safety (>10 million) in relation to the currently estimated level of exposure from its use as a flavouring agent in Europe (0.0002 µg/kg body weight (bw) per day) and in the USA (0.002 µg/kg bw per day).

For *N*-(2,4-dimethoxy-benzyl)-*N*'-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] (*N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide), the NOEL of 100 mg/kg bw per day from a 93-day study in rats (Kot, 2005b) provides an adequate margin of safety (> 33 million) in relation to the currently estimated level of exposure from its use as a flavouring agent in Europe (0.0002 µg/kg bw per day) and in the USA (0.003 µg/kg bw per day). This NOEL is appropriate for the structurally related flavouring agents *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide [FL-no: 16.100] and *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide [FL-no: 16.101], because they are also oxalamides and are expected to be metabolized by similar pathways. For these structurally related flavouring agents, the NOEL of 100 mg/kg bw per day provides an adequate margin of safety (500 million) in relation to the currently estimated levels of exposure to these flavouring agents in both Europe and the USA (0.0002 µg/kg bw per day).

For glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111], the NOEL of 8 mg/kg bw per day for the structurally related substance *N*-ethyl 2-isopropyl-5-methylcyclohexane carboxamide ([FL-no: 16.013] considered in FGE.86) from a 28-day study in rats (Miyata, 1995) provides an adequate margin of safety (> 13 000) in relation to the currently estimated level of exposure from its use as a flavouring substance in Europe (0.6 µg/kg bw/day).

For 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] (*N*-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide), the NOEL of 8.36 mg/kg bw per day for the structurally related *N*-nonanoyl-4-hydroxy-3-methoxybenzylamide ([FL-no: 16.006] considered in FGE.86) from a 90-day study in rats (Posternak et al., 1969) provides an adequate margin of safety (> 400 000) in relation to the currently estimated level of exposure from its use as a flavouring agent in the USA (0.02 µg/kg bw per day).

For cyclopropanecarboxamide, *N*-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]- [FL-no: 16.095], the NOEL of 92 mg/kg bw per day from a 28-day study in rats (Merkel, 2005) provides an adequate margin of

safety (> 180 000) in relation to the currently estimated level of exposure from its use as a flavouring agent in the USA (0.5 µg/kg bw per day).

The evaluations of the 12 substances are summarised in Table 5: Summary of Safety Evaluation by the JECFA (JECFA, 2008a).

7.2. EFSA Considerations

In the previous version of FGE.94, the Panel agreed with the way the application of the Procedure has been performed by the JECFA for 9 of the 12 aliphatic and aromatic amines and amides. For the remaining three substance [FL-no: 16.090; FL-no: 16.095 and FL-no: 16.111] the Panel did not agree and concluded that additional toxicity data are needed before it can be evaluated as a flavouring substance.

In the first revision of FGE.94 additional toxicity data had become available for two substances [FL-no: 16.095 and 16.111].

Based on the new data submitted (Bauter, 2011) for cyclopropanecarboxamide, *N*-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]- [FL-no: 16.095] a NOAEL of 73.3 mg/kg bw/day could be established. When comparing this NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (61 µg *per capita* per day, corresponding to 1 µg /kg bw/day) an adequate margin of safety of 7×10^4 can be calculated.

Based on the new data submitted (Kirkpatrick, 2011) for glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester [FL-no: 16.111] a NOAEL of 75 mg/kg bw/day could be established. When comparing this NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (37 µg *per capita* per day, corresponding to 0.6 µg /kg bw/day) an adequate margin of safety of 12×10^4 can be calculated.

After the publication of FGE.94Rev1 additional toxicity data have become available for the substance [FL-no: 16.090].

Based on the new data (Bauter, 2013b) submitted for 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] a NOAEL of 23.4 mg/kg bw/day could be established. When comparing this NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (1.3 µg *per capita* per day, corresponding to 0,02 µg /kg bw/day) an adequate margin of safety of 1.2×10^6 can be calculated.

Thus the Panel concludes that all 12 substances can be considered to be of no safety concern at their estimated dietary intake based on the MSDI approach.

