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Scientific Opinion of Flavouring Group Evaluation 407 (FGE.407): 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Vittorio Silano, Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Maria Rosaria Milana, André Penninks, Maria de Fátima Tavares Poças, Andrew Smith, Christina Tlustos, Detlef Wölfle, Holger Zorn, Corina-Aurelia Zugravu, Ulla Beckman Sundh, Leon Brimer, Gerard Mulder, Maria Anastassiadou and Wim Mennes

Abstract

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) was requested to deliver a scientific opinion on the implications for human health of the flavouring substance 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid [FL-no: 16.130], in the Flavouring Group Evaluation 407 (FGE.407), according to Regulation (EC) No 1331/2008 of the European Parliament and of the Council. The substance has not been reported to occur in natural source materials of botanical or animal origin. It is intended to be used as both the parent compound and its hemisulfate monohydrate salt as a flavouring substance with modifying properties in specific categories of food. The chronic dietary exposure to the substance estimated using the added portions exposure technique (APET), is calculated to be 882 μg/person per day for a 60-kg adult and 547 μg/person per day for a 15-kg 3-year-old child. There is no concern with respect to genotoxicity. A 90-day dietary administration study in rats showed no adverse effects for doses up to 100 mg/kg body weight (bw) per day, providing an adequate margin of safety. Developmental toxicity was not observed in a study with rats at the dose levels up to 1,000 mg/kg bw per day. The Panel concluded that [FL-no: 16.130] and its hemisulfate monohydrate salt are not expected to be of safety concern at the estimated levels of dietary exposure calculated using the APET approach. This conclusion applies only to the use of the substance as a flavour modifier as requested and when used at the levels as specified for foods from different food categories.

Keywords: 4-amino-5-(3-(isopropylamino)-2, 2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid, FGE.407, [FL-no: 16.130]

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Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of a chemically defined flavouring substance used in or on foodstuffs in the Member States. In particular, EFSA was requested to evaluate 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid [FL-no: 16.130], allocated in the Flavouring Group Evaluation 407 (FGE.407), using the Procedure as referred to in Regulation (EC) No 1334/2008 of the European Parliament and of the Council.

Despite sharing some structural elements with other flavouring substances (e.g. methylquinolines or anthranilates), these are not sufficiently structurally related to the candidate substance. Consequently, the Panel decided to assess this substance on its own.

4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid [FL-no: 16.130] is chemically synthesised and has not been reported to occur in natural source materials of botanical or animal origin.

Specifications

The Panel noted that both the parent compound [FL-no: 16.130] and its hemisulfate monohydrate salt are intended to be added to foodstuffs.

Specifications including complete purity criteria and identity for the materials of commerce for both [FL-no: 16.130] and its salt, have been provided and considered adequate. The candidate substance does not possess chiral centres or double bonds that would give rise to optical or geometrical isomers.

The information provided on the manufacturing process, the composition and the stability of the flavouring substance and its hemisulfate monohydrate salt were considered sufficient.

Use and exposure

4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid and its sulfate salt, [FL-no: 16.130], are intended to be used as flavouring substances with modifying properties[1] in specific food categories.

The chronic dietary exposure to the candidate substance has been estimated using the added portions exposure technique (APET). It is calculated to be 882 μg/person per day (14.7 μg/kg body weight (bw) per day for a 60-kg adult) and 547 μg/person per day (36.4 μg/kg bw per day for a 15-kg 3-year-old child).

The highest acute intake of the candidate substance results from the consumption of non-alcoholic beverages containing 7 mg/kg of the candidate substance consumed by a 60-kg adult and a 15-kg 3-year-old child. This results in an intake of 6.3 mg/person per day (or 105 μg/kg bw per day for a 60-kg adult), and in an intake of 4.0 mg/person per day (or 265 μg/kg bw per day for a 15-kg 3-year-old child).

Absorption, distribution, metabolism and elimination

Absorption, distribution, metabolism and elimination (ADME) studies in rats available for [FL-no: 16.130] and its sulfate salt indicate that both forms are poorly absorbed after oral administration and no significant metabolism is expected in the gastrointestinal tract.

Genotoxicity

[FL-no: 16.130] contains a structural alert for genotoxicity in the form of an amino-substituted aromatic ring. However, the substance gave negative results when tested in a bacterial gene mutation assay and micronucleus assay *in vitro*. Based on these results, the Panel concluded that there is no concern with respect to genotoxicity for the candidate substance [FL-no: 16.130]. Therefore, the safety of the substance was evaluated according to the Procedure for the evaluation of chemically defined flavouring substances.

[1] 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. Official Journal of the European Union, L 354, 34–50.

Systemic toxicity

A 90-day systemic toxicity study in the rat has been performed. Dietary administration of [FL-no: 16.130] to CD rats for 13 weeks at doses up to 100 mg/kg bw per day was well tolerated. Based on the findings in this study, the no-observed-adverse-effect-level (NOAEL) was considered to be 100 mg/kg bw per day, the highest dose tested, in both sexes.

Developmental toxicity

In a developmental toxicity study, rats were administered oral doses of 250–1,000 mg/kg bw per day of the candidate substance. There were no statistically significant differences between the treated and control groups. Therefore, there is no concern for developmental toxicity of [FL-no: 16.130] in rats at dose levels up to 1,000 mg/kg bw per day, the highest dose tested.

Safety assessment for acute exposure

Estimates of maximum acute dietary exposure indicate that this would be about 265 μg/kg bw for a 3-year-old child, which is higher than the acute exposure estimate for an adult (105 μg/kg bw per day). From these data, a margin of exposure of 380 for children can be calculated, which indicates no concern for acute effects, taking into account that the NOAEL was derived from a 90-day toxicity study. In addition, no toxicity was observed at dose levels up to 1,000 mg/kg bw per day in a developmental toxicity study with an exposure period of 14 days.

Safety assessment for long-term exposure

Since no clear structural/metabolic similarity of the candidate substance to flavouring substances in an existing FGE was identified, the Panel proceeded with the individual evaluation of the candidate substance [FL-no: 16.130], according to the EFSA Guidance (EFSA, 2010).

Based on its chemical structure, the substance has been assigned to Cramer class III. The results of studies on metabolism and pharmacokinetics do not allow to conclude that its metabolites are innocuous. Accordingly, the candidate substance is evaluated via the B-side of the Procedure scheme. Based on the comparison of APET with the Cramer class III threshold, a 90-day study and a developmental toxicity study were required and carried out for this substance. Adequate margins of safety of 6,800 for adults and 2,700 for 3-year-old children have been calculated on the basis of the exposure estimates calculated and the NOAEL from the 90-day toxicity study.

Overall, the Panel concluded that using the NOAEL obtained from a 90-day dietary study in rats, there is no safety concern for [FL-no: 16.130], when used as a flavouring substance with modifying properties at the estimated level of dietary exposure calculated using the APET approach and based on the use levels as specified in Appendix B.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008[1] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with the 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.

Regulation (EC) No 1331/2008[2] applies for the evaluation and approval of new flavouring substances.

The applicant has submitted an application for authorisation as a new flavouring substance of the substance: 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid – S9632.

In order for the Commission to be able to consider its inclusion in the Union list of flavourings and source materials (Annex I of Regulation (EC) No 1334/2008), the European Food Safety Authority (EFSA) should carry out a safety assessment of this substance.

1.1.1. Terms of Reference

The European Commission requests EFSA to carry out a safety assessment on: 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid – S9632 [FL-no: 16.130] as a new flavouring substance in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.2. Interpretation of the Terms of Reference

The present scientific opinion FGE.407 covers the safety assessment of the 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid [FL-no: 16.130]. This substance will be evaluated as a flavouring substance with modifying properties[3] (Regulation (EC) No 1334/2008). The Panel noted that the substance is intended to be used as parent compound and hemisulfate monohydrate salt. The Union List[4] would also allow the use of the hemisulfate monohydrate salt under the condition that the parent compound is not of concern.

2. Data and methodologies

The present evaluation is based on data on 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid – [FL-no: 16.130] and its hemisulfate monohydrate salt provided by the applicant in a dossier submitted in support of its application for authorisation as a new flavouring substance. The dossier submitted contained data generated for both the candidate substance and hemisulfate monohydrate salt. The proposed dietary intake of the candidate substance has been calculated by the applicant based on the use and use levels for a wide range of food and beverage categories (see Appendix B). Based on these use levels, the Panel has calculated the intakes in adults and children that were subsequently used in the assessment.

The safety assessment of 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid is carried out by EFSA in accordance with the procedure as lined out in the EFSA scientific opinion 'Guidance on the data required for the risk assessment of flavourings to be used in or on foods' (EFSA, 2010) and the technical report of EFSA 'Proposed template to be used in drafting scientific opinions on flavouring substances (explanatory notes for guidance included)' (EFSA, 2012).

A more thorough explanation on the methodology is given in Appendix F.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

[3] https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_flavourings-guidance_modifying_properties.pdf

[4] See Note 1, Section 2, Part A of Annex I of Regulation (EC) No 1334/2008.

3. Assessment

3.1. Identity of the substance

The candidate substance has been allocated the FLAVIS number (FL-no) 16.130. The name of the flavouring substance is 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid. According to the application, both the hemisulfate monohydrate salt and the parent compound are intended to be added to foodstuffs.

3.2. Organoleptic characteristics

The candidate substance imparts a neutral metallic lingering odour. [FL-no: 16.130] is intended to be used as a substance with flavour-modifying properties.

3.3. Existing authorisations and evaluations

In November 2014, [FL-no: 16.130] was allocated the status 'Generally Regarded As Safe' (GRAS) by the Flavour and Extract Manufactures Associations (FEMAs) expert Panel (FEMA no 4774).

The candidate substance [FL-no: 16.130] and its hemisulfate monohydrate salt (JECFA No 2204.1) were evaluated as flavouring substances by JECFA in 2014 at the 79th session (JECFA, 2014). The Committee concluded that 'on the basis of all of the available evidence, 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)- 2-methylquinoline-3-carboxylic acid and its hemisulfate monohydrate salt would not pose a safety concern at current estimated dietary exposures' (JECFA, 2014).

3.4. Specifications

The specifications of the flavouring substance and its hemisulfate monohydrate are summarised in Table 1.

3.4.1. Information on the configuration of the flavouring substance

The candidate substance [FL-no: 16.130] does not possess chiral centres and does not have geometrical or optical isomers.

3.4.2. Manufacturing process

The compound was chemically synthesised from isopropylamine, 3-hydroxy-2,2-dimethylpropanoic acid, 2-amino-6-fluorobenzonitrile and methylacetoacetate. The process is schematically outlined in Figure 1.

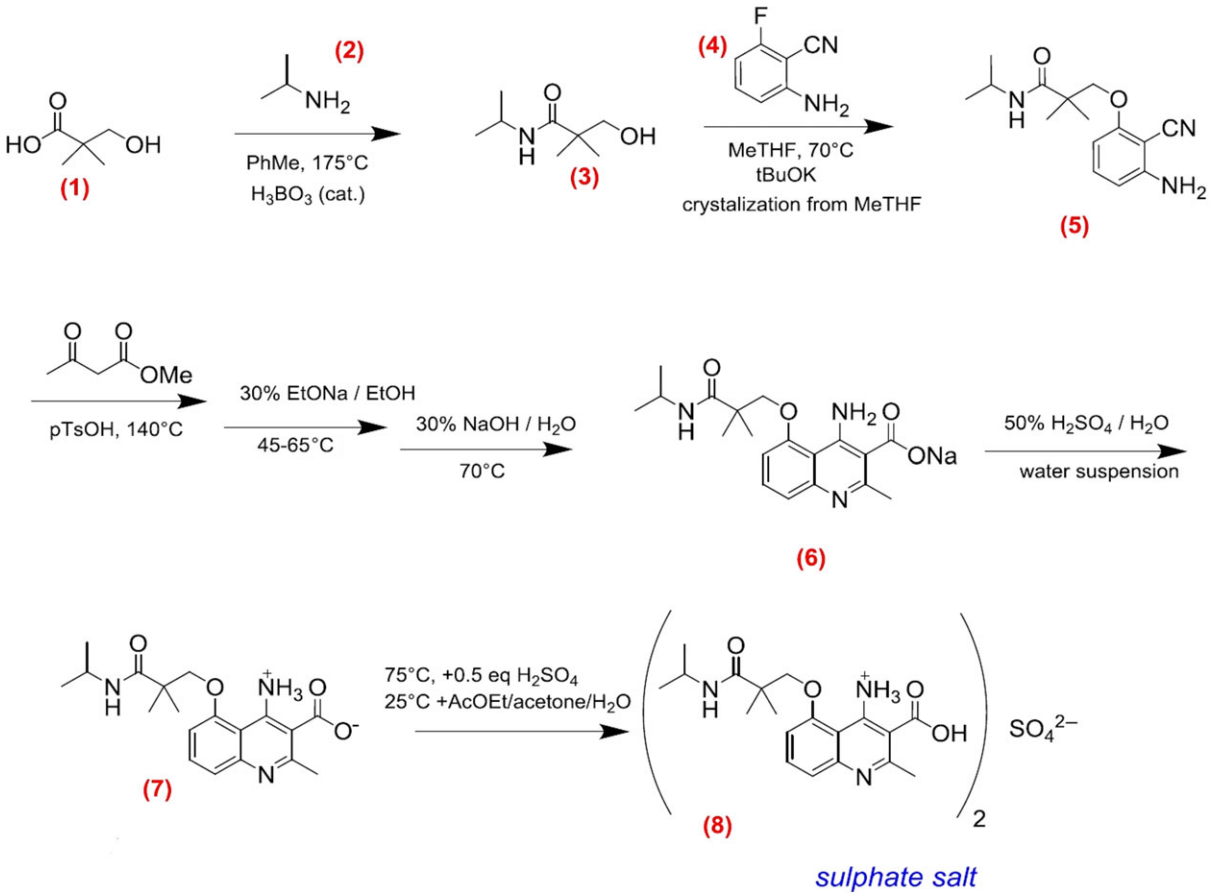
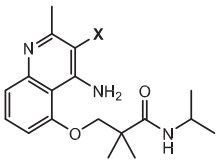


