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Scientific opinion on flavouring group evaluation 414 (FGE.414): 2-hydroxy-4-methoxybenzaldehyde

EFSA Panel on Food Additives and Flavourings (FAF),
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Abstract

The EFSA Panel on Food Additives and Flavourings (FAF) was requested to evaluate the safety of the substance 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] as a new flavouring substance, in accordance with Regulation (EC) No 1331/2008. 2-Hydroxy-4-methoxybenzaldehyde belongs to chemical group 23 (Commission Regulation (EC) No 1565/2000) and is structurally related to the hydroxy- and alkoxy-ring substituted benzyl derivatives evaluated in FGE.52 and in FGE.20Rev4. The Panel considered the structural/metabolic similarity sufficient to evaluate the candidate substance following a group-based approach according to the EFSA Guidance on the data required for the risk assessment of flavourings to be used in or on foods. The information provided on the manufacturing process, the composition and the stability of [FL-no: 05.229] was considered sufficient. From studies carried out with this substance, the Panel concluded that there is no concern with respect to genotoxicity. Based on QSAR evaluation of possible metabolism, and based on information from structurally related substances, various metabolic routes can be anticipated, which only result in the formation of innocuous metabolites. The exposure estimates for [FL-no: 05.229] (24 and 60 μg/person per day for children and adults, respectively) were below the Threshold of Toxicological Concern (TTC) for its structural class (I). Accordingly, toxicity studies are not required and the Panel concluded at step A3 of the Procedure that 2-hydroxy-4-methoxybenzaldehyde is not of safety concern when used as a flavouring substance at the intended uses and use levels. Cumulative exposure estimates for 2-hydroxy-4-methoxybenzaldehyde and three structurally related substances (2.4 and 6.2 mg/kg body weight (bw) per day for adults and children, respectively) are above the TTC for structural class I, but below the ADI (acceptable daily intake) of 0-10 mg/kg bw per day for vanillin, which is one of these structurally related substances. Therefore, the cumulative exposure to these four substances [FL-no: 05.015, 05.018, 05.229 and 09.749] also does not raise a safety concern.

Keywords: 2-hydroxy-4-methoxybenzaldehyde, FGE.414, FL-no: 05.229

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1. Introduction

The present scientific opinion deals with the safety assessment of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] to be used as a new flavouring substance in and on food.

1.1. Background and Terms of Reference as provided by the requestor

Background

The use of flavourings in and on 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 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 of the substance mentioned above as a new flavouring substance in 2020. The application has been examined for administrative completeness and it is considered complete.

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), EFSA should carry out a safety assessment of this substance.

Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment of the substance 2-hydroxy-4-methoxybenzaldehyde (CAS 673-22-3) 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. Existing authorisations and evaluations

2-Hydroxy-4-methoxybenzaldehyde was proposed to be evaluated within the JECFA chemical group 29, i.e. hydroxyl and alkoxy substituted benzyl derivatives (JECFA, 2013), but the evaluation has not yet been conducted.

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] has been evaluated by the Flavour and Extract Manufactures Association (FEMA) expert Panel as 'Generally Regarded As Safe' (GRAS) (FEMA GRAS no 4435, GRAS list 24).

The Panel noted that 2-hydroxy-4-methoxybenzaldehyde is registered in the ECHA database[3] as an 'intermediate' for non-food related purposes under articles 17/18 of the REACH Regulation.

2. Data and methodologies

2.1. Data

The present evaluation is based on data on 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] provided by the applicant in a dossier (Documentation provided to EFSA No. 1) to support its safety evaluation as a food flavouring substance. Additional information was provided by the applicant during the risk assessment process on 21 December 2020 in response to a request from EFSA sent on 3 December 2020 (Documentation provided to EFSA No. 2).

Following a second request for additional data sent by EFSA on 2 March 2021, the applicant requested a clarification teleconference held on 31 March 2021, after which the applicant provided additional data on 22 April 2021 (Documentation provided to EFSA No. 3).

A further request for additional data was sent by EFSA on 9 June 2021 and data were provided on 15 June 2021 (Documentation provided to EFSA No. 4).

[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. OJ L 354, 31.12.2008, p. 34–50.

[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://echa.europa.eu/it/registration-dossier/-/registered-dossier/23928/2/1

2.2. Methodologies

This opinion was prepared following the principles described in the EFSA Guidance of the Scientific Committee on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidance documents from the EFSA Scientific Committee.

The safety assessment of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] was carried out in accordance with the procedure as outlined in the EFSA scientific opinion 'Guidance on the data required for the risk assessment of flavourings to be used in or on foods' (EFSA CEF Panel, 2010) and the EFSA technical report 'Proposed template to be used in drafting scientific opinions on flavouring substances (explanatory notes for guidance included)' (EFSA, 2012).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

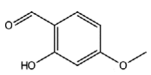
The chemical structure of the candidate substance 2-hydroxy-4-methoxybenzaldehyde is shown in Table 1. The candidate substance has been allocated the FLAVIS number [FL-no: 05.229].

The applicant provided compositional data on two commercial batches (1004445044 and 1004757984) of the candidate substance (Tables 2 and 3). In both batches, the impurities 1-(2-hydroxy-4-methoxyphenyl)ethanone (CAS no. 552-41-0) and methyl-2-hydroxy-4-methoxybenzoate (CAS no. 5446-02-6) were found. These impurities are structurally related to the candidate substance (Tables 1 and 2). In addition, palmitic acid was reported to be present as a minor impurity. The chemical identity of the candidate substance has been confirmed by comparison with NMR reference spectra (^{1}H and ^{13}C) and those of the impurities tentatively by comparison with reference mass spectra. The reported concentrations reflect the peak areas determined via gas chromatographic (GC) analysis (Documentation provided to EFSA No. 2).

In response to a request for clarification by the Panel regarding the differences in the concentrations of 1-(2-hydroxy-4-methoxyphenyl)ethanone in the two investigated batches (< 0.1% in batch 1004445044 and 1.94% in batch 1004757984), the applicant provided compositional data for a third commercial batch (1005087462) of the candidate substance (Table 3). The concentration of 1-(2-hydroxy-4-methoxyphenyl)ethanone in this batch (0.73%) was similar to the concentration reported for batch 1004757984. According to the applicant, the concentration of this impurity in the commercial product is controlled during the production process and the last washing steps ensure that the concentration of this impurity remains below 2% (Documentation provided to EFSA No. 2).

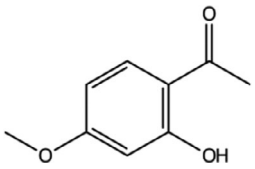
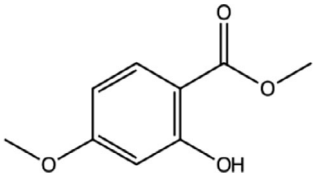
Based on information from European Chemicals Agency (ECHA) website[3], 2-hydroxy-4-methoxybenzaldehyde may contain 1-methyl-2-pyrrolidone (EC no. 212-828-1, CAS no. 872-50-4) as a stabiliser (according to the REACH-registrant of this substance). 1-Methyl-2-pyrrolidone (synonym *N*-methyl-2-pyrrolidone (NMP)) has a harmonised EU classification as a reproductive toxicant (category Repr. 1B). Therefore, the Panel asked the applicant to confirm that 1-methyl-2-pyrrolidone is not used in the manufacturing and/or as an additive/processing aid for 2-hydroxy-4-methoxybenzaldehyde proposed for use as food flavouring as described within the present application dossier. In the response the applicant confirmed that 1-methyl-2-pyrrolidone is not used in the extraction process as a solvent, processing aid, stabiliser nor in any other way in the production of this substance. The Panel considered that 1-methyl-2-pyrrolidone is not expected to be present in the solvents used in the manufacturing process described in the present application dossier. In addition, according to the Scientific Committee on Consumer Safety (SCCS) Opinion on N-methyl-2-pyrrolidone, there are no known natural sources of NMP (SCCS, 2011). Therefore, the Panel concluded that there are no indications that 1-methyl-2-pyrrolidone is present in the flavouring substance 2-hydroxy-4-methoxybenzaldehyde produced according to the procedure described within this application.