CONCLUSION

In Flavouring Group Evaluation 94, Revision 1 (FGE.94Rev1) the EFSA considered a group of 12 flavouring substances consisting of aliphatic and aromatic amines and amides evaluated by the JECFA at the 68th meeting (JECFA, 2007).

The present revision of FGE.94, FGE.94Rev2, includes the assessment of additional toxicity data for 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090].

The Panel concluded that no supporting FGE was available for the substances in the present FGE.

Genotoxicity data from *in vitro* and *in vivo* studies were available for seven [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103 and 16.111] of the 12 flavouring substances evaluated by the JECFA and the results did not indicate any concern for genotoxicity of the substances in this flavouring group.

In FGE.94 the Panel agreed with the way the application of the Procedure has been performed by the JECFA for nine of 12 substances, but for three substances [FL-no: 16.090, 16.095 and 16.111] no adequate NOAEL were available. In the first revision of FGE.94 additional toxicity data had become available for two substances [FL-no: 16.095 and 16.111] and NOAELs to provide adequate margins of safety were derived.

Since then a 90-day study has also been provided for [FL-no: 16.090] and a NOAEL could be established to provide an adequate margin of safety.

For 10 of the 12 substances, use levels have been provided by the Industry. Based on these use levels the mTAMDI figures calculated for nine substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103, 16.104, 16.111 and 17.035] are above the threshold of concern for their structural classes. For these substances more reliable data are needed. On the basis of such data the flavouring substances should be reconsidered using the Procedure. Following this procedure additional toxicological data might become necessary. For the remaining two [FL-no: 16.100 and 16.101] of the 12 substances, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment.

In order to determine whether the conclusion for the JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications are available for all 12 substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035].

Thus, for all 12 substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035] the Panel agrees with JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

SUMMARY OF GENOTOXICITY DATA

Table 2: Genotoxicity Data (*in vitro / in vivo*) evaluated by JECFA (JECFA, 2008a)

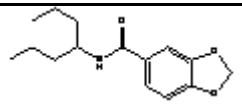
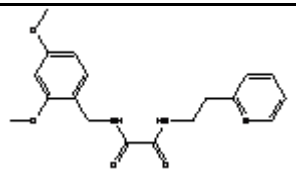
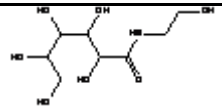
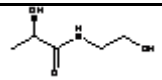
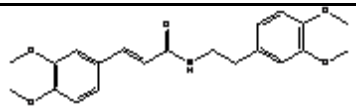
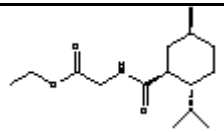
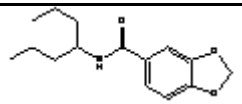
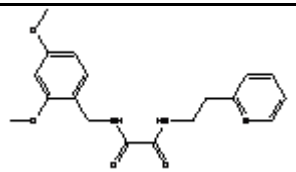
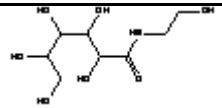
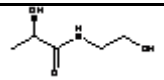
FL-no JECFA -no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
16.098 1767	N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide		Reverse mutation[(a)]	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 21, 62, 190, 560, 1670 or 5000 µg/plate[(b)]	Negative[(c)]	(Zhang, 2004a)
			Reverse mutation[(a)]	*E. coli* WP2uvrA	0, 21, 62, 190, 560, 1670 or 5000 µg/plate[(b)]	Negative[(c)]	(Zhang, 2004a)
			Chromosomal aberration	Chinese hamster ovary cells	0, 21, 62, 190, 560, 1670 or 5000 µg/ml	Negative[(c)]	(Zhang, 2004b)
16.099 1768	N-(2,4-Dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide		Reverse mutation[(a)]	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 21, 62, 190, 560 or 1670 µg/plate	Weakly positive/ negative[(c,d)]	(Zhang, 2005a)
			Reverse mutation[(a)]	*E. coli* WP2uvrA	0, 21, 62, 190, 560, 1670 or 5000 µg/plate	Negative[(c)]	(Zhang, 2005a)
			Chromosomal aberration	Chinese hamster ovary cells	0, 21, 62, 190, 560, 1670 or 5000 µg/plate	Negative[(c)]	(Zhang, 2005b)
16.102 1772	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 3,[(e)] 10,[(e)] 33,[(e)] 100, 333, 1000, 3330 or 5000 µg/plate	Negative[(c)]	(Verspeek-Rip, 2004a)
			Reverse mutation	*E. coli* WP2uvrA	0, 3, 10, 33, 100, 333, 1000, 3330 or 5000 µg/plate	Negative[(c)]	(Verspeek-Rip, 2004a)
16.103 1774	Propanamide, 2-hydroxy-N-(2-hydroxyethyl)-		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 3,[(e)] 10,[(e)] 33,[(e)] 94, 310, 940, 3140 or 4720 µg/plate	Negative[(c)]	(Verspeek-Rip, 2004b)