Figure 1: Production process of [FL-no: 16.130] and its sulphate salt

3.4.3. Stability and decomposition products

The candidate substance is stable in aqueous solution at 40°C at pH 2.8, 4.0 and 7.1 for a period of 4 weeks. The dry compound as well as the sodium, phosphate and sulfate salts are stable at 100°C up to 24 h. At 175°C, thermal degradation may take place. The only decomposition product detected (designated S9379) was formed by decarboxylation and has been fully characterised (for assessment of its genotoxicity, see Section 3.7) (Figure 2).



[FL-no: 16.130] (X=CO_2H)

S9379 (X=H)

Figure 2: [FL-no: 16.130] and the decomposition product formed by decarboxylation

Other trials on use in food product prototypes, such as ready-to-drink coffee beverage (100°C for 8 min), cake (177°C for 30 min) and cookie (204°C for 8 min), showed full stability as measured by the recovery upon quantitative analysis. For hard candies (149°C for 30 min), a lower recovery of 87% was reported for the parent substance. The Panel considered this relatively low recovery as an analytical artefact since for the sulfate salt a recovery of 104% was reported. In addition, no increase in the formation of breakdown products was observed.

For more details on the studies on stability and decomposition products, see Appendix A.

3.4.3.1. Interaction with food components

Trials in which the stability of the candidate substance has been measured in different aqueous media as would be encountered in food and beverage preparations did not indicate any chemical interaction with other food components. Tasting trials conducted with the candidate substance in butterscotch pudding and tropical punch-flavoured beverage did not indicate off-notes, loss of modifying properties or any other signs of interaction with the components of these foods.

3.4.4. Particle size[5]

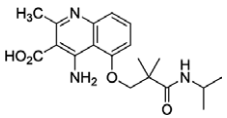
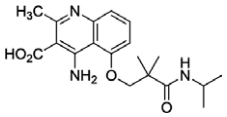
The mean particle size of two batches of the compound measured by laser beam 'Sympatec HELOS H0415 RODOS' was 63 and 81 μm (Senomyx, 2016). The substance is not to be considered as a nanomaterial, as defined by Commission Recommendation 2011/696/EU. The Panel noted that the applicant indicated that the compound will not be used in formulations other than solutions.

3.4.5. Conclusion on specifications

The Panel considered the specifications provided for both the flavouring compound itself [FL-no: 16.130] and for its hemisulfate monohydrate salt, as well as the data on stability of [FL-no: 16.130] and its hemisulfate monohydrate as dry compounds, in solution and in the course of the production of different relevant model products, as sufficient.

[5] Commission Recommendation 2011/696/EU of 18 October 2011 on the definition of nanomaterials. Official Journal of the European Union L257/38-40 on 20.10.2010 (https://ec.europa.eu/research/industrial_technologies/pdf/policy/commission-recommendation-on-the-definition-of-nanomater-18102011_en.pdf)

Table 1: Specifications

FL-no	EU Register Name	Structural formula	JECFA no FEMA no CoE no CAS no EINECS no E no	Odour Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b] Others	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. index[d] Spec. gravity[e]	EFSA comments
16.130	4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid		2204 4774 – 1359963-68-0 – –	White to pale yellow powder. Solid $C_{19}H_{25}N_3O_4$ 359.4	700 mg/L 1,600 mg/L –	n.a. 212–215[f] IR NMR MS > 99%	n.a. n.a.	
	4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid hemisulfate monohydrate salt[g]	 ·1/2·H_2SO_4·H_2O	2204.1 – – 1460210-04-1	Off-white to pale yellow powder. Solid $C_{19}H_{25}N_3O_4$·1/2 H_2SO_4·H_2O 426.5	Soluble Sparingly soluble	n.a. 136–138 UV IR NMR MS > 99% (by HPLC)	n.a. n.a.	

FL-no: FLAVIS no; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; EINECS: European Inventory of Existing Commercial chemical Substances; IR: infrared; NMR: nuclear magnetic resonance; MS: mass spectrometry; UV: ultraviolet; HPLC: high-performance liquid chromatography.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1013.25 hPa (1 atm), if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.
(f): The substance decomposes at 226 ± 2°C.
(g): Synonym: 3-quinolinecarboxylic acid, 4-amino-5-[2,2-dimethyl-3-[(1-methylethyl)amino]-3-oxopropoxy]-2-methyl-, sulfate, hydrate (2:1:2).

Synonym numbers used in the FGE:

S9632: Candidate substance, [FL-no: 16.130]

S1638: Sulfate salt of [FL-no: 16.130]

S9379: Decomposition product formed by decarboxylation of [FL-no: 16.130]

3.5. Structural/metabolic similarity to substances in an existing FGE

Despite sharing some structural elements with other flavouring substances (e.g. methylquinolines or anthranilates), these are not sufficiently structurally related to the candidate substance. Consequently, the Panel decided to assess this substance on its own.

3.6. Exposure assessment

All data necessary for the calculation exposure estimates (i.e. normal and maximum occurrence levels for refined subcategories of foods and beverages) are reported in Appendix B.

3.6.1. Concentration in processed and non-processed foods from natural occurrence

The candidate substance is chemically synthesised and is not expected to occur naturally in food. According to the applicant, there are no reports of its detection in processed foods. Based on the chemical structure of the candidate substance, its formation during food processing is not anticipated.

3.6.2. Non-food sources of exposure

No sources of exposure are known to exist from non-food sources.

3.6.3. Chronic dietary exposure

The exposure assessment to be used in the Procedure for the safety evaluation of the candidate substance is the chronic added portions exposure technique (APET) estimate (EFSA, 2010). The chronic APET has been calculated for adults and children (see Table 2), and these values, expressed per kg body weight (bw), will be used in the Procedure (see Appendix B).

Although the substance is not intended to be used in food categories specifically intended for infants and toddlers, these could still be exposed through consumption of foods from the general food categories, which may contain the substance. However, at present, there is no generally accepted methodology to estimate exposure in these age groups resulting from consumption of foods from the general categories. Exposure of infants and toddlers is currently under consideration by EFSA.

Table 2: APET – Chronic dietary exposure

Chronic APET	Added[a] (μg/kg bw per day)		Other dietary sources[b] (μg/kg bw per day)		Combined[c] (μg/kg bw per day)	
Use level	**Normal**	**Maximum**	**Average**	**Maximum**	**Normal**	**Maximum**
Adults[d]	14.7	n.a.	0	n.a.	14.7	n.a.
Children[e]	36.4	n.a.	0	n.a.	36.4	n.a.

APET: added portions exposure technique; bw: body weight; n.a. not applicable: the chronic APET calculation is based on the combined normal occurrence level.
(a): APET added is calculated on the basis of the amount of flavour added to a specific food category.
(b): APET other dietary sources is calculated based on the natural occurrence of the flavour in a specified food category.
(c): APET combined is calculated based on the combined amount of added flavour and naturally occurring flavour in a specified food category.
(d): For the adult APET calculation, a 60-kg person is considered representative.
(e): For the child APET calculation, a 3-year-old child with a 15 kg bw is considered representative.

3.6.4. Acute dietary exposure

The calculation was based on the maximum use levels and large portion size, i.e. three times standard portion size (see Appendix B).

Although the substance is not intended to be used in food categories specifically intended for infants and toddlers, these could still be exposed through consumption of foods from the general food categories, which may contain the substance. However, at present, there is no generally accepted methodology to estimate exposure in these age groups resulting from consumption of foods from the general categories (Table 3).

Table 3: APET – Acute dietary exposure

Acute APET	Added[(a)] (μg/kg bw per day)		Other dietary sources[(b)] (μg/kg bw per day)		Combined[(c)] (μg/kg bw per day)	
Use level	**Normal**	**Maximum**	**Average**	**Maximum**	**Normal**	**Maximum**
Adults[(d)]	n.a.	105	n.a.	0	n.a.	105
Children[(e)]	n.a.	265	n.a.	0	n.a.	265

APET: added portions exposure technique; bw: body weight; n.a. not applicable: the acute APET calculation is based on the combined maximum occurrence level.
(a): APET added is calculated on the basis of the amount of flavour added to a specific food category.
(b): APET other dietary sources is calculated based on the natural occurrence of the flavour in a specified food category.
(c): APET combined is calculated based on the combined amount of added flavour and naturally occurring flavour in a specified food category.
(d): For the adult APET calculation, a 60-kg person is considered representative.
(e): For the child APET calculation, a 3-year-old child with a 15 kg bw is considered representative.

3.6.5. Cumulative dietary exposure

Not applicable.

3.7. Genotoxicity

The candidate substance [FL-no: 16.130] contains an aminoquinoline structural entity that is considered to be a structural alert for genotoxicity. The candidate substance was tested for genotoxicity *in vitro* and *in vivo* in accordance with the OECD Test Guidelines 471, 473, 474 and 487 (OECD 1997a,b,c, 2014).

Parent compound as well as its sodium salt and S9379 (a decomposition product formed by decarboxylation of [FL-no: 16.130]) did not induce gene mutations in bacterial gene mutation assays in the presence or absence of metabolic activation, using the plate incorporation or pre-incubation methods (BioReliance, 2011; Nucro-Technics, 2011a; WIL Research, 2012c).

The sodium salt of [FL-no: 16.130] did not induce chromosomal aberrations in cultured peripheral human lymphocytes in the presence or absence of metabolic activation (Nucro-Technics, 2011b).

The sulfate salt of [FL-no: 16.130] was negative for the induction of micronuclei *in vitro* in cultured human peripheral lymphocytes in the presence and absence of the exogenous metabolic activation system (BioReliance, 2016).

An *in vivo* bone marrow micronucleus test in mice did not show any induction of micronuclei by the test article [FL-no: 16.130]. The lack of cytotoxicity in the bone marrow cells does not allow a conclusion as whether the test substance or a metabolite reached the bone marrow. Therefore, the result of this study (Nucro-Technics, 2011c) was considered of limited relevance.

The toxicokinetic study in rats shows that the candidate substance is poorly absorbed and rapidly eliminated after oral administration. Considering the low systemic exposure, the *in vivo* micronucleus assay in bone marrow is not considered an appropriate test to investigate the genotoxicity of this substance.

Based on the above mentioned considerations and as there was no indication of genotoxicity in a bacterial gene mutation test and in an *in vitro* mammalian cell micronucleus test, recommended as the basic test battery by the EFSA Scientific Committee (EFSA Scientific Committee, 2011), the Panel considered that there is no concern with respect to genotoxicity for the candidate substance.

For full details, see Appendix C.

3.8. Absorption, distribution, metabolism and elimination

A study was performed to determine the toxicokinetic parameters and oral bioavailability of the candidate substance by either a single intravenous administration or up to 7 days of oral dosing (gavage) in male and female Sprague–Dawley rats (Senomyx, 2011a).

The candidate substance was poorly bioavailable by the oral route (0.5–1.2% of the dose). It is rapidly eliminated after either intravenous ($T_{1/2} < 0.27$ h) or oral administration ($T_{1/2} < 1.2$ h). Systemic exposure to the candidate substance was roughly proportional with dose and was not significantly different between male and female rats on day 7 vs day 1 of dosing. No significant accumulation of the candidate substance was found in plasma after repeated dosing for seven consecutive days.