Table 1: Specifications for 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] as proposed by the applicant

Chemical name (IUPAC)	CAS no EC no EINECS no CoE no JECFA no FL-no FEMA no	Chemical formula MW	Structural formula	Physical form	Solubility data water	ID test	Purity	Impurities	Boiling point[(a)] Melting point[(b)] Specific gravity[(c)] Refractive index[(d)]	Information on the configuration of the flavouring substance
2-Hydroxy-4-methoxybenzaldehyde	673-22-3 211-604-0 – – – 05.229 4435	$C_8H_8O_3$ 152.15		Pale yellow to orange-pink powder	Phosphate buffer, pH 7.1: 943.5 mg/L	GC–MS IR, UV, NMR Sensory test (taste + odour)	> 95%	1-(2-Hydroxy-4-methoxyphenyl)-ethanone < 3%; methyl-2-hydroxy-4-methoxy-benzoate < 1%; palmitic acid < 1%	n.a. 41–43°C n.a. n.a.	Achiral molecule

IUPAC: International Union of Pure and Applied Chemistry; CAS: Chemical Abstract Service; EC: European Commission; EINECS: European Inventory of Existing Commercial chemical Substances; CoE: Council of Europe; JECFA: Joint FAO/WHO Expert Committee on Food Additives; FL-no: FLAVIS number; FEMA: Flavour and Extract Manufactures Association; MW: Molecular Weight; ID: Identity; GC: gas chromatography; MS: mass spectrometry; IR: infrared; UV: ultraviolet; NMR: nuclear magnetic resonance.
(a): At 1,013.25 hPa, if not otherwise stated.
(b): At 973 hPa.
(c): At 20°C, if not otherwise stated.
(d): At 25°C, if not otherwise stated.

Table 2: Chemical structures of impurities structurally related to the candidate substance identified in commercial batches

Chemical name/CAS no.	Chemical structure
1-(2-Hydroxy-4-methoxyphenyl)ethanone (552-41-0)	
Methyl-2-hydroxy-4-methoxybenzoate (5446-02-6)	

CAS: Chemical Abstract Service.

Table 3: Compositional data provided by the applicant on three commercial batches of the candidate substance and on the batch used for genotoxicity testing

Component	Commercial batches			Batch used for genotoxicity testing[a]
	1 (1004445044)	2 (1004757984)	3 (1005087462)	Firsa_OP2-15jan2019
	%			
Water	0.2	0.3	–[b]	2.75
2-Hydroxy-4-methoxybenzaldehyde	99.67	96.48	98.38	95.5
1-(2-Hydroxy-4-methoxyphenyl)ethanone	< 0.1	1.94	0.73	< 0.1
Methyl-2-hydroxy-4-methoxybenzoate	0.11	0.2	0.21	0.13
Palmitic acid	0.14	0.14	0.15	0.22
Linoleic acid	< 0.1	< 0.1	–[b]	0.2
Unknowns not quantified	< 0.1	< 0.1	–[b]	0.69

(a): According to the applicant, the batch corresponds to batch 1004445044 prior to the two last washing steps employed in the manufacturing.
(b): Data not provided.

3.1.2. Organoleptic characteristics

According to the applicant, the candidate substance imparts an odour/flavour with a warm, gourmand, vanilla bean and almond-like note (Documentation provided to EFSA n. 1).

3.1.3. Manufacturing process

The source of the candidate substance is the root bark of *Periploca sepium*.

The isolation procedure involves distillation and extraction steps. After purification (encompassing crystallisation and washing steps) and drying, 2-hydroxy-4-methoxybenzaldehyde is obtained as a crystalline substance with a purity ≥ 95%.

The applicant provided a letter of certificate on the source material and information on the employed solvents (Documentation provided to EFSA No. 1).

3.1.4. Genetically modified organisms

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] is not produced by or from genetically modified organisms.

3.1.5. Solubility and particle size

The applicant reported a water solubility at pH 7.1 of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] of 0.94 g/L at 20 ± 0.5°C, based on the mean of three determinations with the shake flask method, according to the OECD test guideline (TG) 105 (OECD, 1995).

Regarding particle size, the applicant provided a laser diffraction (LD) analysis of one batch of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] and concluded that the material does not contain nanosized particles (i.e. below 100 nm). The Panel noted that LD is not a proper methodology to investigate the presence of nanoparticles and thus to reach any conclusions on this respect (Mech et al., 2020).

The Panel noted that the maximum use levels of the candidate substance [FL-no: 05.229] proposed by the applicant for various food categories, range between 0.05 and 1 mg/kg food. Taking into account the reported water solubility of 0.94 g/L, the Panel considered that the flavouring substance can be reasonably anticipated to be fully dissolved when added to the proposed foods and beverages. Moreover, given the non-polar nature of the candidate substance [FL-no: 05.229] (it is extracted using an organic solvent), in lipophilic media the compound would be even more easily dissolved. Therefore, the Panel considered that the EFSA Guidance on Nanotechnology (EFSA Scientific Committee, 2018) is not applicable and the risk assessment of the candidate substance should be done following the Guidance on risk assessment of Flavourings to be used in or on food (EFSA CEF Panel, 2010).

3.1.6. Proposed specifications

The specifications proposed for 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] by the applicant are shown in Table 1. With regard to the impurity 1-(2-hydroxy-4 methoxyphenyl)ethanone, the Panel noted that lower concentrations (< 2%) than the one indicated in the proposed specifications (i.e. < 3%, see Table 1) are technologically achievable. The Panel noted that the sensory test should not be used as a stand-alone ID test. Otherwise, the Panel found the proposed specifications acceptable.

3.1.7. Methods of analysis in food

No methods of analysis in food have been provided by the applicant.

3.1.8. Stability and fate in food

The stability of 2-hydroxy-4-methoxybenzaldehyde has been tested in aqueous buffer solutions at pH 2, 5 and 7 at 40°C for a time period of up to 28 days (Firmenich S.A., 2019). Under these test conditions, the recovered proportion of 2-hydroxy-4-methoxybenzaldehyde determined by gas chromatography with flame ionization detection (GC-FID) analysis was consistently ≥ 95%. The applicant reported that a 'very slight yellow to red coloration' was observed at pH 5 and 7 and indicated that such colourations are also known from the structurally related vanillin (4-hydroxy-3-methoxybenzaldehyde).