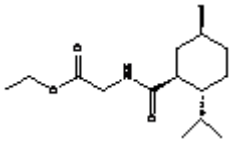
Table 2: Genotoxicity Data (*in vitro / in vivo*) evaluated by JECFA (JECFA, 2008a)

FL-no JECFA -no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	*E. coli* WP2uvrA	0, 3, 10, 33, 94, 310, 940, 3140 or 4720 µg/plate	Negative[(c)]	(Verspeek-Rip, 2004b)
16.090 1777	3-(3,4-Dimethoxyphenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide		Reverse mutation[a]	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	0, 31.6, 100, 316, 1000 or 3160 µg/plate	Negative[(c)]	(Uhde, 2004)
16.111 1776	Glycine, N-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 50, 150, 500, 1500, 2000[(f)], 3000[(f)], 4000[(f)] or 5000[(f)] µg/plate	Weakly positive[(c,g)]	(Thompson, 2005)
			Reverse mutation	*E. coli* WP2uvrA	0, 50, 150, 500, 1500 or 5000 µg/plate	Negative[(c)]	(Thompson, 2005)
In vivo							
16.098 1767	N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide		Micronucelus induction	Swiss albino (CD-1) mice	0, 175, 350 or 700 mg/kg bw[(h)]	Negative	(Pucaj, 2004a)
16.099 1768	N-(2,4-Dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide		Micronucelus induction	Swiss albino (CD-1) mice	0, 200, 400 or 800 mg/kg bw[(h)]	Negative	(Pucaj, 2004b)
16.102 1772	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide		Micronucelus induction	NMRI BR mice	0 or 2000 mg/kg bw[(i)]	Negative	(Buskens, 2004)
16.103 1774	(2R)-2-Hydroxy-N-(2-hydroxyethyl)propanamide		Micronucelus induction	NMRI BR mice	0 or 2000 mg/kg bw[(i)]	Negative	(Buskens, 2004)

(a): Plate incorporation assay and preincubation assay.

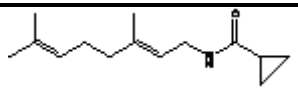
(b): The maximum concentration tested was 1670 µg/plate except for S. typhimurium TA100 in the plate incorporation assay, for S. typhimurium TA98 and TA100 and E. coli WP2uvrA in the preincubation assay without S9 (9000 × g supernatant from rat liver) and for S. typhimurium TA98, TA1535 and TA1537 and E. Coli WP2uvrA in the preincubation assay with S9, because of precipitation.
(c): With and without metabolic activation.
(d): In the plate incorporation assay, S. typhimurium TA1535 tested positive at concentrations of 21, 190 and 1670 µg/plate, but only without S9. In the preincubation assay, S. typhimurium TA100 tested positive only at 62 µg/plate and only with S9.
(e): For S. typhimurium TA100 only.
(f): S. typhimurium TA100 and TA1535 tested without S9 using both plate incorporation and preincubation methods.
(g): Weak incidence of reverse mutation observed in S. typhimurium TA100 and TA1535. All other strains showed no evidence of mutagenicity.
(h): Test material administered via single intraperitoneal injection.
(i): Test material administered via single gavage dose.