Toxicokinetic studies during dietary administration for 90 days confirmed the 7 day study findings.

The bioavailability of the sulfate in the rat after oral dosing was shown to be equivalent to the parent substance.

Qualitative metabolic profiling study of the candidate substance using rat and human hepatic microsomes showed that the candidate substance was not metabolised during the 60-min incubation period (PharmOptima, 2011).

The poor bioavailability of [FL-no: 16.130] is also supported by an excretion study conducted with [FL-no: 16.130] in male and female Sprague–Dawley rats (Senomyx, 2013).

For full details, see Appendix D.

3.9. Toxicity data

A non-good laboratory practice (GLP) 28-day study evaluated the potential toxicity of the candidate substance. The candidate substance was administered in the diet of three groups of five male and five female CD®[Crl:CD®(SD)] rats at the intended dose levels of 10, 30 and 100 mg/kg bw per day. The actual average dose levels were within 5% of the intended dose levels based on feed consumption and measured body weights and concentrations in the feed. Based on a no-observed-adverse-effect-level (NOAEL) of 100 mg/kg bw per day, the doses for the 90-day study were selected (MPI, 2011a).

3.9.1. 90-day dietary systemic toxicity study in rats

In a 90-day study conducted in compliance with the US Food and Drug Administration Toxicological Principles for the Safety of Food Ingredients (similar to the OECD Guideline 408) (MPI, 2011b), the candidate substance was administered in the diet of four groups of twenty male and twenty female CD®[Crl:CD®(SD)] rats at the intended dose levels of 0 (control), 30, 60 and 100 mg/kg bw per day. The vehicle or diet containing test article was available *ad libitum* for 13 weeks. The actual average dose levels were within 5% of the intended dose levels based on feed consumption and measured body weights and concentrations in the feed. There were no test article-related effects noted for any parameter examined. There were no macroscopic or microscopic findings or toxicologically significant organ weight changes noted at any dose level. As a result, the NOAEL following 13 weeks of dietary administration was 100 mg/kg per day, the highest dose level tested, in male and female rats.

3.9.2. Developmental toxicity study in rats

A dose-range study for the developmental toxicity has been performed in rats. Based on the results of this study, the dosage levels of 250, 500 and 1,000 mg/kg bw per day were selected for a definitive embryo/fetal development study of the candidate substance administered orally by gavage to inbred Crl:CD(SD) rats.

A developmental toxicity study was conducted in accordance with the OECD Test Guideline 414 (OECD, 2001), the US FDA Redbook 2000 (Toxicological Principles for the Safety Assessment of Food Ingredients, as updated) and the Guidelines for Reproduction and Development Studies, January 2001 (WIL Research, 2012b).

Based on the lack of test article-related effects at any dosage level, a dosage level of 1,000 mg/kg bw per day, the highest dosage level evaluated, was considered to be the NOAEL for maternal toxicity and embryo/fetal development effects when the candidate substance was administered orally by gavage to inbred Crl:CD(SD) rats.

The results of the toxicity studies are reported in Appendix E.

3.10. Exposure compared to TTC

By comparison of the APET exposure estimate with the threshold of toxicological concern (TTC, or in short threshold of concern) and TTC × 10 (Table 4), it follows from the Procedure (see Appendix F) that for the evaluation of the candidate flavouring substance the results of a 90-day oral toxicity study and a developmental toxicity study are necessary (see Appendix F). These studies have been submitted by the applicant.

Table 4: Summary table on calculated chronic APET and threshold of concern

FL-no	Structural class	Add APET	Add APET[(a)]	Threshold of concern	Threshold of concern × 10
16.130		µg/kg bw per day	µg/person per day		
Adult[(b)]	III	14.7	882	90	900
Child[(c)]		36.4	547		

FL-no: FLAVIS no; APET: added portions exposure technique; bw: body weight.
(a): The APET figure to be used in the Procedure is based on exposure per person per day.
(b): For the adult APET calculation, a 60-kg person is considered representative.
(c): For the child APET calculation, a 3-year-old child with a 15 kg bw is considered representative.

3.11. Procedure for the safety assessment

The Procedure figure is reported in Appendix F.

Based on the genotoxicity data available, the Panel concluded that for the candidate substance [FL-no: 16.130] there is no concern with respect to genotoxicity.

3.11.1. Safety assessment for acute exposure

Estimates of maximum acute dietary exposure indicate that this would be about 0.3 mg/kg bw for a 3-year-old child. The doses of 100 mg/kg bw per day (NOAEL) are well tolerated in rats without adverse effects. From these data, a margin of exposure of 380 for children can be calculated, which indicates no concern for acute effects, taking into account that the NOAEL was derived from a 90-day toxicity study. In a developmental toxicity study with an exposure period of 14 days, no overt maternal toxicity was observed at a dose levels up to 1,000 mg/kg bw per day, which supports this conclusion.

3.11.2. Safety assessment for long-term exposure

For [FL-no: 16.130], as there is no clear structural/metabolic similarity to other flavouring substances evaluated in an existing FGE, the Panel decided to assess this substance through the Procedure for the evaluation of individual flavouring substances (EFSA, 2010) see Appendix F.

3.11.2.1. Procedure steps

Does the candidate substance give rise to concern with respect to genotoxicity?

The candidate substance [FL-no: 16.130] is not considered to be of concern with respect to genotoxicity (see Section 3.7).

3.11.2.2. Step 1

On the basis of its chemical structure, the candidate substance [FL-no: 16.130] is classified in structure class III (Cramer et al., 1978). The TTC for a structural class III substance is 90 µg/person per day.

3.11.2.3. Step 2

Are there data available to demonstrate that the metabolites can be considered innocuous?

The toxicokinetic and metabolism studies carried out on the candidate substance show that metabolism only takes place to a very minor degree. It cannot be concluded that the candidate or the metabolites are innocuous and accordingly the substance proceeds via the B-side of the Procedure.

3.11.2.4. Step B3

Does the dietary exposure exceed the respective Cramer class threshold?

Based on the APET calculation, the chronic exposure to the candidate substance is 882 µg/adult person per day and 547 µg/child per day.

There are no contributions from structurally related flavouring substances to these values.

The chronic APET for both adults and children exceeds the threshold of 90 µg/person per day and accordingly the candidate substance proceeds to step B4.

3.11.2.5. Step B4

Does the dietary exposure exceed the respective Cramer class threshold × 10?

The daily exposure based on the chronic APET is located between the threshold and 10 times the threshold (see Table 4). Following the Procedure (see Appendix F), a 90-day study and a developmental study are required to finalise the safety evaluation of the candidate substance. Both a 90-day study and developmental study have been performed with the candidate substance and these studies provide NOAELs of 100 and 1,000 mg/kg bw per day, respectively (see Section 3.9).

3.12. Margins of safety

The NOAEL of the 90-day oral toxicity study (100 mg/kg bw per day) was considered in the risk assessment of the flavouring substance (see Section 3.9).

For acute exposure, a margin of safety of 380 for children could be derived based on the higher APET of 0.3 mg/kg bw per day for a 3-year-old child, which is considered sufficient.

Based on the higher APET (36.4 μg/kg bw per day) and the NOAEL of 100 mg/kg bw per day, a lowest margin of safety of 2,700 could be derived for long-term exposure in children (Table 5).

Table 5: Summary table of calculated margins of safety

	Study type	NOAEL mg/kg bw/day	Add APET μg/kg bw/day	Margin of safety
Adult	90-Day oral toxicity study in the rat	100	14.7	6,800
Child			36.4	2,700
Adult	Developmental toxicity study in the rat	1,000	14.7	68,000
Child			36.4	27,000

NOAEL: no-observed-adverse-effect-level; APET: added portions exposure technique; bw: body weight.

Based on the Procedure, the Panel concluded that there is no safety concern for the use of [FL-no: 16.130] as a flavouring substance with modifying properties at the estimated level of dietary exposure calculated using the APET approach and based on the use levels in food as specified in Appendix B.

4. Conclusion

The CEF Panel was requested to evaluate 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid [FL-no: 16.130], in the FGE.407 using the Procedure as referred to in Regulation (EC) No 1334/2008 of the European Parliament and of the Council.

Despite sharing some structural elements with other flavouring substances (e.g. methylquinolines or anthranilates), these are not sufficiently structurally related to the candidate substance. Consequently, the Panel decided to assess this substance on its own.

4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid [FL-no: 16.130] is chemically synthesised and has not been reported to occur in natural source materials of botanical or animal origin.

Specifications

The Panel noted that both the parent compound [FL-no: 16.130] and its hemisulfate monohydrate salt are intended to be added to foodstuffs.

Specifications including complete purity criteria and identity for the materials of commerce for both [FL-no: 16.130] and its salt, have been provided and considered adequate. The candidate substance does not possess chiral centres or double bonds that would give rise to optical or geometrical isomers.

The information provided on the manufacturing process, the composition and the stability of the flavouring substance and its hemisulfate monohydrate salt were considered sufficient.

Use and exposure

4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid and its sulfate salt, [FL-no: 16.130], are intended to be used as flavouring substances with modifying properties[6] in specific food categories.

The chronic dietary exposure to the candidate substance has been estimated using the APET. It is calculated to be 882 μg/person per day (14.7 μg/kg bw per day for a 60-kg adult) and 547 μg/person per day (36.4 μg/kg bw per day for a 15-kg 3-year-old child).

The highest acute intake of the candidate substance results from the consumption of non-alcoholic beverages containing 7 mg/kg of the candidate substance consumed by a 60-kg adult and a 15-kg 3-year-old child. This results in an intake of 6.3 mg/person per day (or 105 μg/kg bw per day for a 60-kg adult) and in an intake of 4.0 mg/person per day (or 265 μg/kg bw per day for a 15-kg 3-year-old child).

Absorption, distribution, metabolism and elimination

Absorption, distribution, metabolism and elimination (ADME) studies in rats available for [FL-no: 16.130] and its sulfate salt indicate that both forms are poorly absorbed after oral administration and no significant metabolism is expected in the gastrointestinal tract.

Genotoxicity

[FL-no: 16.130] contains a structural alert for genotoxicity in the form of an amino-substituted aromatic ring. However, the substance gave negative results when tested in a bacterial gene mutation assay and micronucleus assay *in vitro*. Based on these results, the Panel concluded that there is no concern with respect to genotoxicity for the candidate substance [FL-no: 16.130]. Therefore, the safety of the substance was evaluated according to the Procedure for the evaluation of chemically defined flavouring substances.

Systemic toxicity

A 90-day systemic toxicity study in the rat has been performed. Dietary administration of [FL-no: 16.130] to CD rats for 13 weeks at doses up to 100 mg/kg bw per day was well tolerated. Based on the findings in this study, the NOAEL was considered to be 100 mg/kg bw per day, the highest dose tested, in both sexes.

Developmental toxicity

In a developmental toxicity study, rats were administered oral doses of 250–1,000 mg/kg bw per day of the candidate substance. There were no statistically significant differences between the treated and control groups. Therefore, there is no concern for developmental toxicity of [FL-no: 16.130] in rats at the dose levels up to 1,000 mg/kg bw per day, the highest dose tested.

Safety assessment for acute exposure

Estimates of maximum acute dietary exposure indicate that this would be about 265 μg/kg bw for a 3-year-old child, which is higher than the acute exposure estimate for an adult (105 μg/kg bw per day). From these data, a margin of exposure of 380 for children can be calculated, which indicates no concern for acute effects, taking into account that the NOAEL was derived from a 90-day toxicity study. In addition, no toxicity was observed at dose levels up to 1,000 mg/kg bw per day in a developmental toxicity study with an exposure period of 14 days.

Safety assessment for long-term exposure

Since no clear structural/metabolic similarity of the candidate substance to flavouring substances in an existing FGE was identified, the Panel proceeded with the individual evaluation of the candidate substance [FL-no: 16.130], according to the EFSA Guidance (EFSA, 2010).

Based on its chemical structure, the substance has been assigned to Cramer class III. The results of studies on metabolism and pharmacokinetics do not allow to conclude that its metabolites are innocuous. Accordingly, the candidate substance is evaluated via the B-side of the Procedure scheme. Based on the comparison of APET with the Cramer class III threshold, a 90-day study and a

[6] 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 (EC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. Official Journal of the European Union, L 354, 34–50.

developmental toxicity study were required and carried out for this substance. Adequate margins of safety of 6,800 for adults and 2,700 for 3-year-old children have been calculated on the basis of the exposure estimates calculated and the NOAEL from the 90-day toxicity study.