No experimental data on the storage stability of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] and its fate in foods have been provided by the applicant. Taking into account the presence of the same functional groups in the molecule as in vanillin (4-hydroxy-3-methoxybenzaldehyde), the Panel considered that reactions of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] with food constituents would be similar to those described for vanillin (Weerawatanakorn et al., 2015). As potential reaction products of vanillin are not considered to raise a safety concern, this applies also to 2-hydroxy-4-methoxybenzaldehyde.

3.2. Structural/metabolic similarity to flavouring substances in existing FGE

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] belongs to chemical group 23 (Commission Regulation (EC) No 1565/2000)[4] and is structurally related to the group of hydroxy- and alkoxy-ring

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

substituted benzyl derivatives evaluated in FGE.52 (EFSA, 2008) and in FGE.20Rev4 (EFSA CEF Panel, 2012). The Panel considered the structural/metabolic similarity sufficient to evaluate the candidate substance following a group-based approach according to the EFSA Guidance on the data required for the risk assessment of flavourings to be used in or on foods (EFSA CEF Panel, 2010). The applied procedure is given in Appendix A.

3.3. Exposure assessment

3.3.1. Natural occurrence in food

No data reported by the applicant.

A search in the VCF (volatile compounds in food) online database of volatile compounds in food did not give any results on natural occurrence of the substance [FL-no: 05.229] in food (VCF, 2021).

3.3.2. Non-food sources of exposure

According to the applicant, the essential oil from *Periploca sepium* is used as a fragrance ingredient. 2-Hydroxy-4-methoxybenzaldehyde is one of the components of this essential oil. The applicant stated that consumer exposure to 2-hydroxy-4-methoxybenzaldehyde from cosmetics is negligible, but no adequate data to substantiate this statement were provided.

The Panel noted that 2-hydroxy-4-methoxybenzaldehyde (under its synonym p-anisaldehyde) is included in the EFSA Compendium of botanicals[5] as occurring in the roots and underground parts of the plant *Mondia whitei*.

2-Hydroxy-4-methoxybenzaldehyde has been identified in plants, which are used in some Asian countries as 'plant-based traditional medicines' (Rathi et al., 2017).

According to the applicant, 2-hydroxy-4-methoxybenzaldehyde has been reported to occur also in *Aegle marmelos* leaves, *Anacyclus pyrethrum* seed, *Codonopsis pilosula*, *Illicium verum*, *Pimpinella anisum*, *Tribulus terrestris*, *Tylophora indica*. Information on concentrations of 2-hydroxy-4-methoxybenzaldehyde in these plants and on their dietary consumption is not available.

3.3.3. Chronic dietary exposure

The exposure assessment to be used in the Procedure for the safety evaluation of 2-hydroxy-4-methoxybenzaldehyde is the chronic added portions exposure technique (APET) estimate (EFSA CEF Panel, 2010). The chronic APET for [FL-no: 05.229] has been calculated for adults and children (see Table 4), and these values, expressed per kg body weight (bw), will be used in the Procedure (see Appendices A and B). The chronic APET calculation is based on the proposed normal use levels and the standard portion size (see Appendix B).

Based on the information provided by the applicant, the Panel considered that 2-hydroxy-4-methoxybenzaldehyde is not intended to be used in food category 13.2 (foods for infants and young children).

Table 4: APET – chronic dietary exposure as calculated by EFSA

Chronic APET	Added as flavouring substance[(a)]		Other dietary sources[(b)]		Combined[(c)]	
	µg/kg bw per day	µg/person per day	µg/kg bw per day	µg/person per day	µg/kg bw per day	µg/person per day
Adults[(d)]	1	60	0	0	1	60
Children[(e)]	1.6	24	0	0	1.6	24

APET: added portions exposure technique; bw: body weight.
(a): APET Added is calculated on the basis of the normal amount of flavouring added to a specific food category.
(b): APET Other Dietary Sources is calculated based on the natural occurrence of the flavouring in a specified food category.
(c): APET Combined is calculated based on the combined amount of added flavouring and naturally occurring flavouring 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 15 kg bw is considered representative.

[5] Available online: https://www.efsa.europa.eu/en/data-report/compendium-botanicals

3.3.4. Acute dietary exposure

The acute APET calculation for [FL-no: 05.229] is based on the proposed maximum use levels and large portion size (i.e. three times standard portion size) (EFSA CEF Panel, 2010).

Table 5: APET – acute Dietary Exposure as calculated by EFSA

Acute APET	Added as flavouring substance[(a)]		Other dietary sources[(b)]		Combined[(c)]	
	µg/kg bw per day	µg/person per day	µg/kg bw per day	µg/person per day	µg/kg bw per day	µg/person per day
Adults[(d)]	3	180	0	0	3	180
Children[(e)]	7.9	118	0	0	7.9	118

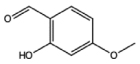
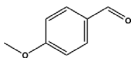
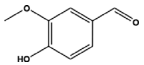
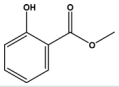
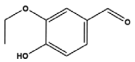
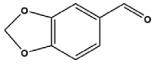
APET: added portions exposure technique; bw: body weight.
(a): APET Added is calculated on the basis of the maximum amount of flavouring added to a specific food category.
(b): APET Other dietary sources is calculated based on the natural occurrence of the flavouring in a specified food category.
(c): APET Combined is calculated based on the combined amount of added flavouring and naturally occurring flavouring 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 15 kg bw is considered representative.

3.3.5. Cumulative dietary exposure

The applicant considered the five flavouring substances in the group of hydroxy- and alkoxy-substituted benzyl derivatives (evaluated in FGE.20 and FGE.52) with the highest annual production volumes, based on the most recent available data from 2015. Using this approach, the substances identified were all evaluated in FGE.52: [FL-no: 05.015, 05.016, 05.018, 05.019 and 09.749]. The annual production volumes (referred to 2015) have been submitted for these five flavouring substances (Table 6).

Average use levels for the five structurally related substances have been submitted based on data from FEMA (documentation provided to EFSA no. 3 and Appendix B). Based on these data, APET exposure estimates were calculated for [FL-no: 05.015, 05.016, 05.018, 05.019 and 09.749]. To calculate the cumulative exposure, the exposure estimate to the structurally related substances of the same structural class (I) according to Cramer et al. (1978) have been added to that of [FL-no: 05.229] resulting in a cumulative exposure of 2,400 µg/kg bw for adults and 6,200 µg/kg bw for children (Table 6). The use of [FL-no: 05.229] as a flavouring substance is not expected to have a significant impact on the overall dietary cumulative exposure.

Table 6: APET estimates for the candidate and the five structurally and metabolically related flavouring substances, in the group of hydroxy- and alkoxy-substituted benzyl derivatives, with the highest poundage data. The cumulative chronic APET is the sum of the APETs of the structural class I substances

FL-no	Union list name	Structural formula	Structural class[a]	APET adult (µg/kg bw per day) (µg/person per day)	APET children (µg/kg bw per day) (µg/person per day)	TTC[b] (µg/person per day)	Annual production volume kg (2015)
05.229	2-Hydroxy-4-methoxybenzaldehyde		I	1.0 60	1.6 24	1,800	200
05.015	4-Methoxybenzaldehyde		I	200 12,000	500 7,400	1,800	6,500
05.018	Vanillin		I	940 56,000	2,400 35,000	1,800	929,000
09.749	Methyl salicylate		I	1,300 78,000	3,300 49,000	1,800	16,800
Cumulative chronic APET				**2,400 150,000**	**6,200 92,000**	**1,800**	
05.019	Ethyl vanillin		II	4,700 280,000	12,000 180,000	540	75,800
05.016	Piperonal		III	52 3,100	130 2,000	90	26,600

APET: added portions exposure technique; bw: body weight.
(a): Determined with OECD Toolbox (version 4.4.1 available at https://qsartoolbox.org/).
(b): TTC: Threshold of toxicological concern for the structural class to which the substance belongs (see Munro et al., 1996).

3.4. Biological and toxicological data

3.4.1. Absorption, distribution and elimination

No experimental data were submitted for the candidate substance [FL-no: 05.229]. However, as discussed in the JECFA report (JECFA, 2002a) and in FGE.20Rev4, hydroxyl- and alkoxy-substituted benzyl derivatives are absorbed from the gut rapidly, from which they are then oxidised in the liver into benzoic acid derivatives and, to a lesser extent, they are reduced to their benzyl alcohol derivatives (JECFA 2002a,b; Adams et al., 2005). Resulting hydroxyl- and alkoxy-benzoic acid derivatives form sulfate, glucuronic acid or glycine conjugates, which are excreted, mainly in the urine.

3.4.2. Metabolism

Metabolism studies on 2-hydroxy-4-methoxybenzaldehyde have not been provided. However, information from *in silico* analysis of possible metabolism of 2-hydroxy-4-methoxybenzaldehyde and biological data on metabolism of structurally related substances have been provided.

3.4.2.1. *In silico* analysis

The applicant provided prediction of liver metabolism obtained with TIMES (Tissue Metabolism Simulator) software.[6]

The Panel checked the predictions reported by the applicant (obtained with TIMES software by OASIS-LMC (Laboratory of Mathematical Chemistry)) with the metabolic simulator of OECD QSAR Toolbox v.4.4. The Panel noted that the Toolbox metabolic simulator is based on the same technology as that of TIMES.

The analysis through the OECD QSAR Toolbox confirmed that the aldehyde group of the candidate substance is predicted to be oxidised into its corresponding acid to form a benzoic acid derivative (2-hydroxy-4-methoxybenzoic acid) or reduced into its corresponding alcohol to form a benzyl alcohol (2-(hydroxymethyl)-5-methoxyphenol). These metabolites are predicted to be conjugated to sulfate, glucuronic acid or amino acids (e.g. glycine), see Appendix D.

3.4.2.2. Information on metabolism of structurally related substances

Oxidised and conjugated products are also known to be formed from a number of similar structures evaluated in FGE.20 and in FGE.52. In particular, 4-hydroxy-3-methoxybenzaldehyde (vanillin [FL-no: 05.018]) and 3-ethoxy-4-hydroxybenzaldehyde (ethyl vanillin [FL-no: 05.019]) have similar functional groups and have been concluded by JECFA and EFSA to be converted to innocuous metabolites. The observed metabolites from these substances support the prediction of the metabolism of 2-hydroxy-4-methoxybenzaldehyde by the OASIS-TIMES software.

Other minor metabolic pathways may comprise oxidative demethylation and decarboxylation (JECFA, 2002a,b; Adams et al., 2005; EFSA CEF Panel, 2012). The products resulting from these conversions can also be anticipated to be readily conjugated and excreted.

Considering the impurities, 1-(2-hydroxy-4-methoxyphenyl)ethanone and methyl-2-hydroxy-4-methoxybenzoate, both have functional groups that are similar to those of 2-hydroxy-4-methoxybenzaldehyde and to substances in FGE.20 and FGE.52; therefore, their conversion to hydroxylated and conjugated products for excretion is also expected. The reported impurities palmitic acid and linoleic acid are fatty acids naturally present in humans, would be expected to be incorporated into endogenous pools and/or oxidised to carbon dioxide.

Conclusion on metabolism

Considering all of the information as summarised above, the Panel concluded that the candidate substance has sufficient structural similarity and predicted metabolic pathway to structures in FGE.52 and FGE.20. Therefore, 2-hydroxy-4-methoxybenzaldehyde can be considered in a group-based evaluation assigned to the group of hydroxy- and alkoxy-substituted benzyl derivatives (evaluated in FGE.20 and FGE.52) and can be anticipated to be metabolised into innocuous products only.

[6] TIMES software, Laboratory of Mathematical Chemistry, Bourgas, Bulgaria http://www.oasis-lmc.org

3.4.3. Genotoxicity

3.4.3.1. *In silico* analysis

The Panel screened the candidate substance and the structurally related impurities shown in Table 2 for the presence of genotoxicity structural alerts using OECD QSAR toolbox. No alerts were found.

3.4.3.2. *In vitro* genotoxicity tests

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] was tested *in vitro* in a bacterial reverse gene mutation assay and in a micronucleus (MN) assay. For these genotoxicity tests, the applicant has selected a batch (Firsa_OP2-15jan2019) which had not been subjected to the last two washing steps regularly applied in the production of the flavouring substance. As shown in Table 3, this resulted in a lower purity of the candidate substance. The Panel noted that the concentration of 1-(2-hydroxy-4-methoxyphenyl)ethanone in the batch employed for the genotoxicity tests was lower than those in two of the investigated commercial batches. This impurity has structural similarity with the candidate substance and has no structural alerts for genotoxicity (see above). Therefore, the Panel concluded that this batch (Firsa_OP2-15jan2019) is acceptable for the genotoxicity testing.

3.4.3.2.1. Bacterial reverse gene mutation assay

A bacterial reverse mutation assay was conducted in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and in *Escherichia coli* WP2 uvrA(pKM101) to assess the mutagenicity of 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] (purity > 95%), both in the absence and in the presence of metabolic activation by Aroclor 1245-induced rat liver postmitochondrial fraction (S9-mix) in two separate experiments using plate incorporation treatment (Gentronix, 2019a). Study design complies with OECD TG 471 (OECD, 1997) and with the GLP principles.

Positive control chemicals and DMSO (as vehicle control) were evaluated concurrently. All tests were evaluated in triplicate plates.

In both experiments, 2-hydroxy-4-methoxybenzaldehyde was tested at concentrations from 1.6 to 5,000 μg/plate with and without S9-mix. Precipitation was observed at the highest concentration tested.

In both experiments, bacteriotoxicity was observed from 500 μg/plate. In *S.* Typhimurium strains excessive toxicity was observed from 1,600 μg/plate and in *E. coli* at 5,000 μg/plate (in the presence and in the absence of S9-mix).

All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests, while vehicle controls were within the historical control ranges. No increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains in the absence or presence of metabolic activation (Gentronix, 2019a). Study results are summarised in Appendix C.

In conclusion, the Panel considered that 2-hydroxy-4-methoxybenzaldehyde did not induce gene mutations in bacteria under these test conditions.

3.4.3.2.2. *In vitro* micronucleus assay

Human peripheral blood lymphocytes from healthy donors were treated with 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] (purity > 95%) at concentrations ranging from 51 to 10,000 μmol/L (equal to 7.8 to 1,521.5 μg/mL). The highest concentration tested was chosen, according to OECD TG 487 (OECD, 2016), at 10,000 μmol/L based on results of a solubility test. The *in vitro* MN assay was carried out according to OECD TG 487 (OECD, 2016) and GLP principles. The cytokinesis block MN assay protocol was applied. Positive controls were cyclophosphamide, mitomycin C and colchicine (Gentronix, 2019b).

Lymphocytes were treated with 2-hydroxy-4-methoxybenzaldehyde in three different test conditions: 3 h treatment in the presence or in the absence of rat liver S9 metabolic activation (from rats treated with Aroclor 1254) and 24 h treatment in the absence of metabolic activation.

The Cytokinesis Block Proliferation Index (CBPI) cytotoxicity data were used to select the concentrations on which to perform the MN analysis.

In the treatment of 3 + 21 h in the presence of S9-mix, the following concentrations were chosen for MN analysis: 1,975 μmol/L, 2,963 μmol/L and 4,444 μmol/L (cytotoxicity of 16%, 33% and 57%, respectively). Precipitation was observed at 10,000 μmol/L.

In the treatment of 3 + 21 h in the absence of S9-mix, the following concentrations were chosen for MN analysis: 1,317 μmol/L, 1,975 μmol/L and 2,963 μmol/L (cytotoxicity of 10%, 38% and 50%, respectively). Precipitation was observed at 10,000 μmol/L.

Since in the first experiment, with treatment for 24 h in the absence of S9-mix, the positive controls did not show the expected results, this part of the experiment was considered invalid and it was repeated.

In the second experiment, 2-hydroxy-4-methoxybenzaldehyde was tested at concentrations ranging from 100 to 800 μmol/L, for 24 h in the absence of S9-mix, no precipitate was observed. Concentrations chosen for MN analysis were: 451.6 μmol/L 496.7 μmol/L, 546.4 μmol/L and 601.1 μmol/L (cytotoxicity of 9%, 30% 38% and 58%, respectively). In this experiment, the positive controls responded as expected. 2-Hydroxy-4-methoxybenzaldehyde did not increase the MN cell frequency compared to vehicle (DMSO) controls in any of the test conditions.

The Panel concluded that 2-hydroxy-4-methoxybenzaldehyde did not increase MN cell frequency under the test conditions of this study.

Since 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] did not induce gene mutations or structural or numerical chromosomal aberrations *in vitro*, there was no requirement to test the candidate substance *in vivo* (EFSA Scientific Committee, 2011).

Summary of the results are reported in Appendix C.

3.4.3.3. Conclusions on genotoxicity

Based on the experimental data on the candidate substance, the Panel concluded that [FL-no: 05.229] does not raise concern for genotoxicity.

Considering the structural similarities of the impurities (1-(2-hydroxy-4-methoxyphenyl)ethanone and methyl-2-hydroxy-4-methoxybenzoate) to [FL-no: 05.229], and to the structurally related substances evaluated in FGE.52 and FGE.20Rev4 and taking into account the absence of structural alerts for genotoxicity from the *in silico* analysis, the Panel concluded that there was no concern for genotoxicity for these impurities.

3.4.4. Toxicological data

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] has not been tested in toxicity studies.

Considering the structural/metabolic similarity with the group of hydroxy- and alkoxy-substituted benzyl derivatives evaluated in FGE.52 and in FGE.20Rev4 and the application of a group-based evaluation, the toxicological data summarised in table 7 can be used for the evaluation of the candidate substance.

Table 7: Key toxicity studies with substances structurally related to [FL-no: 05.229]

Substance name FL-no JECFA no	NOAEL ADI (mg/kg bw per day)	Study
4-Hydroxy-3-methoxybenzaldehyde (Vanillin) 05.018 889	1,000 0-10	2-year study in rats (JECFA, 1968, 2002b) 1,000 mg/kg is the highest dose tested, no adverse effects reported (Hagan et al., 1967)
Methyl-2-hydroxybenzoate (Methyl salicylate) 09.749 899	50 0-0.5	2-year study in dogs (JECFA, 1968, 2002b) Dose levels: 50, 150, 350 mg/kg bw. The groups at the 2 higher doses had enlarged liver and enlarged hepatic cells. Animals at 150 mg/kg had growth retardation (Webb and Hansen, 1963)

ADI: acceptable daily intak; FL-no: FLAVIS number; JECFA: Joint FAO/WHO Expert Committee on Food Additives; NOAEL: no observed adverse effect level; bw: body weight.

Developmental/Reproductive Toxicity Studies

In FGE.20Rev4, the EFSA CEF Panel has considered reproductive toxicity studies with vanillin [FL-no: 05.018], ethyl-vanillin [FL-no: 05.019] and piperonal [FL-no: 05.016] performed by Vollmuth et al., 1990. No fetal toxicity was reported; however, since these studies were only described in an abstract with limited details, the Panel concluded that they cannot contribute to the evaluation of [FL-no: 05.229].

3.5. Application of the Procedure

Because 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] does not raise a concern for genotoxicity and on the basis of its structural similarity to flavouring substances that have already been evaluated by EFSA previously in FGE.20Rev4 and FGE.52, it is appropriate to evaluate the use of [FL-no: 05.229] as a flavouring substance following the stepwise evaluation procedure for grouped substances as outlined in the 'Guidance on the data required for the risk assessment of flavourings to be used in or on foods' (EFSA CEF Panel, 2010) and Appendix A.

Step 1

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] is allocated to structural class I.

Step 2

Based on data from structurally related substances evaluated in FGE.52 and in FGE.20Rev4 and on an *in silico* analysis of possible metabolism it can be predicted that 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] is metabolised to innocuous products, only.

Step A3

The conditions of use result in an exposure of the substance to amounts that are below the TTC for structural class I (< 1,800 μg/person per day), both for adults and for children. Therefore, 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] is not expected to be of safety concern.

3.6. Assessment of Acute, Combined and Cumulative exposure

Similar to the exposure estimates for chronic exposure, the APET estimates for acute exposure are below the TTC for structural class I (related to subchronic toxicity). Therefore, these acute exposure estimates do not raise a safety concern, either, since acute toxicity is associated with levels of exposure that are at least equal to or higher than those that are associated with subchronic toxicity.

Since data on exposure from other sources were not provided, the combined exposure estimates (see Tables 4 and 5) fully reflect the APET estimates for use as flavouring substance. The combined exposure estimates will not be addressed as such.

The estimates of cumulative exposure to the candidate substance and the structurally related structural class I substances [FL-no: 05.015, 05.018 and 09.749] are above the TTC for structural class I (1,800 μg/person per day). However, the cumulative exposure estimates (see Table 6) for adults and children are 2,400 and 6,200 μg/kg bw per day, respectively. These estimates are below the ADI of 0–10 mg/kg bw per day (JECFA, 2002a,b) for vanillin [FL-no: 05.018]. The Panel noted that approximately 40% of the cumulative exposure estimate comes from the APET estimate for vanillin. The cumulative exposure to these four substances does not raise a safety concern, either. The Panel noted that the contribution of [FL-no: 05.229] to the cumulative exposure is negligible (< 0.1%).

4. Discussion

The European Commission requested EFSA to carry out the safety assessment of the substance 2-hydroxy-4-methoxybenzaldehyde (CAS no. 673-22-3) as a new flavouring substance in accordance with Regulation (EC) No 1331/2008. EFSA allocated 2-hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] to Flavouring Group Evaluation 414 (FGE.414) and used the procedure as referred to in Regulation (EC) No 1334/2008.

The candidate substance has structural similarity to flavouring substances evaluated by EFSA in FGE.20 and its revisions and by JECFA, as considered by EFSA in FGE.52. Consequently, the Panel decided to assess the candidate substance in FGE.414 on the basis of its structural similarity to the flavouring substances of these two FGEs.

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] is a substance known to occur naturally and is obtained by extraction and purification from plant material. It has not been reported to occur in food.

Specifications including complete purity criteria and identity for the material of commerce have been provided and considered adequate. The candidate substance does not possess chiral centres and does not have geometrical isomers. The information provided on the manufacturing process, the composition and the stability of the flavouring substance was considered sufficient.

2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229] is intended to be used as a flavouring substance and adequate information on uses and use levels has been provided, as specified in Appendix B. The substance is not intended to be used in food for infants and young children. The chronic dietary exposure to the candidate substance has been estimated using the APET method. The chronic APET exposure estimates are 1 and 1.6 μg/kg bw per day (60 and 24 μg/person per day) for adults and children (15-kg bw; 3-years-old), respectively. The acute APET exposure estimates are 3 and 7.9 μg/kg bw per day (180 and 118 μg/person per day, for adults and children respectively).

No substance-specific information on absorption, distribution, metabolism and elimination (ADME) of [FL-no: 05.229] has been submitted. However, based on QSAR evaluation of possible metabolism and based on information from structurally related substances in FGE.20 and FGE.52, it can be anticipated that 2-hydroxy-4-methoxybenzaldehyde will be rapidly absorbed, metabolised and excreted. Anticipated routes of metabolism include: (i) oxidation or reduction of the aldehyde function to yield the corresponding carboxylic acid (benzoic acid) or benzyl alcohol derivatives; (ii) demethylation of the methoxy group to yield a free aromatic hydroxy group or (iii) possibly oxidation and decarboxylation of the aldehyde group to yield another free aromatic hydroxy group. The hydroxy groups and the carboxylic acid functions may be further conjugated, e.g. with sulfate or glucuronic acid. For the benzoic acid derivatives also conjugation with amino acids, in particular glycine, is an important route of biotransformation. All metabolic routes are anticipated to result in the formation of innocuous metabolites.

The applicant submitted adequate studies to investigate the genotoxic potential of 2-hydroxy-4-methoxybenzaldehyde. From the data, the Panel concluded that there is no concern with respect to genotoxicity of the flavouring substance [FL-no: 05.229]. A similar conclusion was reached previously for the flavouring substances in FGE.20 and FGE.52.

No studies on (sub)-chronic toxicity or reproductive and developmental studies were submitted for [FL-no: 05.229]. However, the substance is anticipated to be metabolised to innocuous substances, only. In addition to this, the exposure estimates of this substance (24 and 60 μg/person per day for children and adults, respectively) were below the TTC for its structural class I (i.e. 30 μg/kg bw per day or 1800 μg/person per day). Accordingly, toxicity studies are not required and the Panel concluded at step A3 of the evaluation Procedure that 2-hydroxy-4-methoxybenzaldehyde is not of safety concern when used as a flavouring substance at the intended uses and use levels as specified in Appendix B. For the structurally related substance vanillin, JECFA (2002a,b) has derived an ADI of 0-10 mg/kg bw per day and comparison of the APET exposure estimates of [FL-no: 05.229] with this ADI supports the conclusion of the FAF Panel.

Cumulative exposure estimates for 2-hydroxy-4-methoxybenzaldehyde plus three structurally related substances (2.4 and 6.2 mg/kg bw per day for adults and children, respectively) are above the TTC for structural class I, but below the ADI of 0-10 mg/kg bw per day for the structurally related substance vanillin. Therefore, the cumulative exposure to these four substances also does not raise a safety concern.

5. Conclusions

Overall, the Panel concluded that there is no safety concern for [FL-no: 05.229], when used as a flavouring substance at the estimated level of dietary exposure calculated using the APET approach based on the intended uses and use levels as specified in Appendix B. This assessment is only applicable if the food flavouring is isolated from *Periploca Sepium* using methodologies giving rise to a final product with purity and residue levels described in this opinion. The Panel also concluded that the cumulative exposure to [FL-no: 05.229] and three structurally related substances does not raise a safety concern.

Documentation as provided to EFSA

1) Dossier 'Complete technical dossier for a new flavouring ingredient.' July 2020. Submitted by Firmenich S.A.
2) Additional information received on 21 December 2020, submitted by Firmenich S.A in response to a request from EFSA (3 December 2020).
3) Additional information received on 22 April 2021, submitted by Firmenich S.A in response to a request from EFSA (2 March 2021).

4) Additional information received on 15 June 2021, submitted by Firmenich S.A in response to a request from EFSA (9 June 2021).
5) Firmenich S.A., 2019. Stability test of 2-Hydroxy-4-methoxybenzaldehyde according to the Protocol for Stability Test of Flavor & Fragrance Materials. Submitted by Firmenich S.A.
6) Gentronix, 2019a. 2-Hydroxy-4-methoxybenzaldehyde: Genetic Toxicity Evaluation using a Bacterial Reverse Mutation Test in Salmonella typhimurium LT2 Strains TA1535, TA1537, TA98 and TA100, and Escherichia coli WP2 uvrA/pKM101. Gentronix study number AME00606. 11 April 2019. Unpublished report submitted by Firmenich S.A.
7) Gentronix, 2019b. 2-Hydroxy-4-methoxybenzaldehyde: Genetic Toxicity Evaluation using a Micronucleus Test in Human Lymphocyte Cells. Gentronix study number MNT00607. 4 June 2019. Unpublished report submitted by Firmenich S.A.

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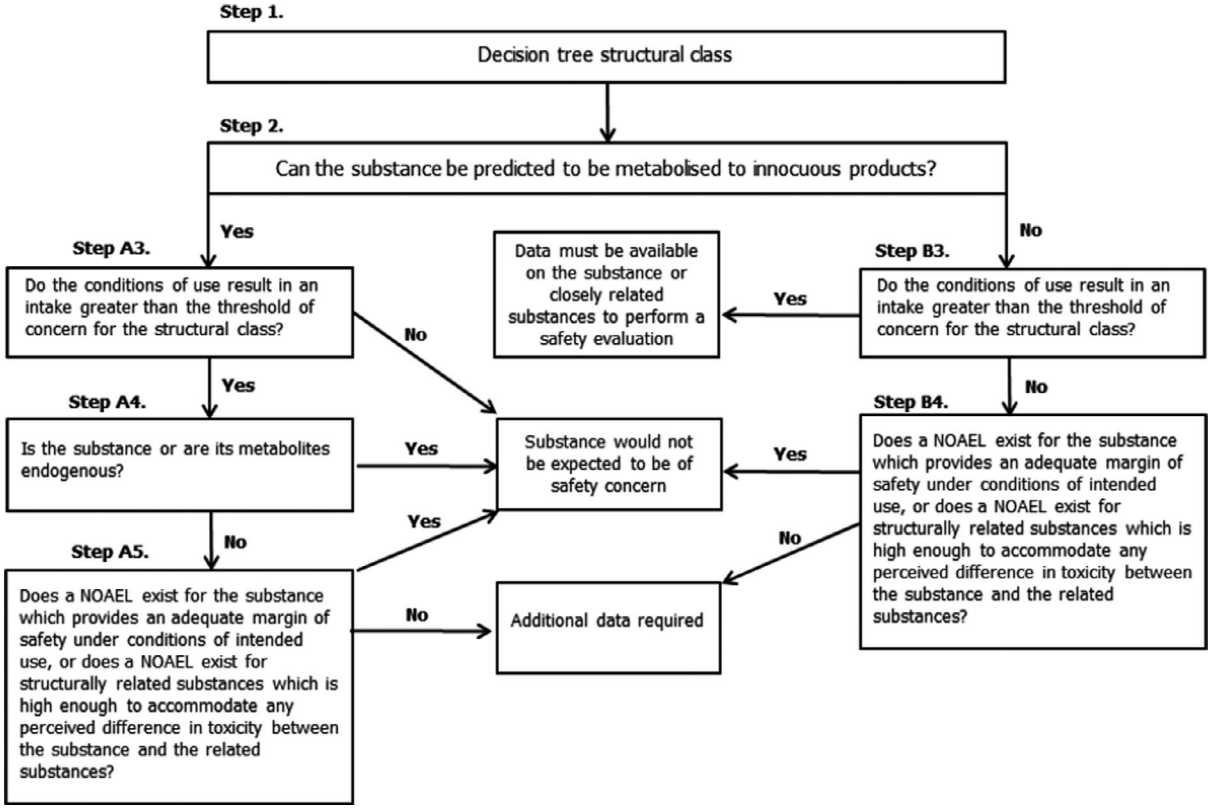
Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and elimination
APET	added portions exposure technique
BMDL	lower confidence limit of the benchmark dose
bw	body weight
CAS	Chemical Abstract Service
CBPI	Cytokinesis Block Proliferation Index
CoE	Council of Europe
DMSO	dimethylsulfoxide
EC	European Commission
EINECS	European Inventory of Existing Commercial chemical Substances
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavour and Extract Manufactures Association
FGE	Flavouring Group Evaluation
FLAVIS	Flavour Information System database
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detection
GLP	Good Laboratory Practice
GRAS	Generally Regarded As Safe
ID	Identity
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD	laser diffraction
MN	micronucleus

MS	mass spectrometry
MW	Molecular Weight
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
QSAR	quantitative structure–activity relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCCS	Scientific Committee on Consumer Safety
SPET	single portion exposure technique
TG	Test Guideline
TIMES	tissue metabolism simulator
TTC	Threshold of Toxicological Concern
VCF	volatile compounds in food
WHO	World Health Organization

Appendix A – Procedure for the safety evaluation of chemically defined flavouring substances



Note: BMDL may be used instead of NOAEL.

Figure A.1: Procedure applied for the safety evaluation of [FL-no: 05.229] according to EFSA Guidance on the data required for the risk assessment of flavourings to be used in or on foods (EFSA CEF Panel, 2010)

Appendix B – Food categories and use levels provided for the candidate and five supporting substances

Table B.1: Food categories and use levels (only those food categories are included for which use levels were provided). Portion sizes are according to the EFSA Guidance on the data required for the risk assessment of flavourings to be used in or on foods (EFSA CEF Panel, 2010) and deviate occasionally from those specified by the applicant

CODeX code	Food categories[a]	Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)						
			Normal	Maximum	Normal	Normal	Normal	Normal	Normal
Flavouring substances FL-no:			**05.229[c]**		**05.015[d]**	**05.016[d]**	**05.018[d]**	**05.019[d]**	**09.749[d]**
01.1	Milk and dairy-based drinks	200	0.08	0.3			221	1398	
01.2	Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks)	200	0.1	0.3					
01.3	Condensed milk and analogues (plain)	70	0.08	0.3					
01.4	Cream (plain) and the like	15	0.1	0.3					
01.5	Milk powder and cream powder and powder analogues (plain)	30	0.15	0.3					
01.6	Cheese and analogues	40	0.03	0.3					
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)	125	0.1	0.5	27	9	27	12	29
01.8	Whey and whey products, excluding whey cheeses	200	0.1	0.3					
02.1	Fats and oils essentially free from water	15	0.2	0.6			96	0.06	
02.2	Fat emulsions mainly of type water-in-oil	15	0.2	0.6					
02.3	Fat emulsions mainly of type water-in-oil, including mixed and/or flavoured products based on fat emulsions	15	0.2	0.6					
02.4	Fat-based desserts excluding dairy-based dessert products of category 1.7	50	0.3	0.6					
03.0	Edible ices, including sherbet and sorbet	50	0.03	0.1					
04.1.2	Processed fruit	125	0.05	0.2					
04.1.2.5	Jams, jellies, marmalades	30	0.05	0.2					
05.1	Cocoa products and chocolate products, including imitations and chocolate substitutes	40	0.13	0.5					

CODeX code	Food categories[a]	Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)						
			Normal	Maximum	Normal	Normal	Normal	Normal	Normal
Flavouring substances FL-no:			**05.229[c]**		**05.015[d]**	**05.016[d]**	**05.018[d]**	**05.019[d]**	**09.749[d]**
05.2	Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4	30	0.2	0.6	53	16	247	57	1942
05.3	Chewing gum	3	0.5	1	18	60	82	37	2108
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	35	0.2	0.8	0.3	2.5	597	140	
06.1	Whole, broken or flaked grain, including rice	200	0.05	0.2					
06.2	Flours and starches (including soya bean powder)	30	0.05	0.2					
06.3	Breakfast cereals, including rolled oats	30	0.1	0.3			353	270	
06.4	Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pastas and noodles)	200	0.05	0.2					
06.5	Cereal and starch based desserts (e.g. rice pudding, tapioca pudding)	200	0.1	0.3	29	5.41	48	18	190
06.6	Batters (e.g. for breading or batters for fish or poultry)	30	0.2	0.4					
06.7	Pre-cooked or processed rice products, including rice cakes (Oriental type only)	200	0.1	0.3					
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	0.1	0.4					
07.1	Bread and ordinary bakery wares	50	0.1	0.3	70	13	74	42	141
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	80	0.2	0.5	70	13	74	42	141
08.2	Processed meat, poultry and game products in whole pieces or cuts	100	0	0		0.1	2	3.9	
10.2	Egg products	100	0.1	0.3					
10.4	Egg-based desserts (e.g. custard)	125	0.13	0.3					
11.1	Refined and raw sugar	10	0.5	1					

CODeX code	Food categories[a]	Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)						
			Normal	Maximum	Normal	Normal	Normal	Normal	Normal
Flavouring substances FL-no:			**05.229[c]**		**05.015[d]**	**05.016[d]**	**05.018[d]**	**05.019[d]**	**09.749[d]**
11.2	Brown sugar excluding products of food category 11.1	10	0.5	1					
11.3	Sugar solutions and syrups, and (partially) inverted sugars, including molasses and treacle, excluding products of food category 11.1	30	0.5	1					
11.4	Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings)	30	0.5	1					
11.5	Honey	15	0	1					
11.6	Table-top sweeteners, including those containing high-intensity sweeteners	1	0.05	1					
12.2	Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	1	0	0				13	
12.4	Mustards	15	0.01	0.05					
12.6	Sauces and like products	30	0.01	0.05			358	102	200
12.9	Protein Products	15	0.15	0.4					
13.3	Dietetic foods intended for special medical purposes (excluding food products of category 13.1)	200	0.15	0.3					
13.4	Dietetic formulae for slimming purposes and weight reduction	200	0.15	0.3					
13.5	Dietetic foods (e.g. supplementary foods for dietary use), excluding products of food categories 13.1, –13.4 and 13.6	200	0.15	0.3					
13.6	Food supplements	5	0.15	0.3					
14.1	Non-alcoholic ('soft') beverages	300	0.03	0.1	20	6.61	40	17	67
14.2.1	Beer and malt beverages	300	0.02	0.1					
14.2.2[e]	Cider and perry	300	0.02	0.1					
14.2.3[e]	Grape wines	150	0.03	0.1					
14.2.4[e]	Wines (other than grape)	150	0.05	0.1					

CODeX code	Food categories[a]	Standard portions[b] (g)	Occurrence level as added flavouring substance (mg/kg)						
			Normal	Maximum	Normal	Normal	Normal	Normal	Normal
Flavouring substances FL-no:			**05.229[c]**		**05.015[d]**	**05.016[d]**	**05.018[d]**	**05.019[d]**	**09.749[d]**
14.2.5[e]	Mead	150	0.1	0.2					
14.2.6[e]	Distilled spirituous beverages containing more than 15% alcohol	30	0.1	0.2					
14.2.7[e]	Aromatised alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	300	0.1	0.2	10	4	30	5	41
15.1	Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)	30	0	0			200		
15.2	Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit)	30	0.05	0.15			200		
15.3	Snacks – fish based	30	0	0			200		
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	300	0.05	0.1					

(a): Most of the categories reported are the sub-categories of Codex GSFA (General Standard for Food Additives, available at http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf) used by the JECFA in the SPET technique (FAO/WHO, 2008).
(b): For Adults. In case of foods marketed as 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 the 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): The candidate substance in FGE.414.
(d): Supporting substances for which only normal use levels have been provided.
(e): Food categories from 14.2.2 to 14.2.7 according to sub-categories of Codex GSFA (http://www.fao.org/gsfaonline/foods/index.html).

Appendix C – Genotoxicity studies

Table C.1: Summary of *in vitro* genotoxicity data for [FL-no: 05.229]

Chemical name FL-no	Test system in vitro	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
2-Hydroxy-4-methoxybenzaldehyde [FL-no: 05.229]	Bacterial Reverse Mutation	*Salmonella* Typhimurium TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2 uvrA (pKM101)	1.6–5,000 μg/plate[(a),(b)]	Negative	Gentronix (2019a)	Study performed in accordance with OECD TG 471 and in compliance with GLP
	Micronucleus Induction	Human peripheral blood lymphocytes	1,317–2,963 μmol/L[(c)] 1,975–4,444 μmol/L[(d)] 451.6–601.1 μmol/L[(e)]	Negative	Gentronix (2019b)	Study performed in accordance with OECD TG 487 and in compliance with GLP; the given concentrations are those for the cultures that were scored for micronuclei

FL-no: FLAVIS number; OECD: Organisation for Economic Co-operation and Development; GLP: Good Laboratory Practice.
(a): With and without metabolic activation.
(b): Two experiments performed using the plate incorporation method.
(c): 3 h incubation with 21 h recovery period, without metabolic activation.
(d): 3 h incubation with 21 h recovery period, with metabolic activation.
(e): 24 h incubation with no recovery period, without metabolic activation.

Appendix D – Anticipated routes of metabolism for 2-hydroxy-4-methoxybenzaldehyde

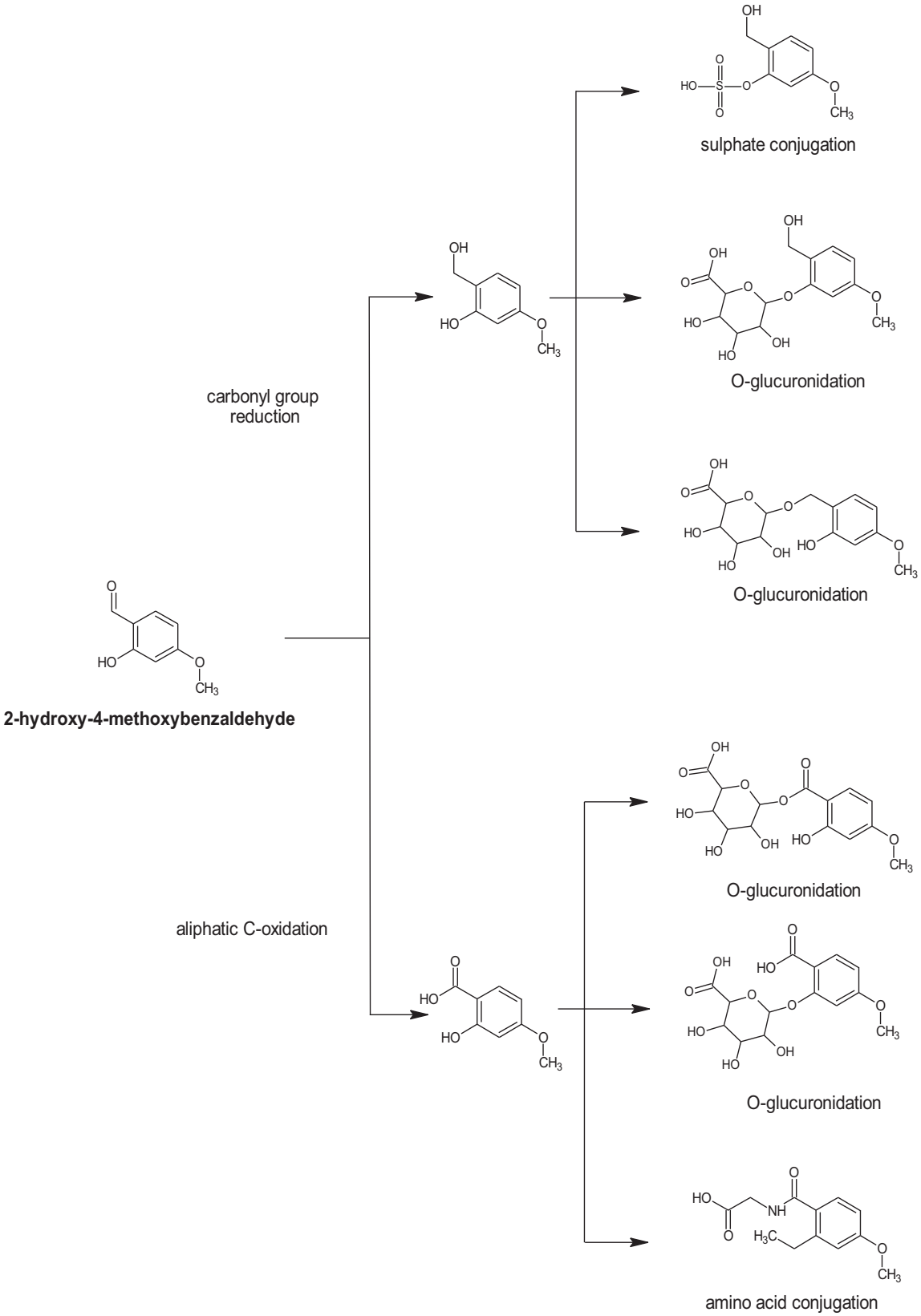


Figure D.1: Anticipated routes of metabolism for 2-hydroxy-4-methoxybenzaldehyde