Table 3: Additional Genotoxicity Data on *N*-[(ethoxycarbonyl)methyl]-p-menthane-3-carboxamide

FL-no JECFA-no	EU Register name JECFA name	Structural formula	Test System	Test Object	Route	Dose	Reported Result	Reference
16.111 1776	Glycine, N-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester		Mouse Lymphoma	L5178Ytk+/- mouse lymphoma cells	Oral	42.03 to 672.5 µg/ml 84.06 to 1008.75 µg/ml	Negative[(a)] Negative[(b)]	(Flanders, 2006)
			Mouse Lymphoma	L5178Ytk+/- mouse lymphoma cells	Gavage	10.51 to 504.38 µg/ml 42.03 to 672.5 µg /ml	Negative[(a)] Negative[(b)]	(Flanders, 2006)

(a): Without metabolic activation.
(b): With metabolic activation.

Table 4: Additional Genotoxicity Data on *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
16.095 1779	Cyclopropanecarboxamide, N-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]-		Reverse mutation	*S. typhimurium* TA97a, TA98, TA100, TA1535	0, 5, 10, 50, 100, 500, 1000, 2000, 2500 or 5000 µg/plate	Negative[(a)]	(Next Century Incorporated, 2004)
			Reverse mutation	*E. coli* WP2uvrA	0, 50, 100, 500, 1000 or 2000 µg/plate	Negative[(a)]	(Next Century Incorporated, 2004)

(a): With and without metabolic activation.

SUMMARY OF SAFETY EVALUATIONS

Table 5: Summary of Safety Evaluation by the JECFA (JECFA, 2008a)

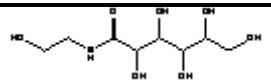
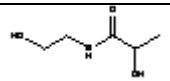
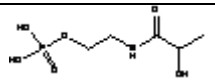
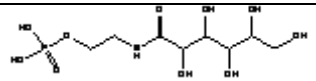
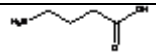
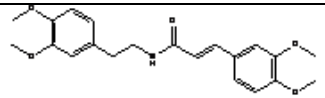
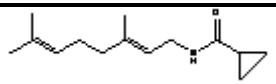
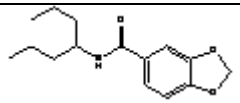
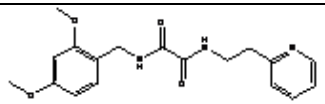
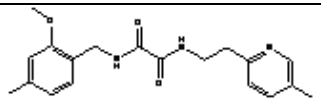
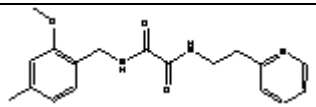
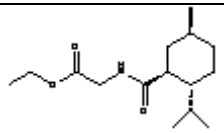
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
16.102 1772	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide		24 13	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.
16.103 1774	Propanamide, 2-hydroxy-N-(2-hydroxyethyl)-		24 10	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.
16.104 1775	2-[(2-Hydroxypropanoyl)amino]ethyl dihydrogen phosphate		12 5	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.
16.105 1773	(2R,3S,4S,5R)-2-[(2,3,4,5,6-Pentahydroxyhexanoyl) amino]ethyl dihydrogen phosphate		12 3	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.
17.035 1771	4-Amino-butyric acid		0.12 0.1	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.
16.090 1777	3-(3,4-Dimethoxyphenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide		1.3 1	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.

Table 5: Summary of Safety Evaluation by the JECFA (JECFA, 2008a)

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)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
16.095 1779	Cyclopropanecarboxamide, N-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]-		61 31	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.
16.098 1767	N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide		0.012 0.1	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.
16.099 1768	N-(2,4-Dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide		0.012 0.2	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.
16.100 1769	N1-(2-Methoxy-4-methylbenzyl)-N2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide		0.012 0.01	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.
16.101 1770	N1-(2-Methoxy-4-methylbenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide		0.012 0.01	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.
16.111 1776	Glycine, N-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl] carbonyl]-, ethyl ester.		37 34	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.