Overall, the Panel concluded that using the NOAEL obtained from a 90-day dietary study in rats, there is no safety concern for [FL-no: 16.130], when used as a flavouring substance with modifying properties at the estimated level of dietary exposure calculated using the APET approach and based on the use levels as specified in Appendix B.

Documentation provided to EFSA

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3) MPI, 2011a. S9632: A 4-week Dietary Administration Toxicity Study in Rats (MPI Study No. 1646-010).
4) MPI, 2011b. S9632: A 13-week Dietary Administration Toxicity Study in Rats (MPI Study No. 1646-011).
5) Nucro-Technics, 2011a. Bacterial Reverse Mutation Assay of S3333 (Sodium Salt of S9632) (Nucro-Technics Project No. 245063).
6) Nucro-Technics, 2011b. In Vitro Mammalian Chromosome Aberration Test of S3333 (Sodium Salt of S9632) in Human Lymphocytes. (Nucro-Technics Project No. 245062).
7) Nucro-Technics, 2011c. In Vivo Mouse Micronucleus Test of S9632. (Nucro-Technics Project No. 245046).
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9) Senomyx, 2011a. Single IV and Repeated Dose 7-Day Oral PK Study with S9632 in Sprague-Dawley Rats.
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12) Senomyx, 2016. Responses to Request for Additional Information Application for authorisation of a flavouring substance, 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid – [FL-no: 16.130], submitted under Commission Implementing Regulations (EC) Nos 872/2012, 234/2011 and 1334/2008 of the European Parliament and Council. EFSA-Q-2015-00244. Including Appendix A–L.
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15) WIL Research, 2012c. Salmonella/Mammalian Microsome Assay-Summary Results for MBR12-210D.

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Abbreviations

ADME	absorption, distribution, metabolism and elimination
APET	added portions exposure technique
bw	body weight
CAS	Chemical Abstract Service
CBPI	cytokinesis-blocked proliferation index
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DMSO	dimethyl sulfoxide
EINECS	European Inventory of Existing Commercial chemical Substances
FAO	Food and Agriculture Organization of the United Nations
FDA	US Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS	Flavour Information System database
FL-no	FLAVIS number
FOB	functional observation battery
GLP	good laboratory practice
GMP	good manufacturing practice
GRSA	Generally Regarded As Safe
GSFA	General Standard for Food Additives
IR	infrared
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
HPBL	human peripheral blood lymphocytes
HPLC	high-performance liquid chromatography
LC	liquid chromatography
MA	motor activity
MS	mass spectrometry
NCE	normochromatic erythrocytes
NMR	nuclear magnetic resonance
NOEL	no-observed-effect-level
NOAEL	no-observed-adverse-effect-level
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocytes
RCG	relative cell growth
RMI	relative mitotic index
RTD	ready-to-drink
SPET	single portion exposure technique

TTC	toxicological threshold of concern
USDA	United Stated Department of Agriculture
UV	ultraviolet
WHO	World Health Organization

Appendix A – Production process and decomposition products

Process description:

3-Hydroxy-2,2-dimethylpropanoic acid ((1) in Figure A.1) is reacted with isopropylamine (2) in solution in 2-methyltetrahydrofuran at 175°C affording 3-hydroxy-*N*-isopropyl-2,2-dimethylpropanamide (3). The so obtained 3-hydroxy-*N*-isopropyl-2,2-dimethylpropanamide was deprotonated by potassium *tert*-butoxide in Me-THF and the resulting alkoxide was reacted with 2-amino-6-fluorobenzonitrile (4). The resulting 3-(3-amino-2-cyanophenoxy)-*N*-isopropyl-2,2-dimethylpropanamide was purified by crystallisation (5).

Then, 3-(3-amino-2-cyanophenoxy)-*N*-isopropyl-2,2-dimethylpropanamide is reacted with methyl acetoacetate at moderate temperature, then cyclised with an excess of sodium ethylate in ethanol to the intermediate which was directly saponified into sodium 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylate using sodium hydroxide in water (6).

The insoluble 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid is isolated by precipitation by treatment of sodium 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylate with sulfuric acid in water (7 = [FL-no: 16.130]).

Using sulfuric acid, the 4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2- methylquinoline-3-carboxylic acid is transformed into its sulfate salt, 3-carboxy-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinolin-4-aminium sulfate, which is purified by crystallisation from a solvent mixture (8). The decarboxylated S9379 was not detected (Senomyx, 2016).

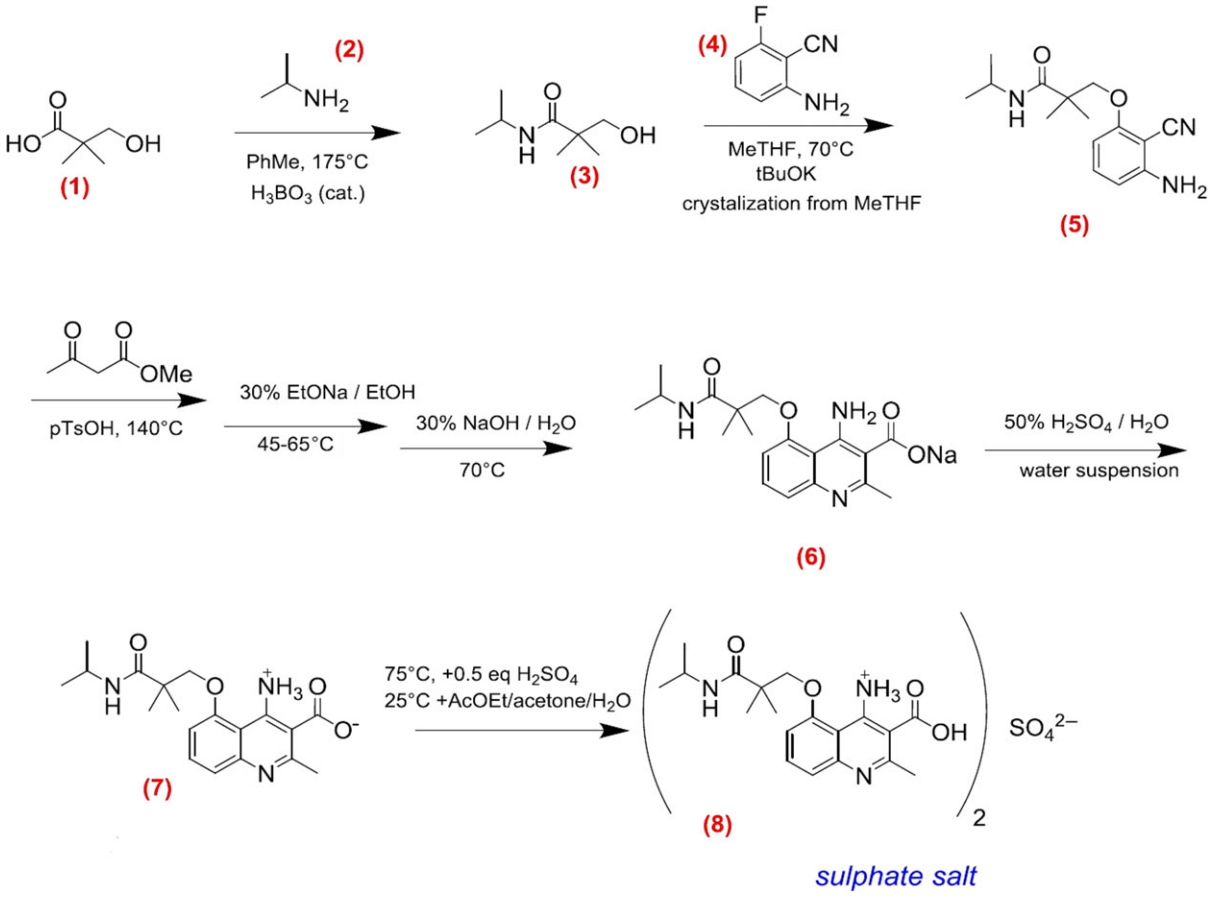


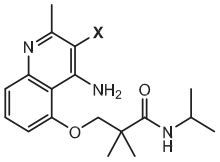
Figure A.1: Process details for the synthesis of [FL-no: 16.130] and its sulfate

Information on decomposition products when processed in various product prototypes

[FL-no: 16.130] was found to be stable in model cake, cookie and candy product prototypes under typical processing conditions as indicated by the high recovery of [FL-no: 16.130] and the absence or minimal presence of the likely decomposition products.

Dry powder stability of [FL-no: 16.130] *and its sulfate salt*

As a dry powder, [FL-no: 16.130] and its sulfate salt were found to be stable at 100°C for at least 24 h (Senomyx, 2016). During heating of the dry powder at 175°C, a decarboxylated form of [FL-no: 16.130], referred to as S9379; see structure below) was formed as the single detectable degradant (Senomyx, 2016).



[FL-no: 16.130] (X=CO_2H)

S9379 (X=H)

The above decarboxylation product was also observed as a minor impurity of [FL-no: 16.130] during the synthesis of various batches of [FL-no: 16.130] (Senomyx, 2016) including the good manufacturing practice (GMP) batch used for the 90-day and developmental toxicity studies (overall purity 99%, S9379 0.4%). The identity of this impurity was confirmed by direct comparison with an authentic sample by liquid chromatography/mass spectrometry (LC/MS) (Senomyx, 2016). The sulfate salt of [FL-no: 16.130] also produces minor amounts of S9379 under similar conditions (overall purity 99.9%, S9379 < limit of detection of 0.02%).

Evaluation of [FL-no: 16.130] *stability in product prototypes*

The food product categories that were identified to be processed at temperatures higher than 100°C were confectionery, breakfast cereals and snacks. Breakfast cereals and snacks are processed at a wide range of times and temperatures. Model products were selected that could serve as surrogates that are representative of the most extreme temperature and time conditions (Pariza et al., 1998). Three key products were evaluated for the potential loss of [FL-no: 16.130] during processing: cookies, yellow cake and a hard candy. In addition, we have included a ready-to-drink (RTD) coffee beverage as a typical heat-processed (retort) beverage.

The stability of [FL-no: 16.130] was evaluated in these surrogate product prototypes in order to determine the recovery of the starting compound and whether the degradation product S9379 formed in the dry powder under extreme conditions is formed during processing of representative products. The batch of [FL-no: 16.130] used for the cookies, cake and candy studies already contained ~ 0.4% of S9379 as an impurity formed during the synthesis of this material. Therefore, the amounts of S9379 found in the cookie, cake and candy studies include a portion present in the starting material. This was the same batch used for the safety studies. The identified degradation product measured in product prototypes was either below the level of quantification or minimally formed in all cases.

a. Evaluation of the stability of [FL-no: 16.130] in a cookie prototype

The stability of [FL-no: 16.130] (overall purity 99%, S9379 0.4%) was evaluated in a cookie prototype baked at 204°C for 8 min. The per cent [FL-no: 16.130] remaining, as well as the presence of the breakdown product (S9379), was evaluated by liquid chromatography with tandem mass spectrometry (LC–MS/MS) (Senomyx, 2016). No statistically significant loss of [FL-no: 16.130] was observed (nominal concentration, 5.0 mg/kg; measured concentration, 5.1–5.8 mg/kg). The measured concentration of S9379 was 0.043 mg/kg, equivalent to 0.9% of the [FL-no: 16.130] nominal level. Overall, the vast majority of [FL-no: 16.130] remained intact through the process of preparation and baking, as indicated by the high percentage of [FL-no: 16.130] recovered, as well as the low level of potential degradant measured.

In another test, [FL-no: 16.130] sulfate salt (overall purity 99.9%, S9379 < limit of detection of 0.02%) was added to another similar sugar cookie ingredient mix and baked at 177°C for 10 min (i.e. lower temperature than above but 2 min longer). The recovery of [FL-no: 16.130] sulfate salt (18 mg/kg) was found to be 100% (RSD = 6.7%, N = 3). The concentration of the potential degradant S9379 was 0.02 mg/kg, corresponding to 0.1% of the nominal [FL-no: 16.130] sulfate salt concentration.

b. Evaluation of the stability of [FL-no: 16.130] in a cake prototype

The stability of [FL-no: 16.130] (overall purity 99%, S9379 0.4%) was evaluated in a yellow cake prototype baked at 177°C for 30 min. The per cent [FL-no: 16.130] remaining, as well as the presence

of the degradant (S9379) was evaluated by LC–MS/MS (Senomyx, 2016). No statistically significant loss of [FL-no: 16.130] was observed (nominal concentration, 5.0 mg/kg; measured concentration, 5.1–6.1 mg/kg). The measured concentration of S9379 was 0.041 mg/kg, equivalent to 0.82% of the [FL-no: 16.130] nominal level. Overall, the vast majority of [FL-no: 16.130] remained intact through the process of preparation and baking, as indicated by the high percentage of [FL-no: 16.130] recovered, as well as the low level of the potential degradant measured.

c. Evaluation of the stability of [FL-no: 16.130] in a hard candy prototype

The stability of [FL-no: 16.130] (overall purity 99%, S9379 0.4%) was evaluated in a hard candy prototype, which involved heating to a boil until the mixture reached 149°C (~30 min) followed by cooling. The per cent [FL-no: 16.130] remaining, as well as the presence of the potential degradant (S9379), was evaluated by LC–MS/MS (Senomyx, 2016). The measured [FL-no: 16.130] represents 87% of the nominal concentration at 15 mg/kg (RSD = 2.8%, n = 3). The measured concentration of S9379 was 0.14 mg/kg, equivalent to 0.94% of the [FL-no: 16.130] nominal level. Overall, the majority of [FL-no: 16.130] remained intact through the process of preparation and cooking, as indicated by the percentage of [FL-no: 16.130] recovered, as well as the low level of potential degradant measured.

In another test, [FL-no: 16.130] sulfate salt (overall purity 99.9%, S9379 < limit of detection of 0.02%) was used while the candy ingredients and cooking conditions kept the same. The recovery of [FL-no: 16.130] sulfate salt (nominal concentration at 24 mg/kg) was found to be 104% (RSD = 5%, N = 6). The concentration of the potential degradant S9379 was 0.04 mg/kg, corresponding to 0.4% of the nominal sulfate salt concentration.

In summary, [FL-no: 16.130] was found to be stable in model cake, cookie and candy product prototypes under typical processing conditions. The amount of potential breakdown product (S9379) measured represented < 1% of the corresponding nominal [FL-no: 16.130] concentration (Table A.1 below). Considering that S9379 was already present as an impurity at the level of ~ 0.4% in the batch of [FL-no: 16.130] used in these stability studies, the true level of S9379 generated in the preparation and processing for the prototypes is less than the level indicated by the measured S9379 concentration.

Table A.1: Summary of the analysis of [FL-no: 16.130] and the potential degradant (S9379) in product prototypes. The percentage in a parenthesis for the degradant is the % of the nominal concentration represented by the measured degradant

Product prototype	[FL-no: 16.130] Nominal concentration, mg/kg	[FL-no: 16.130] Measured concentration, mg/kg	[FL-no: 16.130] Mean measured concentration, mg/kg	Decarboxylation product, mg/kg (% of [FL-no: 16.130])
Cake	5	5.1–6.1	5.8	0.041 (0.82)
Cookie	5	5.1–5.8	5.4	0.043 (0.86)
Candy	15	12.7–13.4	13.1	0.14 (0.94)

FL-no: FLAVIS number.

d. Evaluation of [FL-no: 16.130] stability after retort in a ready to drink coffee beverage

In order to further evaluate the stability of [FL-no: 16.130] in aqueous products, the stability of [FL-no: 16.130] sulfate salt (overall purity 99.9%, S9379 < limit of detection of 0.02%) was evaluated under retort processing conditions in a RTD coffee beverage, which also included a follow-on shelf-life study phase (Senomyx, 2016).

Three retort coffee beverage formulas were prepared: 1) RTD coffee beverage prototype containing 4% sucrose (no modifier), 2) RTD coffee beverage containing 4% sucrose and 10 mg/kg [FL-no: 16.130] sulfate salt and 3) RTD coffee beverage containing 10 mg/kg [FL-no: 16.130] sulfate salt (no sucrose). Each formula was packaged in 200 × 390 g foil retort pouches (16 ounce) and processed in a still, steam air overpressure retort to commercial sterility.

Samples were stored at 4°C or under accelerated conditions of 30°C, with analysis at 0, 1, 2, 3, 4, 5 and 6 months. It was assumed that 1 month in accelerated storage is equal to 2 months in ambient storage. Samples were evaluated for pH, visually, by sensory and the concentration of compound was confirmed by LC/MS.

No changes were observed in pH, visual inspection or sensory under any condition. The analytical results demonstrated no significant degradation of [FL-no: 16.130] during the processing conditions or during storage at 4°C (control) or 30°C (accelerated) for up to 6 months (Senomyx, 2016).

Appendix B – Use levels and exposure calculations

Table B.1: Normal and maximum occurrence levels for refined categories of foods and beverages

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[d] (mg/kg)	
			Normal	Maximum	Average[e]	Maximum	Normal	Maximum
01.1	Milk and dairy-based drinks	200	2.2	6.6			2.2	6.6
01.2	Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks)	200						
01.3	Condensed milk and analogues (plain)	70						
01.4	Cream (plain) and the like	15	8	30			8	30
01.5	Milk powder and cream powder and powder analogues (plain)	30	12	30			12	30
01.6	Cheese and analogues	40						
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)	125	3.5	10			3.5	10
01.8	Whey and whey products, excluding whey cheeses	200						
02.1	Fats and oils essentially free from water	15	8	30			8	30
02.2	Fat emulsions mainly of type water-in-oil	15	8	30			8	30
02.3	Fat emulsions mainly of type water-in-oil, including mixed and/or flavoured products based on fat emulsions	15	8	30			8	30
02.4	Fat-based desserts excluding dairy-based dessert products of category 1.7	50						
03.0	Edible ices, including sherbet and sorbet	50	8.9	30			8.9	30
04.1.1	Fresh fruit	140						
04.1.2	Processed fruit	125	3.5	10			3.5	10
04.1.2.5	Jams, jellies, marmalades	30	10	30			10	30
04.2.1	Fresh vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed	200						
04.2.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter) and nuts and seeds	200						
04.2.2.5	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter)	30	12	30			12	30

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[d] (mg/kg)	
			Normal	Maximum	Average[e]	Maximum	Normal	Maximum
05.1	Cocoa products and chocolate products, including imitations and chocolate substitutes	40	10	30			10	30
05.1.3	Cocoa-based spreads, including fillings	30						
05.2	Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4	30	14.9	30			14.9	30
05.3	Chewing gum	3	30	300			30	300
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	35	12.7	30			12.7	30
06.1	Whole, broken or flaked grain, including rice	200						
06.2	Flours and starches (including soya bean powder)	30						
06.3	Breakfast cereals, including rolled oats	30	14.9	45			14.9	45
06.4	Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pastas and noodles)	200						
06.5	Cereal- and starch-based desserts (e.g. rice pudding, tapioca pudding)	200	2.2	10			2.2	10
06.6	Batters (e.g. for breading or batters for fish or poultry)	30						
06.7	Precooked or processed rice products, including rice cakes (Oriental type only)	200						
06.8	Soya bean products (excluding soya bean products of food category 12.9 and fermented soya bean products of food category 12.10)	100						
07.1	Bread and ordinary bakery wares	50						
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	80	5	15			5	15
08.1	Fresh meat, poultry and game	200						
08.2	Processed meat, poultry and game products in whole pieces or cuts	100	4.4	15			4.4	15
08.3	Processed comminute meat, poultry and game products	100	4.4	15			4.4	15
08.4	Edible casings (e.g. sausage casings)	1						
09.1.1	Fresh fish	200						
09.1.2	Fresh molluscs, crustaceans and echinoderms	200						
09.2	Processed fish and fish products, including molluscs, crustaceans and echinoderms	100						

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[d] (mg/kg)	
			Normal	Maximum	Average[e]	Maximum	Normal	Maximum
09.3	Semipreserved fish and fish products, including molluscs, crustaceans and echinoderms	100						
09.4	Fully preserved, including canned or fermented, fish and fish products, including molluscs, crustaceans and echinoderms	100						
10.1	Fresh eggs	100						
10.2	Egg products	100						
10.3	Preserved eggs, including alkaline. salted and canned eggs	100						
10.4	Egg-based desserts (e.g. custard)	125	3.5	15			3.5	15
11.1	Refined and raw sugar	10						
11.2	Brown sugar excluding products of food category 11.1	10						
11.3	Sugar solutions and syrups, and (partially) inverted sugars, including molasses and treacle, excluding products of food category 11.1.3 (soft white sugar, soft brown sugar, glucose syrup, dried glucose syrup, raw cane sugar)	30	14.8	30			14.8	30
11.4	Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings)	30	14.8	30			14.8	30
11.5	Honey	15						
11.6	Table-top sweeteners, including those containing high-intensity sweeteners	1						
12.1	Salt and salt substitutes	1						
12.10	Protein products other than from soybeans	15						
12.2	Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	1						
12.3	Vinegars	15						
12.4	Mustards	15	8	30			8	30
12.5	Soups and broths	200	2.2	10			2.2	10
12.6	Sauces and like products	30						
12.7.a	Salads 120 g (e.g. macaroni salad, potato salad) excluding cocoa- and nut-based spreads of food categories	120						
12.7.b	Sandwich spreads (20 g), excluding cocoa- and nut-based spreads of food categories	20						

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[d] (mg/kg)	
			Normal	Maximum	Average[e]	Maximum	Normal	Maximum
12.8	Yeast and like products	1						
12.9	Soybean-based seasonings and condiments	15						
12.9.1	Fermented soya bean products (e.g. miso)	40						
12.9.2	Soybean sauce	15						
12.9.3	Fermented soybean sauce	15						
13.2. a	Complementary foods for infants and young children: Dry instant cereals (with or without milk), including pasta	110						
13.2. b	Complementary foods for infants and young children: Meat-based or fish-based dinner	170						
13.2. c	Complementary foods for infants and young children: Dairy-based dessert	110						
13.2. d	Complementary foods for infants and young children: Vegetables, potatoes, broth, soups, pulses	170						
13.2. e	Complementary foods for infants and young children: Biscuits and cookies	20						
13.2. f	Complementary foods for infants and young children: Fruit purée	110						
13.2. g	Complementary foods for infants and young children: Fruit juice	120						
13.2. h	Milk for young children	200						
13.3	Dietetic foods intended for special medical purposes (excluding food products of category 13.1 'Infant formulae, follow-up formulae and other formulae for special medical purposes for infants')	200						
13.4[f]	Dietetic formulae for slimming purposes and weight reduction	200						
13.5	Dietetic foods (e.g. supplementary foods for dietary use), excluding products of food categories 13.1 (Infant formulae, follow-up formulae and other formulae for special medical purposes for infants), 13.2–13.4 and 13.6	200						
13.6	Food supplements	5						
14.1a	Coffee powder	12						
14.1b	Drinks mix powders	30						
14.1c	Other non-alcoholic ('soft') beverages (expressed as liquid)	300	1.4	7			1.4	7

Food categories[a]		Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)		Occurrence level from other sources[c] (mg/kg)		Combined occurrence level from all sources[d] (mg/kg)	
			Normal	Maximum	Average[e]	Maximum	Normal	Maximum
14.2.1[f]	Beer and malt beverages	300	1.4	7			1.4	7
14.2.2[f]	Cider and perry	300						
14.2.3[f]	Grape wines	150	2.8	10			2.8	10
14.2.4[f]	Wines (other than grape)	150						
14.2.5[f]	Mead	150	2.8	10			2.8	10
14.2.6[f]	Distilled spirituous beverages containing more than 15% alcohol	30	14.5	30			14.5	30
14.2.7[f]	Aromatised alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	300						
15.1	Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)	30	12	30			12	30
15.2	Processed nuts, including coated nuts and nut mixtures (with, e.g. dried fruit)	30						
15.3	Snacks – fish based	30	12	30			12	30
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	300						

(a): Most of the categories reported are the subcategories of Codex GSFA (General Standard for Food Additives, available at http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf) used by JECFA in the SPET technique (FAO/WHO, 2008). In the case of category 13.2 (complementary foods for infants and young children), further refined categories have been created so that a specific assessment of dietary exposure can be performed in young children.

(b): For Adults. In case of foods marketed as a powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of the standard dilution factors established by JECFA (FAO/WHO 2008):

- 1/25 for powder used to prepare water-based drinks such as coffee, containing no additional ingredients,
- 1/10 for powder used to prepare water-based drinks containing additional ingredients such as sugars (ice tea, squashes, etc.),
- 1/7 for powder used to prepare milk, soups and puddings,
- 1/3 for condensed milk.

(c): As natural constituent and/or developed during the processing and/or as carry-over resulting from their use in animal feed.

(d): As added flavouring or from other sources. The normal and maximum combined occurrence levels of the substance will be assessed by the applicant either by adding up occurrence levels from added use to that from other sources or by expert judgement based on the likelihood of their concomitant presence. This will be done both for normal use levels and for maximum use levels.

(e): In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amount will be considered (e.g. for the category 'Fresh fruit' 04.1.1., the normal concentration will be the median concentration observed in all kinds of fruit where the flavouring substance is known to occur).

(f): The subcategories 14.2.1–14.2.7 ('alcoholic beverages') and the subcategory 13.4 ('dietetic formulae for slimming purposes and weight reduction') are *a priori* not consumed by children.

Calculation of the Dietary Exposure – APET

Chronic Dietary Exposure

Adults ('Added Portions Exposure Technique' [APET][7]).

On the Basis of Normal Occurrence Level from Added Flavourings

The APET is calculated by adding the highest contribution from one portion of food and one portion of beverages:

Solid Food: The maximum intake of 447 μg/person per day will be from food categories 5.2 (Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4; 30 g/day) or 6.3 (Breakfast cereals, including rolled oats; 30 g/d) with the normal combined occurrence level of 14.9 mg/kg food.

Beverage: The maximum intake of 435 μg/person per day will be from category 14.2.6 (Distilled spirituous beverages containing more than 15% alcohol; 30 g/d) with the normal combined occurrence level of 14.5 mg/kg food.

The total APET will be 882 μg/person per day corresponding to 14.7 μg/kg bw per day for a 60-kg person.

Children (3-year-old child of 15-kg body weight)

Food subcategories resulting in the highest potential dietary exposure:

Solid Food: The maximum intake will be 282 μg/child per day from food categories 5.2 (Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4; 30 g/day) or 6.3 (Breakfast cereals, including rolled oats; 30 g/d) with the normal combined occurrence level (14.9 mg/kg food), taking into account a portion size reduction factor of 0.63 for children (EFSA, 2010).

Beverage: The maximum intake of 265 μg/child per day will be from category 14.1c (Other non-alcoholic ('soft') beverages (expressed as liquid); 300 g/d) with the normal combined occurrence level of 1.4 mg/kg food, taking into account a portion size reduction factor of 0.63 for children (EFSA, 2010).

The total APET will be 547 μg/child per day corresponding to 36.4 μg/kg bw per day for a 15 kg child.

Conclusion

The total APET values are 14.7 μg/kg bw per day for 60-kg adults and 36.4 μg/kg bw per day for 15-kg children. In terms of per capita intake, the adult value of 882 μg/day is the higher.

Acute Dietary Exposure

The calculation was based on the maximum use levels and large portion size, i.e. three times standard portion size (see Table B.1).

Although the substance is not intended to be used in food categories specifically intended for infants and toddlers, these could still be exposed through consumption of foods from the general food categories, which may contain the substance. However, at present there is no generally accepted methodology to estimate exposure in these age groups resulting from consumption of foods from the general categories.

Adults

On the basis of maximum occurrence level of 7 mg/kg for food categories 14.1c (Other non-alcoholic ('soft') beverages (expressed as liquid) or 14.2.1 (Beer and malt beverages) and a large portion size of 900 g/d (3 × 300 (standard portion size for both food categories mentioned)), the highest acute exposure level for adults is 6,300 μg/person per day, which is equivalent to 105 μg/kg bw per day.

Children (3-year-old child of 15-kg body weight)

On the basis of maximum occurrence level of 7 mg/kg for food categories 14.1c (Other non-alcoholic ('soft') beverages) (expressed as liquid) and a large portion size of 900 g/d (3 × 300

[7] The APET has been calculated based on the occurrence levels in the food subcategories reported in the above table, with the exclusion of categories 13.2 (complementary foods for infants and young children).

(standard portion size for the food categories mentioned)), and taking into account a portion size reduction factor of 0.63 for children (EFSA, 2010), the highest acute exposure level for a child is 3,970 μg/person per day, which is equivalent to 265 μg/kg bw per day.

Conclusion

The highest[8] acute exposure value for [FL-no: 16.130] value is 265 μg/kg bw per day, derived from the scenario of a 3-year-old child with a 15-kg body weight.

Cumulative Dietary Exposure to [FL-no: 16.130]

There is no other flavouring substance structurally and metabolically related to [FL-no: 16.130]. Moreover, the candidate substance is chemically synthesised and is not expected to occur naturally in food. Consequently, the cumulative dietary exposure estimate is not applicable in this case.

[8] The highest value obtained among adults and children of all ages.

Appendix C – Genotoxicity

Theoretical considerations

With reference to the structure alerts for genotoxicity (Ashby and Tennant, 1991; Benigni et al., 2008), [FL-no: 16.130] contains a structural alert for genotoxicity in the form of an amino-substituted aromatic ring. In as much as quinoline and some alkyl quinolines are known to be metabolised to give N-oxides (EFSA, 2008), this structural element constitutes a structural alert for genotoxicity (Benigni et al., 2008).

In vitro studies

Bacterial reverse mutation assay

A preliminary study was carried out in *Salmonella* Typhimurium strains, TA98 and TA100 in the presence and absence of metabolic activation (S9-mix). The candidate substance [FL-no: 16.130] (purity > 98%) was tested in *S.* Typhimurium strains TA98 and TA100 with a plate incorporation method at the following concentrations: 0, 1.5, 5.0, 15, 50, 150, 500, 1,500 and 5,000 μg/plate using dimethyl sulfoxide (DMSO) as a solvent. 2-Nitrofluorene 1 μg/plate was included as a positive control. Duplicate plates were used for each concentration. No precipitates were observed at any concentrations. Therefore, the test article was evaluated at the maximum recommended concentration for a soluble non-toxic compound. The treatment with [FL-no: 16.130] did not result in bacterial toxicity or mutagenic activity. Significant increases in the number of revertant colony counts were observed in the concurrent positive control compared to the corresponding negative control, confirming the sensitivity of the test system and the activity of the S9 mix. The candidate substance [FL-no: 16.130] was not mutagenic in TA98 and TA100 under the test conditions of this study (BioReliance, 2011).

The sodium salt of the candidate substance (purity > 99%) was evaluated for its potential to induce point mutations in *S.* Typhimurium strains, TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA* (Nucro-Technics, 2011a). The study was performed according to the OECD Test Guideline 471 (OECD, 1997a). The concentrations of [FL-no: 16.130] sodium salt investigated for both the plate incorporation and pre-incubation tests, were 0, 51, 130, 320, 800, 2,000 and 5,000 μg/plate. The lowest concentration of 51 μg/plate was not investigated in experiments with metabolic activation and for strain TA1535 without metabolic activation (OECD, 1997a). At the end of the incubation period, precipitate was not visible at any concentration. Therefore, the test article was completely soluble in the test system. No toxicity was observed at any concentration including the highest of 5,000 μg [FL-no: 16.130] sodium salt per plate. Therefore, the test article was evaluated at the maximum recommended concentration for a soluble non-toxic compound (OECD, 1997a).

In both the plate incorporation and pre-incubation test, with or without metabolic activation, [FL-no: 16.130] sodium salt did not induce any increase in revertants over the concurrent negative controls.

The negative controls (DMSO) for each tester strain were within the historical negative control data.

All concurrent positive controls (for experiments without S9-mix: sodium azide, 2-nitrofluorene, methyl methanesulfonate and 9-aminoacridine; for experiments with S9-mix: benzo[α]pyrene and 2-aminoanthracene, cyclophosphamide monohydrate) induced at least a 3.1-fold increase in colony counts per plate when compared to the corresponding negative controls and were at levels similar to the historical positive control data.

The sodium salt of [FL-no: 16.130] was not mutagenic to *S.* Typhimurium strains TA98, TA100, TA1535, TA1537 and *E. coli* strain, WP2 *uvrA*, under the test conditions of this study.

Bacterial reverse mutation assay with S9379

An Ames assay was performed on S9379, a decomposition product formed by decarboxylation of [FL-no: 16.130] (4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline). S9379 was tested in *S.* Typhimurium strains, TA98 and TA100 in the presence or absence of metabolic activation (S9-mix) at 1, 5, 10, 50, 100, 500, 1,000 and 5,000 μg/plate. The plate incorporation method was applied. The positive controls without metabolic activation were 2-nitrofluorene and sodium azide. The positive control with metabolic activation for both strains was 2-aminoanthracene. DMSO was used as the vehicle control. Precipitates were not observed in either strain with or without metabolic activation at concentrations up to 5,000 μg/plate. Cytotoxicity (reduction in the background lawn) was observed at 5,000 μg/plate in both strains without metabolic activation; the highest

concentration evaluated for mutagenicity in the absence of metabolic activation was 1,000 μg/plate. There was no increase in the number of revertant colonies as compared with the vehicle control in either strain with or without S9-mix. It was concluded that S9379 was not mutagenic under the test conditions of this study (WIL Research, 2012c).

In vitro micronucleus assay

The purpose of the study by BioReliance (2016) was to evaluate the potential of the sulfate salt (S1638) of the candidate substance [FL-no: 16.130], or its potential metabolites, to induce micronuclei in human peripheral blood lymphocytes (HPBL). The study was performed according to the OECD Test Guideline 487 (OECD, 2014).

[FL-no: 16.130] sulfate salt was tested in cultured human peripheral lymphocytes from a single young healthy female donor using cytokinesis-block methodology both in the presence and absence of an exogenous metabolic activation system (S9-mix, rat liver S9 from rats induced with Aroclor™ 1254). Cells were treated for 4 h (and 20 h recovery period) with and without S9-mix or for 24 h without S9-mix. S1638 was dissolved in *N*-methyl-2-pyrrolidone that was used as a vehicle control, a further negative control of untreated cells was included. A preliminary toxicity assay was carried out at nine concentrations ranged from 0.2 to 2,000 μg/mL. The levels of cytotoxicity, measured as cytokinesis-blocked proliferation index (CBPI) relative to the vehicle control, at the highest concentration tested were 31% and 20% in 4-h exposure groups with and without S9, respectively, and 45% in 24-h exposure group with S9-mix. Based on these results, the concentrations chosen for the micronucleus assay ranged from 100 to 2,000 μg/mL for all three treatment conditions.

In the micronucleus assay, cytotoxicity (55 ± 5% CBPI relative to the vehicle control) was not observed at any concentration tested at 4 + 20 h with or without S9-mix. Cytotoxicity was observed at the highest concentration tested (2,000 μg/mL) in cells treated for 24 h. The concentrations selected for evaluation of micronuclei were 500, 1,000 and 2,000 μg/mL for all three treatment conditions. No significant or concentration-dependent increases in micronuclei induction were observed in any treatment with or without S9-mix ($p > 0.05$; Fisher's Exact and Cochran-Armitage tests) (BioReliance, 2016).

The Panel concluded that [FL-no: 16.130] sulfate salt was negative for the induction of micronuclei in HPBL in the presence and absence of metabolic activation.

Chromosomal aberration test

The sodium salt of the candidate substance [FL-no: 16.130] (purity > 99%) was investigated in a chromosomal aberration test for its potential to induce structural chromosomal aberrations in primary human lymphocytes (Nucro-Technics, 2011b). The study was performed according to the OECD Test Guideline 473 (OECD, 1997b).

Duplicate cultures of human lymphocytes were treated with [FL-no: 16.130] sodium salt under three different conditions: 3-h exposure in the absence or presence of metabolic activation (S9-mix); 20-h exposure in the absence of S9-mix.

The concentrations of [FL-no: 16.130] sodium salt tested for the 3-h treatment without S9-mix were 0, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 μg/mL.

The concentrations of [FL-no: 16.130] sodium salt tested for the 3-h treatment with S9-mix and 20-h treatment without S9-mix were 0, 62.5, 125, 250 and 500 μg/mL.

The vehicle control was DMSO. Positive controls were mitomycin C, used at 0.5 and 1.0 μg/mL as a positive control for 3-h treatment and 0.1 and 0.2 μg/mL for 20-h treatment in the absence of S9-mix. Cyclophosphamide, was used at 10, 20 and 40 μg/mL as a positive control for treatment with S9-mix.

Only the highest exposure concentration of 500 μg [FL-no: 16.130] sodium salt per mL produced precipitates in the treatment medium at the beginning of the treatment period. Therefore, the test article was tested at the limit of solubility (OECD, 1997b). The pH and osmolality of all treated cultures were well within the normal physiological ranges.

For all conditions, cells were harvested 20 h after the initiation of the treatment, approximately 2 h before harvesting, Colcemid® was added to cultures at 0.1 μg/mL to arrest cells in metaphase.

Based on the relative cell growth (RCG) and the relative mitotic index (RMI), only the highest three concentrations were selected for chromosomal aberration analysis for each treatment condition. At least 100 metaphase cells from each culture were examined.

The treatment of 3 h without S9-mix, at 0, 125, 250 and 500 μg/mL, resulted in 3.0, 3.5, 2.0 and 2.5% of cells with chromosomal aberrations, respectively. Endoreduplicated cells were not observed at any of the analysed concentrations. A low incidence of polyploid cells (0.5%) was detected at 250 μg/mL.

The treatment of 3 h with S9-mix at 0, 125, 250 and 500 μg/mL resulted in 3.5, 5.0, 4.0 and 2.5% of cells with chromosomal aberrations, respectively. Endoreduplicated cells were not observed at any of the analysed concentrations. A low incidence of polyploid cells (0.5%) was detected for 50 μg/mL.

The treatment of 20 h without S9-mix at 0, 125, 250 and 500 μg/mL, resulted in 1.5, 2.0, 1.0 and 3.0% of cells with structural chromosomal aberrations, respectively. Polyploid and endoreduplicated cells were not observed for any of the analysed concentrations.

All concurrent positive controls induced significant numbers ($P < 0.001$) of cells with chromosomal aberrations.

The percentage of cells with structural or numerical chromosomal aberrations in the [FL-no: 16.130] sodium salt-treated HPBL cultures was not statistically significantly increased relative to solvent control at any concentration tested in the presence or absence of S9-mix. The positive and solvent controls fulfilled the requirements for a valid test.

The Panel concluded that the chromosomal aberration test was negative. The *in vitro* genotoxicity studies are summarised in Table C.1.

In vivo studies

Micronucleus assay

The candidate substance, [FL-no: 16.130] (purity > 99%), was tested in a mouse bone marrow micronucleus assay (Nucro-Technics, 2011c). The assay was performed in accordance with the OECD Test Guideline 474 (OECD, 1997c).

In the range-finding study, three groups of Swiss Albino (CD-1) mice, each containing two males and two females, were administered with [FL-no: 16.130], through oral gavage, at 500, 1,000 or 2,000 mg/kg bw. The test article was suspended in 1% aqueous methyl cellulose. Based on the results of this preliminary study, the dose levels of 500, 1,000 and 2,000 mg/kg bw per day were chosen for the main study. Since there was no substantial difference in toxicity between genders, only male mice were used for the main study. The range-finding study was not conducted under GLP conditions.

In the main study, five groups of Swiss Albino (CD-1) mice were dosed via gavage with: 0, 500, 1,000 or 2,000 mg [FL-no: 16.130]/kg bw or with 70 mg cyclophosphamide/kg bw (positive control).

Animals from each group were sacrificed at 24, 36 or 48 h after dosing (seven animals per time point). For each sacrificed animal, bone marrow was recovered and pooled from both femora. Bone marrow smears were prepared, fixed and stained (May-Grunwald/Giemsa) for evaluation. Two thousand polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. In addition, the number of normochromatic erythrocytes (NCE) with micronuclei was scored. The PCE/NCE ratio within 200 cells was determined for each animal. The number of PCEs with micronuclei was analysed for statistically significant differences (at $p \leq 0.05$) using Kruskal–Wallis one-way analysis of variance on ranks. The analysis was carried out for each dose level at each of the 3 time points. No statistically significant decrease in the PCE/NCE ratio was observed at any concentration tested. There were no statistically significant differences in the number of PCE with micronuclei between the test article (all three dose levels) and the negative control group. Statistically significant differences ($p \leq 0.05$) at 24, 36 and 48 h in the number of PCEs with micronuclei in the positive control group when compared to both the negative control group were observed.

Based on the above results, the test article, [FL-no: 16.130], did not induce micronuclei in the mouse micronucleus test at the dose levels up to 2,000 mg/kg bw, administered by a single oral gavage to mice. Due to the lack of cytotoxicity in the bone marrow cells or any other demonstration of bone marrow exposure, the results of this study have to be considered of limited relevance (Table C.2).

Table C.1: Summary of *in vitro* genotoxicity studies

Test material	Test system	Test object	Concentration	Result	Reference	Comments
[FL-no: 16.130]	Bacterial gene mutation assay	*Salmonella* Typhimurium TA98, TA100	0, 1.5, 5.0, 15, 50, 150, 500, 1,500 and 5,000 μg/plate[(a)]	Negative	BioReliance, 2011	The candidate substance was tested only in two *S.* Typhimurium strains, with and without metabolic activation
[FL-no: 16.130] sodium salt	Bacterial gene mutation assay	*S.* Typhimurium TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	0, 51, 130, 320, 800, 2,000 and 5,000 μg/plate[(a),(b)]	Negative	Nucro-Technics, 2011a	Study compliant with the OECD guideline 471. Test performed both with the plate incorporation and pre-incubation methods, and with and without metabolic activation
[FL-no: 16.130] sodium salt	Chromosomal aberration test	Human peripheral blood lymphocytes	0, 62.5, 125, 250 and 500 μg/mL[(c),(d)] 0, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 μg/mL[(e)]	Negative	Nucro-Technics, 2011b	Study compliant with the OECD guideline 473. Test performed with and without metabolic activation
S9379, decomposition product formed by decarboxylation of [FL-no: 16.130]	Bacterial gene mutation assay	*S.* Typhimurium TA98, TA100	1, 5, 10, 50, 100, 500, 1,000 and 5,000 μg/plate[(a)]	Negative	WIL Research, 2012c	S9379 was tested only in two *S.* Typhimurium strains, with and without metabolic activation. Cytotoxicity (reduction in the background lawn) was observed at 5,000 μg/plate in both strains without metabolic activation
[FL-no: 16.130] sulfate salt	Micronucleus test	Human peripheral blood lymphocytes	500, 1,000 and 2,000 μg/mL[(f),(g),(h)]	Negative	BioReliance, 2016	Study compliant with the OECD guideline 487. Test performed with and without metabolic activation. Cytotoxicity was observed at 2,000 μg/mL in the non-activated 24-h exposure group

FL-no: FLAVIS number.
(a): With and without metabolic activation.
(b): Test performed both with the plate incorporation and pre-incubation methods.
(c): 3-h treatment with S9-mix.
(d): 20-h treatment without S9-mix.
(e): 3-h treatment without S9-mix.
(f): 4-h treatment with S9-mix.
(g): 4-h treatment without S9-mix.
(h): 24-h treatment without S9-mix.

Table C.2: Summary of *in vivo* genotoxicity studies

Test material	Test system	Test object	Route	Dose	Result	Reference	Comments
[FL-no: 16.130]	Micronucleus test	Male and female CD-1 mice	Gavage	0, 500, 1,000 and 2,000 mg/kg bw	Negative	Nucro-Technics, 2011c	Study compliant with OECD guideline 474. No evidence of bone marrow exposure

FL-no: FLAVIS number.

Appendix D – Absorption, distribution, metabolism and elimination

A short-term study was performed to determine the toxicokinetic parameters and oral bioavailability of the candidate substance by either a single intravenous administration or up to 7 days of oral dosing (gavage) in male and female Sprague–Dawley rats (Senomyx, 2011a).

The candidate substance was poorly bioavailable by the oral route (%F = 0.53–1.19%) and rapidly eliminated after either intravenous ($T_{1/2}$ < 0.3 h) or oral administration ($T_{1/2}$ < 1.2 h). At 100 mg/kg per day, the combined mean C_{max} on day 7 was 154 ng/mL (0.428 μM) and the combined mean $AUC_{0–24}$ was 232 h*ng/mL. Based on AUC_{last} and C_{max}, the exposure to the candidate substance in plasma was roughly proportional with dose. Exposure (AUC_{last}) to the candidate substance was not significantly different in either male or female rats on day 7 vs day 1 of dosing. No significant accumulation of the candidate substance was found in plasma after repeated dosing for seven consecutive days.

In order to further evaluate its toxicokinetics after dietary administration, a 90-day feeding study was carried out in CD®[Crl:CD®(SD)] rats on the candidate substance (MPI, 2011b). There were no consistent differences in the toxicokinetic parameters calculated for male and female rats on day 7 or day 90. Systemic exposure, as estimated by $AUC_{0–24}$ and C_{max}, increased in approximate proportion to the increase in dose between 30 and 100 mg/kg per day. Combined mean T_{max} values ranged from 1.5 to 3 h on day 7 and from 1.5 to 7.5 h on day 90. Combined mean T_{max} was generally longer at 60 and 100 mg/kg per day than at 30 mg/kg per day on day 90. Consistent with results from the 7-day pharmacokinetic study in rats at 100 mg/kg per day, the combined mean C_{max} on day 7 was 114 ng/mL (0.317 μM) and the combined mean $AUC_{0–24}$ was 1,040 h*ng/mL. Systemic exposure to [FL-no: 16.130] was higher on day 90 than on day 7. Combined mean accumulation ratios ranged from 1.1 to 2.8 for $AUC_{0–24}$ and ranged from 1.8 to 5.2 for C_{max}.

A qualitative metabolic profiling study of the candidate substance was performed using rat and human hepatic microsomes (PharmOptima, 2011). Based on concerted and detailed analysis of the full scan mass spectra of study samples, the candidate substance was not metabolised by the rat or human microsomes during the 60-min incubation period. Mass chromatograms were generated for the common Phase I transformations of M+16, M+32, M−16, M−32, M+18, M−18, M−42 and M−44 (decarboxylation) and M−141 (O-dealkylation of the 2,2-dimethyl-*N*-(propan-2-yl)-propanamide moiety). Full scan and mass chromatograms were examined in detail and printed to support that no metabolism was observed above the level of 0.1% of the candidate substance. No Phase I biotransformations of the candidate substance were observed in either the rat or human microsomal incubation samples to the level of 0.1%.

In order to exclude the possibility that [FL-no: 16.130] is undergoing presystemic metabolism by the gut microflora, the current study investigated the amount of [FL-no: 16.130] recovered in the faeces and urine following a single oral dose (PO) in male and female rats (Senomyx, 2013).

Rats were orally dosed with 10 mg/kg of [FL-no: 16.130], and faeces and urine were collected at four time points, 8, 24, 48, and 72 h after dosing. Diluted faecal extracts and urine samples were analysed by liquid chromatography with mass spectrometry (LC–MS/MS). An average of 86.2% from male rats and 91.4% from female rats of [FL-no: 16.130] was recovered from faeces and urine over the combined 72-h collection period. The vast majority of the compound was excreted during the 8–24-h time interval, the majority of which was recovered from the faeces (75.5–99.5% in faeces vs 0.1–0.6% in urine). These results support that [FL-no: 16.130] is poorly absorbed and mainly excreted unchanged.

Oral bioavailability of sodium salt of [FL-no: 16.130] in rats

As the bacterial reverse mutations tests for the candidate substance were carried out on the sodium salt of the candidate substance, the oral bioavailability of this salt was compared to that of the candidate substance. As part of this study, the bioavailabilities of the phosphate and sulfate salt forms of the candidate substance were compared as well (Senomyx, 2011b). Four groups of four male Sprague–Dawley rats were treated by gavage with a single dose of 30 mg/kg of either the free acid of [FL-no: 16.130] or its sodium salt or its phosphate salt or its sulfate salt in 1% methylcellulose. Blood samples were taken from a jugular catheter at approximately 15, 30 min, 1, 2, 4, 8 and 24 h after gavage administration. Plasma samples were analysed using LC–MS/MS with an internal standard. The plasma AUC_{last} and C_{max} of the sodium salt relative to that of the parent candidate substance were 89.4% and 99.4%, respectively (after correcting for differences in molecular weight). Similarly, the plasma AUC_{last} and C_{max} of the phosphate salt relative to that of the candidate substance were 112%

and 99.9%, respectively. Finally, the plasma AUC_{last} and C_{max} of the sulfate salt was relative to that of the parent candidate substance were 136% and 114%, respectively. Based on AUC_{last}, Cmax and T_{max} data, all three salt forms of the candidate substance are not significantly different and considered to be bioequivalent to the candidate substance in terms of systemic exposure.

Appendix E – Toxicity

28-day range-finding toxicity study in rats

The purpose of this (non-good laboratory practice (GLP)) study was to evaluate the potential toxicity of the candidate substance, [FL-no: 16.130] (purity > 99%), in rats after dietary administration for 28 days (MPI, 2011a) in order to select doses for a 13-week subchronic toxicity study in rats.

Three treatment groups of five male and five female CD®[Crl:CD®(SD)] rats were administered the test article at the intended dose levels of 10, 30 and 100 mg/kg body weight (bw) per day. The actual average dose levels were within 5% of the intended dose levels, based on feed consumption and measured body weights and concentrations in the feed. One additional group of five animals/sex served as the control and received the vehicle diet. The vehicle or test article diet was available *ad libitum* for 28 consecutive days.

Observations for morbidity, mortality and the availability of food and water were conducted twice daily for all animals. Observations for clinical signs were conducted weekly. Body weights were measured and recorded on days −1, 1, 4, 5, 11, 14, 21 and 28. Food consumption was measured and recorded on days 4, 7, 11, 14, 21 and 28, and compound consumption was calculated. Ophthalmoscopic examinations were conducted pretest and prior to the terminal necropsy. Blood and urine samples for clinical pathology evaluations were collected from all animals prior to the terminal necropsy. At study termination, necropsy examinations were performed and only the liver was microscopically examined for animals at 0 and 100 mg/kg per day.

No test article-related effects were noted for any parameter examined. One female at 100 mg/kg bw per day exhibited mildly increased lymphocytes but being an isolated incidence this was not considered related to test article administration. One male at 100 mg/kg bw per day exhibited markedly increased bile acids and mild to moderate increases of aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, and sorbitol dehydrogenase. These are all related to liver function and injury but because this was an isolated occurrence they were believed to be incidental in this animal and not test article related. Group mean spleen weights were increased in males of the 100 mg/kg bw per day group which was the result of one animal having a spleen weight approximately twice that of the other animals of the group; this increased spleen weight was not considered to be test article related. As a result, the no-observed-effect-level (NOEL) following 28 days of dietary administration was set at 100 mg/kg bw per day, the highest dose level tested, in male and female rats. In case any of the above described effects in single animals at the highest dose (100 mg/kg bw per day) actually represents the first sign of a systematic toxicity, this must be expected to be disclosed in the below described 90-day study where 100 mg/kg bw per day is chosen as the highest dose administered.

Repeated 90-day oral toxicity study in rats

A 90-day feeding study was carried out in rats on the candidate substance, [FL-no: 16.130] (purity > 99%), in order to evaluate its subchronic toxicity in rats after dietary administration for 13 weeks (90 days) (MPI, 2011b).

The study is based on the US Food and Drug Administration (FDA) Toxicological Principles for the Safety of Food Ingredients and conducted in accordance with the FDA GLP.

The candidate substance was administered in the diet to four groups of 20 male and 20 female CD®[Crl:CD®(SD)] rats at the intended dose levels of 0 (control), 30, 60 and 100 mg/kg bw per day. The actual average dose levels were within 5% of the intended dose levels based on feed consumption and measured body weights and concentrations in the feed. The vehicle or diet containing test article was available *ad libitum* for 13 weeks.

Additionally, one control group of three animals/sex and three treated groups of six animals/sex per group served as part of the toxicokinetic study reported in Appendix D.

Observations for morbidity, mortality and the availability of food and water were conducted twice daily for all animals. Cage-side clinical observations were conducted once daily for main study. Functional Observation Battery (FOB) and Motor Activity (MA) evaluations were conducted prior to testing and again at the same time of day during the 13th week of test article administration for all animals. Body weights were measured and recorded on day 1 and then weekly throughout the study. Food consumption was measured and recorded weekly. Compound consumption was calculated weekly on all main study animals. Neurobehavioral examinations were conducted prior to initiation of test

article administration and then weekly during study. Ophthalmoscopic examinations were conducted pretest on all animals and prior to scheduled necropsy.

Samples for haematology and clinical chemistry evaluations were collected from 10 animals/sex per group on days 14 and 45, and again prior to termination. Urinalysis and samples for coagulation evaluations were collected prior to termination only. At study termination, necropsy examinations were performed and organ weights were recorded for all animals, excluding animals from the toxicokinetic groups, and appropriate organ weight ratios were calculated (relative to body and brain weights). Microscopic examination of fixed haematoxylin and eosin-stained paraffin sections were performed on sections of tissues from the control and high-dose (100 mg/kg bw per day) groups.

There were no test article-related effects noted for any parameter examined. There were no macroscopic or microscopic findings or toxicologically significant test article-related organ weight changes noted at any dose level. As a result, the no-observed-adverse-effect-level (NOAEL) following 13 weeks of dietary administration was 100 mg/kg per day, the highest dose level tested, in male and female rats. Formulation analysis demonstrated that the formulation preparation method produced homogeneous preparations (RSD < 20%).

The results of the toxicokinetic part of this study are reported in Appendix D.

Dose range-finding study for a developmental toxicity test in rats

The objective of the study was to determine the dosage levels of the candidate substance, [FL-no: 16.130] (purity > 98.9%), to be used in a definitive developmental toxicity study conducted in accordance with the OECD Guidelines for Testing of Chemicals Guideline 414 (Prenatal Developmental Toxicity Study), (WIL Research, 2012a).

The candidate substance in 1% methyl cellulose [400 cps] was administered orally by gavage to four groups of eight bred female Crl:CD(SD) rats twice daily (approximately 4 h apart) from gestation days 6 through 20. The total daily dosage levels were 125, 250, 500 and 1,000 mg/kg bw per day administered at a dosage volume of 10 mL/kg per dose. A concurrent control group composed of eight bred females received the vehicle on a comparable regimen. The females were approximately 14 weeks of age at the initiation of dose administration. All animals were observed twice daily for mortality and morbidity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 21, a laparohysterectomy was performed on each female. The uteri, placentas and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external malformations and developmental variations.

All females survived to the scheduled necropsy on gestation day 21. There were no remarkable maternal clinical or macroscopic findings noted at any dosage level. Mean body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights and food consumption in the 125, 250, 500 and 1,000 mg/kg bw per day groups were unaffected by test article administration. Intrauterine growth and survival were unaffected by test article administration at all dosage levels tested. A single low-weight fetus in the 1,000 mg/kg bw per day group was noted with craniorachischisis, microphthalmia, gastroschisis, tarsal flexure, bent tail and anal atresia. There were no other external malformations or external developmental variations noted in this study.

There were no remarkable maternal clinical or macroscopic findings and mean maternal body weight, body weight gain and food consumption were unaffected by test article administration at all dosage levels evaluated. Additionally, intrauterine growth and survival and fetal morphology were unaffected by test article administration at all dosage levels tested. Based on the results of this study, the dosage levels of 250, 500 and 1,000 mg/kg bw per day were selected for a definitive embryo/fetal development study of the candidate substance administered orally by gavage to inbred Crl:CD(SD) rats.

Developmental toxicity (prenatal) study in rats

The objective of the study was to determine the potential of the candidate substance, [FL-no: 16.130] (purity > 98.9%), to induce developmental toxicity after maternal exposure from implantation to 1 day prior to expected parturition, to characterise maternal toxicity at the exposure levels tested and to determine a NOAEL for maternal and developmental toxicity (WIL Research, 2012b). The study was conducted in general accordance with the OECD Test Guideline 414 (OECD, 2001) and the US FDA Redbook 2000 (Toxicological Principles for the Safety Assessment of Food Ingredients, as updated)

(FDA, 2007) and the Guidelines for Reproduction and Development Studies, January 2001 (WIL Research, 2012b).

The test article, [FL-no: 16.130], in 1% methylcellulose [400 cps] was administered orally by gavage to three groups of 25 bred female Crl:CD(SD) rats twice daily (approximately 4 h apart) from gestation days 6 through 20. The total daily dosage levels were 250, 500 and 1,000 mg/kg bw per day administered at a dosage volume of 10 mL/kg per dose. A concurrent control group composed of 25 bred females received the vehicle (1% methylcellulose [400 cps]) on a comparable regimen. The females were approximately 14 weeks of age at the initiation of dose administration. All animals were observed twice daily for mortality and morbidity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 21, a laparohysterectomy was performed on each female. The uteri, placentas and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations.

All females survived to the scheduled necropsy on gestation day 21. No test article-related clinical or macroscopic findings were noted at any dosage level. Mean body weights, body weight gains, gravid uterine weights and food consumption were unaffected by test article administration at all dosage levels. No test article-related findings were noted on intrauterine growth and survival and fetal morphology at any dosage level. The mean litter proportion (per cent per litter) of late resorptions in the 500 mg/kg per day group (2.8% per litter) was above the maximum mean value in the laboratories historical control data (0.5% per litter); however, this did not occur in a dose-related manner, the value was primarily due to 1 litter with 64% late resorptions. The difference from the concurrent control group was not statistically significant.

Based on the lack of test article-related effects at any dosage level, a dosage level of 1,000 mg/kg per day, the highest dosage level evaluated, was considered to be the NOAEL for maternal toxicity and embryo/fetal development effects when the candidate substance was administered orally by gavage to inbred Crl:CD(SD) rats (Table E.1).

Table E.1: Summary of toxicity studies

FL-no	Test material	Species; Sex No/group	Route of administration	Dose level (mg/kg bw per day)	Duration (days)	Result (mg/kg bw per day)	Reference	Comments
16.130		Rat Crl:CD(SD); M+F 5/4	Diet	0, 10, 30 and 100	28	100	(MPI, 2011a)	Performed in accordance with the US Food and Drug Administration Toxicological Principles for the Safety of Food Ingredients (FDA, 2007)
		Rat Crl:CD(SD); M+F 20/4	Diet	0, 30, 60 and 100	90	100 M 100 F	(MPI, 2011b)	Performed in accordance with the US Food and Drug Administration Toxicological Principles for the Safety of Food Ingredients (FDA, 2007) and FDA Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58
		8 Female Crl:CD(SD) rats	Gavage	0, 125, 250, 500 and 1,000	Gestation days 6 through 20	1,000	(WIL Research, 2012a)	
		25 female Crl:CD(SD) rats	Gavage	0, 250, 500 and 1,000	Gestation days 6 through 20	Maternal 1,000 Fetal 1,000	(WIL Research, 2012b)	OECD TG 414 (OECD, 2001) and US Food and Drug Administration Toxicological Principles for the Safety of Food Ingredients (FDA, 2007)

bw: body weight; F: Female; FL-no: FLAVIS number; M: Male.

Appendix F – Procedure for the evaluation of a new flavouring substance (Figure F.1)

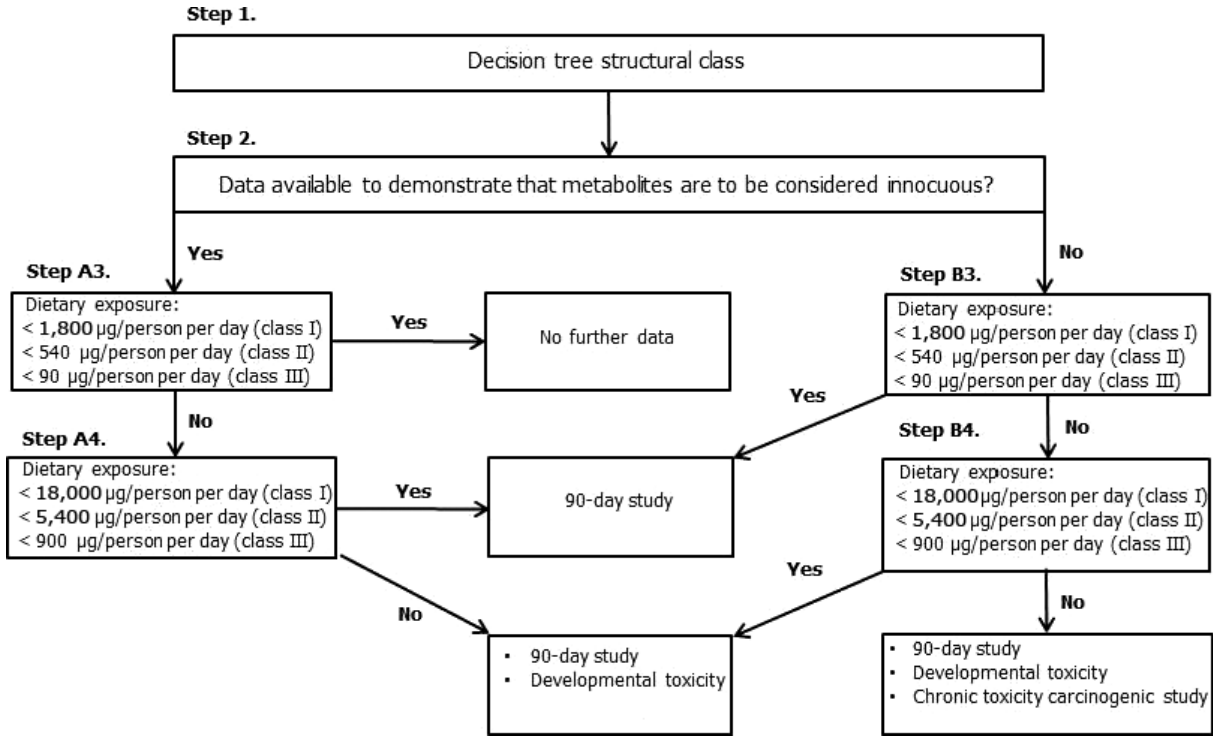


Figure F.1: The stepwise procedure for the evaluation of a new flavouring substance according to Commission Regulation (EC) No 1334/2008