(a): EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365)= µg/capita/day.
(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.

REFERENCES

Bauter MR, 2011. A repeated dose 90-day dietary study in rats. *N*-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide. Product Safety Labs. Study no. 31351. September 16, 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2013a. 3-(3,4 Dimethoxy-phenyl-N-[2-(3,4 dimethoxyphenyl)-ethyl]-acrylamide: palability/toxicity study: a 14-day dietary study in rats. Product Safety Labs. Study no. 33518. June 20, 2013. Unpublished report submitted by Flavour Industry to FLAVIS Secretariat.

Bauter MR, 2013b. 3-(3,4 Dimethoxy-phenyl)-N-[2-(3,4 dimethoxyphenyl)-ethyl]-acrylamide: a 90-day dietary study in rats. Product Safety Labs. Study no. 35184. June 20, 2013. Unpublished report submitted by Flavour Industry to FLAVIS Secretariat.

Buskens CAF, 2004. Micronucleus test in bone marrow cells of the mouse with qugar. Test facility: NOTOX B.V. 14 October 2004. Unpublished report submitted by EFFA to FLAVIS secretariat.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetics Toxicology 16(3), 255-276.

EFFA (European Flavour and Fragrance Association), 2006. Transfer files for FGE.94 concerning [FL-no: 16.104 and 16.105]. Unpublished data from EFFA to FLAVIS Secretariat.

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

EFFA (European Flavour Association), 2014. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 13 January 2014. Information on substances in FGE.74Rev3 [FL-no: 12.238, 12.239 and 12.291], FGE.78Rev2 [FL-no: 01.004, 01.018, 01.019, 01.040, 01.045 and 01.061] and FGE.91Rev2 [FL-no: 12.038 and 12.085]. FLAVIS/8.219.

EFSA (European Food Safety Authority), 2004. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food (AFC) on a request from the Commission related to Flavouring Group Evaluation 03 (FGE.03): Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes, and an orthoester of formic acid, from chemical groups 1 and 2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal 2004, 107, 1-59.

Flanders L, 2006. L5178Y TK +/- mouse lymphoma assay. WS-5 Pure. Safepharm Laboratories. Project no. 2197/0005. August 16, 2006. Unpublished report submitted by Flavour Industry to FLAVIS Secretariat.

Flavour Industry, 2004. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-94.

Flavour Industry, 2006a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-94.

Flavour Industry, 2006b. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-94.

Flavour Industry, 2006c. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-94.

Flavour Industry, 2007. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-94.

Flavour Industry, 2008. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-40/A-94.

Flavour Industry, 2012. Unpublished information submitted by Flavour Industry to EFSA and the FLAVIS Secretariat. A-94Rev1 [FL-no: 16.102].

Flavour Industry, 2013. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-94rev1 [FL-no: 16.090]. FLAVIS/8.204.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

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

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Sixty-seventh Meeting. Rome, 20-29 June 2006, Summary and Conclusions. Issued 7 July 2006.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2007. Evaluation of certain food additives. Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 947. Geneva, 19-28 June 2007.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008a. Safety evaluation of certain food additives and contaminants. Sixty-eight Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 59. IPCS, WHO, Geneva 2008. http://whqlibdoc.who.int/publications/2008/9789241660594_eng.pdf (May 2008).

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008b. JECFA Online Edition ”Specification for Flavourings” http://www.fao.org/ag/agn/jecfa-flav/search.html (May, 2008).

Kirkpatrick JB, 2011. WS-5: A 90-day oral gavage toxicity study with a 14-day recovery period in Sprague-Dawley rats (OECD 408). WIL Research Laboratories. Study no. WIL-14070. September 1, 2011. Unpublished report submitted by Flavour Industry to FLAVIS Secretariat.

Kot K, 2005a. 90-day dietary toxicity study with S807 in rats. Unpublished report prepared by Covance Laboratories, Vienna, VA, USA for the Flavor and Extract manufacturers Association, Washington, DC, USA. Study No. 7563-101. 31 January 2005. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Kot K, 2005b. 90-day dietary toxicity study with S336 in rats. Unpublished report prepared by Covance Laboratories, Vienna, VA, USA for the Flavor and Extract manufacturers Association, Washington, DC, USA. Study No.7563-103. 31 January 2005. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Merkel DJ, 2005. Subchronic toxicity study (28-day dietary study in rats). Unpublished report prepared by the Product Safety Laboratories, Dayton, NJ, USA for the Flavor and Extract manufacturers Association, Washington, DC, USA. Study No. 16600. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Miyata K, 1995. Summary of 28-day repeated dose oral toxicity study of WS-3. Unpublished report to the Flavor Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Next Century Incorporated, 2004. Proprietary protection. 04-237: Bacterial reverse mutation test: plate incorporation and preincubation method for solids. Stirparo, B.S. Project no. 04-09-006. December 1, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Poet TS, Wu H, Soelberg J and Hinderliter P, 2005. The hydrolysis of WS-5 in artificial pancreatic juice and rat liver homogenate. Unpublished report prepared by Battelle, Pacific Northwest Division, Richland, WA, USA, for the Flavor and Extract Manufacturers Association, Washington, DC, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium (Battelle Project No. 49599).

Posternak NM, Linder A and Vodoz CA, 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. Food and Cosmetics Toxicology 7, 405-407.

Pucaj K, 2004a. Compound S807 *in vivo* mouse micronucleus test. Nucro-Technics Project no. 142257. December 20, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Pucaj K, 2004b. Compound S336 *in vivo* mouse micronucleus test. Unpublished report prepared by Nucro-Technics, Scarborough, Ontario, Canada, for the Flavor and Extract manufacturers Association, Washington, DC, USA. Nucro-Technics Project no. 142258. 20 December 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

SCF (Scientific Committee for Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Sipes G and Kong W, 2012. Report on the lack of hydrolysis of n-benzonitrile-p-menthan-3-carboxamide by rat or human microsomes. University of Arizona College of Medicine, Department of Pharmacology. 24 February 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Thompson PW, 2005. WS5 Pure: reverse mutation assy "Ames test" using *Salmonella typhimurium* and *Escherichia coli*. Unpublished report prepared by SafePharm Laboratories, Derbyshire, United Kingdom, for the Flavor and Extract Manufacturers Association, Washington, DC, USA. (SPL Project No. 1044/068). 29 June 2005. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Uhde H, 2004. Mutagenicity study of rubenamin in the *Salmonella typhimurium* reverse mutation assay (*in vitro*). Laboratory of Pharmacology and Toxicology KG. Report no. 18432/2/04. December 20, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Verspeek-Rip CM, 2004a. Evaluation of the mutagenic activity of qugarin the *Salmonella typhimurium* reverse mutation assay and the Escherichia reverse mutation assay (with independent repeat). Unpublished report prepared by NOTOX B.V.'s-Hertogenbosch, Netherlands for the Flavor and Extract Manufactures Association, Washington, DC, USA. (NOTOX, C.M. Project no. 414989). 13 October 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Verspeek-Rip CM, 2004b. Evaluation of the mutagenic activity of Q salt in the *Salmonella typhimurium* reverse mutation assay and the Escherichia reverse mutation assay (with independent repeat). NOTOX, C.M. Project no. 414967. 14-10-04. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Zhang B, 2004a. Bacterial reverse mutation test of S807. Benzo[1,3]dioxole-5-carboxylic acid (1-propyl-butyl)-amide. Nucro-Technics. Project no. 142259. December 20, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Zhang B, 2004b. Chromosome aberration test of S807 in cultured Chinese hamster ovary cells. Benzo[1,3]dioxole-5-carboxylic acid (1-propyl-butyl)-amide. Nucro-Technics. Project no. 142262. December 20, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Zhang B, 2005a. Bacterial reverse mutation test of S336. N-(2,4-dimethoxy-benzyl)-N´-(2-pyridin-2-yl-ethyl)-oxalamide. Nucro-Technics Project no. 142261. December 20, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Zhang B, 2005b. Chromosome aberration test of S336 in cultured chinese hamster ovary cells. N-(2,4-dimethoxy-benzyl)-N´-(2-pyridin-2-yl-ethyl)-oxalamide. Nucro-Technics Project no. 142260. December 20, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

APPENDIX

Appendix A. Use Levels and mTAMDI

Normal and maximum use levels provided by the Flavour Industry (EC, 2000; EFFA, 2006; Flavour Industry, 2004; Flavour Industry, 2006a; Flavour Industry, 2006b; Flavour Industry, 2006c; Flavour Industry, 2007; Flavour Industry, 2008) in accordance with the Commission Regulation (EC) No 1565/2000 (EC, 2000).

The normal and maximum use levels are shown in Table A.1. Based on these normal use levels mTAMDI figures can be calculated (see Table A.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**
16.090	3 10	- -	5 10	- -	- -	10 20	- -	10 50	- -	- -	- -	- -	5 20	- -	3 20	5 25	10 30	5 10
16.095	0,01 0,05	0,01 0,05	0,1 0,5	- -	- -	1 5	- -	0,1 0,5	0,01 0,05	0,01 0,05	- -	- -	0,01 0,05	- -	1 5	1 5	0,01 0,05	0,01 0,05
16.098	1 3	2 4	- -	1 3	1 3	- -	- -	1 2	1 3	1 3	- -	- -	5 10	- -	- -	- -	5 10	1 3
16.099	1 3	2 4	- -	1 3	1 3	- -	- -	1 2	1 3	1 3	- -	- -	5 10	- -	- -	- -	5 10	1 3
16.102	50 200	50 200	50 200	- -	- -	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200
16.103	50 200	50 200	50 200	- -	- -	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200	50 200
16.104	5 15	- -	5 15	5 15	- -	5 15	- -	- -	- -	- -	- -	- -	5 15	- -	5 15	5 15	5 15	- -
16.105	- -	5 15	- -	- -	- -	- -	- -	- -	5 15	- -	- -	- -	5 15	- -	- -	- -	5 15	- -
16.111	10 300	10 200	10 150	10 200	10 200	10 200	10 50	20 200	10 100	10 100	- -	- -	- -	20 300	10 50	10 400	50 350	10 200
17.035	30 100	30 100	20 100	- -	- -	30 100	30 100	50 300	20 200	- -	- -	- -	- -	30 200	30 200	40 300	20 100	30 100

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)
16.102	2,3,4,5,6-Pentahydroxy-*N*-(2-hydroxyethyl)-hexanamide	24	13	27000	I	1800
16.103	Propanamide, 2-hydroxy-*N*-(2-hydroxyethyl)-	24	10	27000	I	1800
16.104	2-[(2-Hydroxypropanoyl)amino]ethyl dihydrogen phosphate	12	5	2700	I	1800
16.105	(2R,3S,4S,5R)-2-[(2,3,4,5,6-Pentahydroxyhexanoyl)amino]ethyl dihydrogen phosphate	12	3	870	I	1800
17.035	4-Amino-butyric acid	0.12	0.1	18000	I	1800
16.090	3-(3,4-Dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide	1.3	1	3000	III	90
16.095	Cyclopropanecarboxamide, *N*-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]-	61	31	380	III	90
16.098	*N*-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide	0.012	0.1	470	III	90
16.099	*N*-(2,4-Dimethoxy-benzyl)-*N*'-(2-pyridin-2-yl-ethyl)-oxalamide	0.012	0.2	470	III	90
16.100	*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide	0.012	0.01	ND	III	90
16.101	*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide	0.012	0.01	ND	III	90
16.111	Glycine, *N*-[[(1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl]carbonyl]-, ethyl ester.	37	34	7400	III	90

ND: No intake data available

ABBREVIATIONS

BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
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
HPRT	Hypoxanthine Phosphoribosyl transferase
ID	Identity
IP	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	Micronucleated Binucleate cells
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level

NTP	National Toxicology Program
PCE	Polychromatic erythrocyte
RI	Replication Index
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation