Safety Assessment: Outcome of assessment on approval of 2-Hydroxy-4-methoxybenzaldehyde for use as a new flavouring

Reference number RP1466

Food Standards Agency (FSA) and Food Standards Scotland (FSS)

Regulated Product Dossier Assessment

Assessment finalised: 15/11/2023

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Summary

An application was submitted to the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in February 2022 from Firmenich S.A. (Meyrin, Switzerland) for the approval of use of 2-hydroxy-4-methoxybenzaldehyde as a flavouring substance in or on food (Application reference: RP1466). This Application is being considered within the food flavourings regime.

The Applicant intends to use the Candidate Substance as a flavouring ingredient in accordance with Article 4 of assimilated Regulation (EC) No 1334/200.
The Candidate Substance (2-hydroxy-4-methoxybenzaldehyde; CAS 673-22- 3) is a pale yellow to orange–pink solid powder that imparts an odour/flavour with a vanilla bean almond-like note at ambient conditions.
To support the FSA and FSS in undertaking this safety assessment the Additives, Enzymes and other regulated products Joint Expert Group (AEJEG) were asked to provide their risk assessment advice and conclusions on the information provided within the dossier and the supplementary information from the applicant. The AEJEG concluded that 2-hydroxy-4-methoxybenzaldehyde is safe under the proposed conditions of use. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) also reviewed the AEJEG safety assessment agreeing with the conclusions of the AEJEG.

The views of the AEJEG and COT have been taken into account in this safety assessment which represents the conclusions of the FSA and FSS on the Candidate Substance (2-hydroxy-4-methoxybenzaldehyde) as a flavouring ingredient.

1. Introduction

The FSA and FSS have undertaken a safety assessment for the approval of use (Application reference: RP1466) of 2-hydroxy-4-methoxybenzaldehyde from, Firmenich S.A. (Meyrin, Switzerland) ("the Applicant") under the food flavouring legislation. The Applicant intends to request the approval of the Candidate Substance as a flavouring substance in or on food. To support the safety assessment by FSA and FSS, the AEJEG has provided advice to the FSA and FSS, outlined in this document.

The dossier was evaluated in line with Article 3 of assimilated Regulation 1331/2008 (Reg 1331/2008) which established a common authorisation procedure for food additives, food enzymes and food flavourings. This, and the guidance put in place by EFSA for food flavourings applications, has formed the basis and structure for the assessment (EFSA, 2010).

With thanks to the members of the AEJEG during the course of the assessment who were; Dr Allain Bueno, Dr Claude Lambré, Dr Claire Stephenson; Dr Martin Rose, Dr Olwenn Martin and Professor Qasim Chaudry.

Following review and advice by the AEJEG at their meeting in December 2022, further information (RFI) was requested from the Applicant in order to address information gaps in the dossier and complete the risk assessment. The RFI sought information on specifications, presence of impurities, stability of the flavourings in food, existing authorisations and risk assessments, proposed use levels and dietary exposure assessment.

This document describes the conclusions and advice of the AEJEG assessment on the safety of the use of 2-hydroxy-4-methoxybenzaldehyde as a flavouring substance in or on food in line with the assimilated Regulation No 1331/2008 and assimilated Regulation No. 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods at a maximum level of 1 mg/kg. The AEJEG concluded that Candidate Substance (2-hydroxy-4 methoxybenzaldehyde (CAS 673-22-3)) is metabolised to innocuous products, so

the safety assessment was carried out through the AEJEG's procedure for assessing food flavourings, leading to the conclusion that the threshold of toxicological concern (TTC) should be used to assess the safety of the compound. The respective TTC for the Candidate Substance is 1800 µg/person/day (Cramer Class 1).

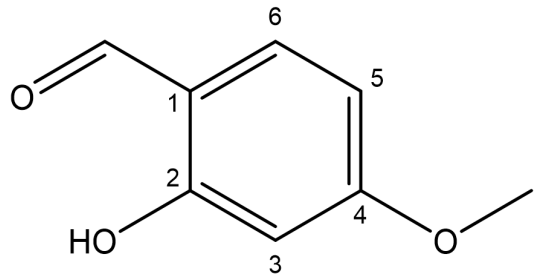
A dietary exposure assessment was performed based on the variety of foods to which the Candidate Substance will be added. The estimated human exposure to the Candidate Substance, is 36-120 µg/day, which is substantially below the TTC for Class I substances (1800 µg/day). The applicant estimated exposure using the Maximized Survey-Derived Daily Intake (MSDI) method based on annual production of 200 kg. This resulting exposure estimate was 28.5 µg/person/day, which corresponds to 0.00048 mg/kg/day. The AEJEG agreed that the critical estimated exposures are for a 1–3-year-old child, at 5.83 µg /kg bw/day for chronic exposure and 11.8 µg /kg bw day for acute exposure. Members noted that since these exposures are below the Cramer I TTC threshold of 30 µg /kg bw/day and 2-hydroxy-4-methoxybenzaldehyde is a Cramer class I compound (i.e. predicted to be of low toxicity), that the proposed uses do not represent a dietary risk for the consumer.

2. Assessment

2.1 Identity and characterisation

2-Hydroxy-4-methoxybenzaldehyde (chemical structure presented in Figure 1) is isolated from the root bark *of Periploca sepium*. The Applicant stated that "This substance is not produced by or from genetically modified organisms (GMOs)" The Applicant has supplied the following information: International Union of Pure and Applied Chemistry (IUPAC): 2-Hydroxy-4-methoxybenzaldehyde; CAS Registry Number: 673-22-3; EC number: 211-604-0; Flavor and Extract Manufacturers Association (FEMA) Generally recognized as safe (GRAS): 4435 (GRAS List 24).

Structural Formula:



Chemical Formula: $C_8H_80_3$

Molecular Weight: 152.15

Figure 1: Chemical structure of 2-Hydroxy-4-methoxybenzaldehyde as presented by the Applicant.

The Applicant has supplied upon request a statement from their quality department on residues and contaminants on the control of contaminants, pesticides, and heavy metals.

2.2 Particle Size Information

This information was provided by the Applicant in full. The Candidate Substance is produced as a powder by crystallisation. The information supplied showed that: “the maximal size of the particles is 435 µm, 50% of the particles have a size > 81 µM, (x50 = 81 µm, median distribution value) and less than 1% of the particles have a size < 4.5 µm.”

2.3 Manufacturing Process

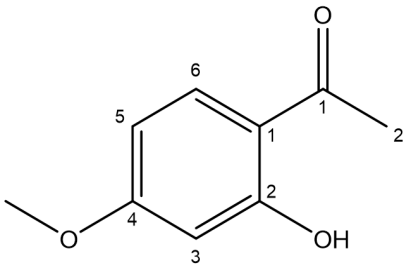
The Applicant deemed the manufacturing process confidential, as such non-confidential information provided by the Applicant has been presented. 2-Hydroxy-4-methoxybenzaldehyde is a natural flavouring substance that is found in the root of numerous varieties of plants. Pure 2-hydroxy-4-methoxybenzaldehyde is extracted

from the *Periploca sepium* plant. Members of the AEJEG were satisfied overall with the information received on the manufacturing process of the Candidate Substance.

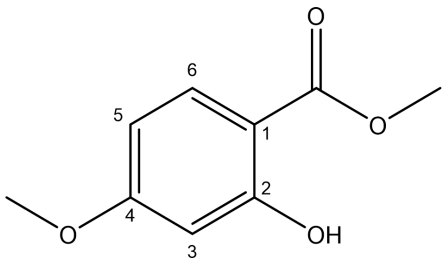
2.4 Presence of Impurities

Information on impurities was provided (Figure 2) as part of this Application. In the production process, 3 impurities were clearly identified. The Applicant provided the composition of the 2-hydroxy-4-methoxybenzaldehyde (and hence the presence and level of impurities) in percentage terms, without suppling exact amounts. The AEJEG further considered the presence of impurities and requested analytical results for 1-(2-hydroxy-4-methoxyphenyl) ethanone at < 3%. The Applicant argued that the impurity 1-(2-hydroxy-4-methoxyphenyl) ethenone is a close structural analogue of the candidate substance 2-hydroxy-4-methoxybenzaldehyde. Neither molecule has any alerts for genotoxicity, and they are not expected to be metabolized into substances of concern (DEREK Nexus v.2.5.2 (Build 5, July 2022), OASIS TIMES v2.30.1.11). Based on its chemical structure, 1-(2-hydroxy-4-methoxyphenyl) ethanone (CAS 552-41-03) is classified in structure as Cramer Class I (Cramer et al., 1978), meaning that the TTC for this compound is 1800 µg/person/day.

1) 1-(2-Hydroxy-4-methoxyphenyl)ethanone (CAS 552-41-0): <3%



2) Methyl-2-hydroxy-4-methoxybenzoate (CAS 5446-02-6): <1 %



3) Palmitic acid (CAS 57-10-3): < 1%

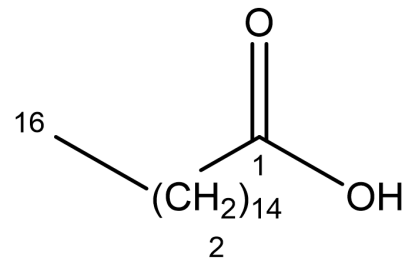


Figure 2: List of impurities as presented by the Applicant.

The Applicant concluded that based on 1) the in-silico predictions and 2) the negative in vitro genotoxicity tests (OECD 471 & OECD 487) results obtained on the Candidate Substance, which is a close structural analogue, the impurity 1-(2-hydroxy-4-methoxyphenyl) ethanone presents no safety concern with respect to genotoxicity.

The Applicant added: as the impurity 1-(2-hydroxy-4-methoxyphenyl) ethanone is present at a maximal concentration of 3% in the Candidate Substance, exposure to it from the use of the Candidate Substance will be close to two orders of magnitude (> 33 times) below the exposure to the Candidate Substance. Therefore, the presence of the impurity 1-(2-hydroxy-4-methoxyphenyl) ethanone (CAS 552-41-03) at a maximal concentration of 3% in the Candidate Substance does not present any significant safety concern.

The AEJEG was satisfied with the information provided by the Applicant and considered that based on the information provided, the presence of contaminants would not pose a risk to health.

2.5 Stability of the additive on food

Full details on the stability of 2-hydroxy-4-methoxybenzaldehyde were provided. The Applicant stated that the compound is stable at 40°C for up to one month. Other trials on use in foods did not indicate any chemical interaction with other food components. Tasting trials carried out on different foods containing the Candidate

Substance did not indicate off-notes, reduced efficacy, or any other signs of degradation or of interaction with the components of these foods over time. Members of the AEJEG were satisfied with the information received by the Applicant.

2.6 Existing authorisations and risk assessments

EFSA 2021 has carried out a safety assessment of this substance to allow the European Commission to consider its inclusion in the Union list of flavourings and source materials (Annex I of Regulation (EC) No 1334/2008). EFSA concluded that the substance “is not of safety concern when used as a flavouring substance at the intended uses and uses levels”. The Flavour and Extract Manufactures Association (FEMA) expert Panel has evaluated 2-hydroxy-4-methoxybenzaldehyde [Flavouring) FL-no: 05.229] and has concluded that the substance is: ‘Generally Regarded as Safe’ (GRAS) (Adams et al. (2005) The 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 1\z7/18 of the REACH Regulation. JECFA (The Joint FAO/WHO Expert Committee on Food Additives) has not yet evaluated 2-hydroxy-4-methoxybenzaldehyde.
Members of the AEJEG considered and took into account the information received on the existing authorisations and risk assessment section.

2.7 Dietary exposure assessment

2.7.1 Non-food sources of exposure

The Applicant explained that though *Periploca sepium* is used as a natural ingredient in the fragrance industry, consumer exposure via dermal or inhalation routes is considered negligible because exposure is an order of magnitude lesser that its threshold of toxicological concern (Cramer Class I). The Applicant stated that: “this substance has been identified as a natural constituent in numerous plant varieties some of which have been reported to be consumed as “plant-based traditional medicines” in some Asian countries including India and China [Rathi et al., 2017 in the Reference List]”. No consumption patterns in Europe have been documented, no documents were supplied on consumption data from outside Europe. The FSA / FSS requested more information on food source of exposure from other parts of the world.

The Applicant replied and reinforced that in other parts of the world, the substance is used either as a flavouring or as a chemical intermediate/precursor for the synthesis of other materials (SciFinder). It is present in various chemical inventories (e.g. Canadian Domestic Substances List (NDSL) Canada, The Toxic Substances Control Act (TSCA) US, Korean (Ecommerce)EC). The Applicant stated that no data were available for non-food sources of exposure in other regions of world. Members of the AEJEG considered the information received satisfactory.

The Applicant has estimated exposure to 2-hydroxy-4-methoxybenzaldehyde from its use as a flavouring in food using the APET (Added Portions Exposure Technique). The FSA's Exposure Assessment Team (EAT) noted that the Applicant stated that the flavouring is not intended to be used in food for toddlers (1 – 3 years) and infants. However, if the flavouring is proposed to be used in food categories that infants and toddlers are expected to consume e.g., milk and milk products, processed fruit, spread, cereals etc., then intake should be expected for infants and toddlers. The EAT team noted that this is different from use in infant specific/complementary foods, for which the applicant has stated use is not proposed. A request for additional information (RFI) was sent to clarify this point. The Applicant replied that the exposure assessment was conducted according to the referenced document (EFSA Guidance 2010) using the data required for the risk assessment of flavourings to be used in or on foods (EFSA Journal 2010; 8 (6):1623). The Applicant noted that "there is no regulatory requirement to conduct exposure assessments for children of bodyweight lower than 15 kg for other food categories. Exposure assessment for children three years old and older with a 15 kg bw was conducted". The Applicant invited the AEJEG to consider the documentation shared to answer EFSA's request for additional information.

The AEJEG noted errors in some of the numbers reported in the spreadsheet (APET Calculation sheet) resulting in some higher exposures than those provided by the Applicant (e.g., Acute Toddler Exposure at Maximum Use Level MUL = 5.4 µg /kg bw/day rather than the 4.17 µg /kg bw/day stated by the Applicant). Furthermore, the AEJEG noted errors in the selection of the worst-case food category for infants. On this basis the Applicant was requested to provide an updated calculation

spreadsheet correcting any errors in the calculated exposures, and to update the selection of food categories giving the highest exposure or otherwise providing a justification for the selection of an alternative food group. An updated APET calculation table (Table 1), providing the use levels in food categories (FCs) was provided, evaluated, and approved by the AEJEG.

Members agreed that the critical estimated exposures were for a 1–3-year-old child, at 5.83 µg /kg bw/day for chronic exposure and 11.8 µg /kg bw/day for acute. Members noted that, since these exposures are below the Cramer I TTC threshold of 30 µg /kg bw/day and 2-hydroxy-4-methoxybenzaldehyde is a Cramer class I compound, the proposed uses do not represent a dietary risk for the consumer.

Table 1. Revised APET calculations.

	Added as flavourings substance	**Added as flavourings substance**	**Other dietary sources**	**Other dietary sources**	**Combined**	**Combined**
Chronic APET	µg/kg bw/day	µg /capita/day	µg /kg bw/day	µg /capita/day	µg /kg bw/day	µg /capita/day
Adults	1.0	60.0	0	0	1.0	60.0
Children (3y)	1.6	24.6	0	0	1.6	24.6
Young children (1y/10Kg)	2.5	24.6	0	0	2.5	24.6
Acute APET	µg /kg bw/day	µg /capita/day	µg /kg bw/day	µg /capita/day	µg /kg bw/day	µg /capita/day
Adults	3.1	187.5	0	0	3.1	187.5
Children (3y)	7.9	118.1	0	0	7.9	118.1
Young Children (1y/10Kg)	11.8	118.1	0	0	11.8	118.1

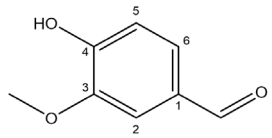
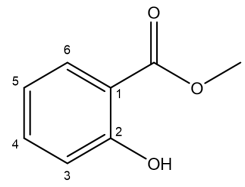
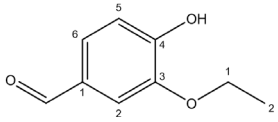
2.7.2 Cumulative Dietary Exposure

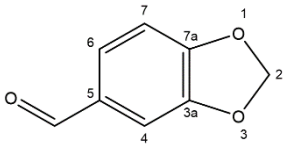
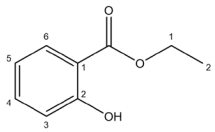
2.7.2.1 Structurally and metabolically related flavouring substances

EFSA in 2010 (updated in 2021) provided guidance on assessing the cumulative effect of flavouring ingredients in the same FGE to ensure that the capacity of the

organism to metabolize them will not be “exceeded”. EFSA concluded that the Candidate Substance should be considered a member of FGE.52. The AEJEG agreed with this categorisation. In Table 2 the Applicant has listed the five substances in FGE.52, which are structurally similar to the Candidate Substance, 2-hydroxy-4-methoxybenzaldehyde (4-methoxysalicylaldehyde), with the highest poundage data and individual MSDI values.

Table 2. Structurally and metabolically related flavouring substances with highest MSDI values

Identified flavouring substance. (FL.no, EU register name Structural Formula)	**EU MSDI (µg/capita/day) (Annual production volume – 2015)**	**USA MSDI (µg/capita/day) (Annual production volume - 2015)**
FL-no: 05.018 Vanillin (4-Hydroxy-3-methoxybenzaldehyde) 	132563 (929000 kg)	118905 (831000 kg) *
FL-no: 09.749 Methyl salicylate (Methyl-2-hydroxybenzoate) 	2397 (16800 kg)	17905 (44000 kg)
FL-no: 05.019 Ethyl Vanillin (3-Ethoxy-4-hydroxybenzaldehyde) 	10816 (75800 kg)	52983 43000 (kg)

Identified flavouring substance. (FL.no, EU register name Structural Formula)	EU MSDI (µg/capita/day) (Annual production volume – 2015)	USA MSDI (µg/capita/day) (Annual production volume - 2015)
FL-no: 05.016 Piperonal (1,3-Benzodioxole-5-carboxaldehyde) 	3795 (26600 kg)	6541 (3200 kg)
FL-n°: 09.748 Ethyl salicylate (Ethyl-2-hydroxybenzoate) 	184 (1290 kg)	4.2 (1700kg)

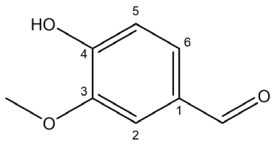
*Production volume from 2005.

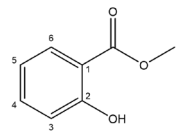
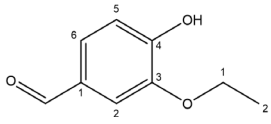
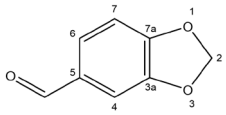
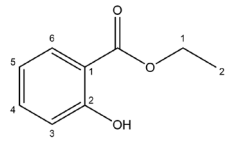
The five substances with the original highest MSDI values were identified from EFSA and WHO publications (EFSA 2008 and 2012b, WHO 2002) by the Applicant, but the values in Table 2 were updated to represent more recent (2015) annual production volumes for these chemicals. The Applicant reiterated that: “Vanillin, methyl salicylate, piperonal and ethyl salicylate are all synthesized at relatively large quantities in both the EU and US (Table 2) which supports that background exposure of humans to these substances from dietary exposure from natural sources is negligible by comparison”. In Table 3, normal occurrence levels for these substances used as added flavouring substances have been retrieved. In Table 4, an APET in adults and children has been calculated, considering each of these high poundage substances from one solid and one beverage source.

Table 3. Occurrence levels for flavouring substances structurally and metabolically related to the Candidate Substance in FGE.52 with the highest MSDI value, taken from thegoodscentscompany.com

FL-No	EU Register name	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)						
		01.0	02.0	03.0	05.0	07.0	14.1	14.2
05.018	Vanillin	0-120	0-0.2	0-95	0-270	0-220	0-63	-
05.019	Methyl salicylate	0-27	-	0-27	0-8400	0-54	0-59	-
05.016	Ethyl vanillin	0-74	-	0-47	0-200	0-63	0-20	0-100
08.132	Piperonal	-	-	-	500 2000	-	300 500	300 500
08.133	Ethyl salicylate	-	-	-	500 2000	-	300 500	300 500

Table 4. Added and combined APET for flavouring substances structurally and metabolically related to the Candidate Substance with the highest MSDI values.

Substance (FL.no, EU register name Structural Formula)	Added APET (mg/kg/bw)	Other dietary sources (mg/kg/day)	Combined APET (mg/kg/day)
FL-no: 05.018 4-Hydroxy-3-methoxybenzaldehyde (Vanillin)  	Adult: 0.58 Child: 2.33	Estimated to be negligible compared with exposure from flavours	Adult: 0.58 Child: 2.33
FL-no: 09.749 Methyl-2-hydroxybenzoate (Methyl salicylate)	Adult: 5.89 Child: 23.57	Estimated to be negligible compared with	Adult: 5.89 Child: 23.57

Substance (FL.no, EU register name Structural Formula)	Added APET (mg/kg/bw)	Other dietary sources (mg/kg/day)	Combined APET (mg/kg/day)
		exposure from flavours	
FL-no: 05.019 3-Ethoxy-4-hydroxybenzaldehyde (Ethyl Vanillin) 	Adult: 0.63 Child: 2.53	Not present in nature and estimated to be negligible compared with exposure from flavours	Adult: 0.63 Child: 2.53
FL-no: 05.016 1,3-Benzodioxole-5-carboxaldehyde (Piperonal) 	Adult: 3.83 Child: 15.33	Estimated to be negligible compared with exposure from flavours	Adult: 3.83 Child: 15.33
FL-no: 09.748 Ethyl-2-hydroxybenzoate (Ethyl-salicylate) 	Adult: 3.83 Child: 15.33	Estimated to be negligible compared with exposure from flavours	Adult: 3.83 Child: 15.33

In Table 5 the Applicant estimated the potential cumulative dietary exposure to the five structurally and metabolically related substances in FGE.52 with the highest MSDI values within one day in adults and children. The applicant added up the APET of these 5 “high poundage substances”.

Table 5. Total combined cumulative dietary exposure to structurally and metabolically related substances in FGE.52

Substance (FL.no, EU register name Structural Formula)	**Added apet (mg/kg/bw)**	**Other dietary Sources (mg/kg/bw)**	**Combined apet (mg/kg/bw)**
2-Hydroxy-4-methoxybenzaldehyde	Adult: 0.0006 Child: 0.0015	Undocumented from other dietary sources	Adult: 0.0006 Child: 0.0015
Total dietary cumulative dietary exposure from the five highest poundage substances (FGE.52)	Adult: 14.76 Child: 59.09	Estimated to be negligible compared with exposure from flavours	Adult: 14.76 Child: 59.09
Total			Adult: 14.76 Child: 59.09

The Applicant concluded that; “cumulative dietary exposure to 2-hydroxy-4-methoxybenzaldehyde was calculated to be 0.6 µg /kg bw/day for adults and 1.5 µg /kg bw/day for children”. The non-food sources of exposure to 2-hydroxy-4-methoxybenzaldehyde such as use in cosmetics or household consumer products are negligible and were considered to be zero by the Applicant.

The total dietary cumulative exposure from the Candidate Substance plus the five highest poundage substances (FGE.52) was for Adults: 14.76 (mg/kg bw/day) and Children: 59.09 (mg/kg bw/day).

In response to an EFSA request for additional information, the Applicant stated that exposure to 2-hydroxy-4-methoxybenzaldehyde in children will be 11,519 times lower than to other FGE.52 reference substances and that therefore will present no identifiable risk from dietary exposure.

The FSA/FSS asked for two further clarifications from the Applicant: A) although the Applicant stated categories like milk and dairy-based drinks, cereal, and starch-based desserts (e.g., rice pudding, tapioca pudding) produce the highest exposure, for beverages and solid food, respectively, the categories 13.3 (Dietetic foods for medical purposes), 13.4 (Dietetic foods for slimming purposes and weight reduction), and 13.5 (Dietetic foods (supplementary)) result in higher exposures. B). How the different food categories contribute to exposure portion sizes and although occurrence levels are presented no exposure estimates were presented for the categories.

The Applicant replied to both questions: A) The Applicant invited the AEJEG to consider the documentation shared with EFSA to answer a request for additional information. In particular, where a complete table of levels in food applications with corresponding exposure estimates was supplied and determination of exposure using the APET method were recalculated. B) The Applicant stated that new exposure data are now available. The new information provided contained usual and maximal levels of 2-hydroxy-4-methoxybenzaldehydes with corresponding exposures.

Overall, the AEJEG considered all the information on the exposure assessment satisfactory.

2.8 Biological and toxicological data

2.8.1 Genotoxicity studies

2.8.1.1 *In vitro* genotoxicity studies

Genotoxicity of 2-hydroxy-4-methoxybenzaldehyde was investigated using two endpoints: a GLP (good laboratory practice) bacterial reverse mutation assay (Organization for Economic Cooperation and Development (OECD TG 471), updated 1997) and an in vitro micronucleus test (OECD 487, 2016). Samples used were analysed by GC-MS prior to the studies to ensure they met the proposed specification. The 2010 EFSA flavouring guidance states “a test for induction of gene mutations in mammalian cells (preferably the mouse lymphoma tk assay) with colony

sizing; OECD Guideline 476" should be performed as required by the Scientific Committee (EFSA 2010). The Applicant stated that "bacterial reverse mutation test covers gene mutation endpoint, and the *in vitro* micronucleus test covers both structural and numerical chromosome aberration endpoints". Both tests provided negative results.

A) GLP BACTERIAL REVERSE MUTATION ASSAY

2-Hydroxy-4-methoxybenzaldehyde (Batch: Firsa_OP2_15jan2019, 95.5% pure 2-hydroxy-4-methoxybenzaldehyde) was evaluated for its mutagenic potential by measuring its ability to induce reverse mutations in *Salmonella typhimurium* LT2 strains TA1535, TA1537, TA98 and TA100, and *Escherichia coli* strain WP2uvrA/pKM101 incubated with or without liver S9 fractions from Aroclor 1245-induced rats at concentrations of up to 5000 µg/plate [OECD 471 (1997)]. The results showed no significant increase in numbers of revertant (histidine or tryptophan-independent, respectively) colonies with any of the five indicator strains either in the presence or absence of S9 mix. As the result was clearly negative, a confirmatory test was not carried out. The Applicant concluded that 2-hydroxy-4-methoxybenzaldehyde was not mutagenic using a plate incorporation method for *Salmonella typhimurium* LT2 strains TA1535, TA1537, TA98 and TA100, and *Escherichia coli* WP2 strain uvrA/pKM101, either in the presence or absence of S9 mix, when tested under the conditions used in this assay.

B) IN VITRO MICRONUCLEUS TEST

The potential of 2-hydroxy-4-methoxybenzaldehyde (Batch: Firsa_OP2_15jan2019, 95.5% pure 2-hydroxy-4-methoxybenzaldehyde) to induce micronucleus formation was tested by the Applicant in the *in vitro* micronucleus test with manual scoring on microscope slides. The test system was scheduled in accordance with the OECD Guideline 487 (2016): human lymphocytes were treated with the Candidate Substance at concentrations up to and including 10,000 µM for 3 hours in both the absence and presence of an *in vitro* activation system based on S9 fraction (liver S9 mix from Aroclor 1254-induced rat). Neither statistically significant nor dose-dependent increases in micronuclei were observed at any dose in treatment groups with or without S9.

The Applicant noted that “these results are consistent with the negative results reported for many in vitro genotoxicity studies conducted with the 5 structurally similar FGE.52 compounds (see WHO Series 48, 2014).”

Members of the AEJEG were satisfied with the information provided.

2.8.1.2 *In vivo* genotoxicity studies

The Applicant has not tested the Candidate Substance with an *in vivo* model for genotoxicity because as recommended by the EFSA Scientific Committee (EFSA 2010), no additional *in vivo* genotoxicity studies need be conducted if a Candidate Substance is negative in the two *in vitro* studies cited in the previous section. Additionally, the Applicant stated that: “three of the substances with similar structures (vanillin, ethyl vanillin, and piperonal) have been tested with different in vivo models with no evidence of genotoxicity (see WHO Series 48).” The Applicant concluded that the substance has no genotoxic potential. All the studies were considered satisfactory by the AEJEG.

2.9 Examination for structural/ metabolic similarity to a substance in an existing FGE

The Applicant has not performed metabolism studies on the substance, stating that “both JECFA and EFSA have concluded that the substances in FGE.52 are rapidly and completely metabolized to innocuous substances that are produced endogenously (WHO Series 48; JECFA 2002; EFSA 2008).” They anticipated that because of its structural similarity, 2-hydroxy-4-methoxybenzaldehyde will be oxidized into salicylate and benzoic acid derivatives by hepatic hydrolytic enzymes (Figure 3).

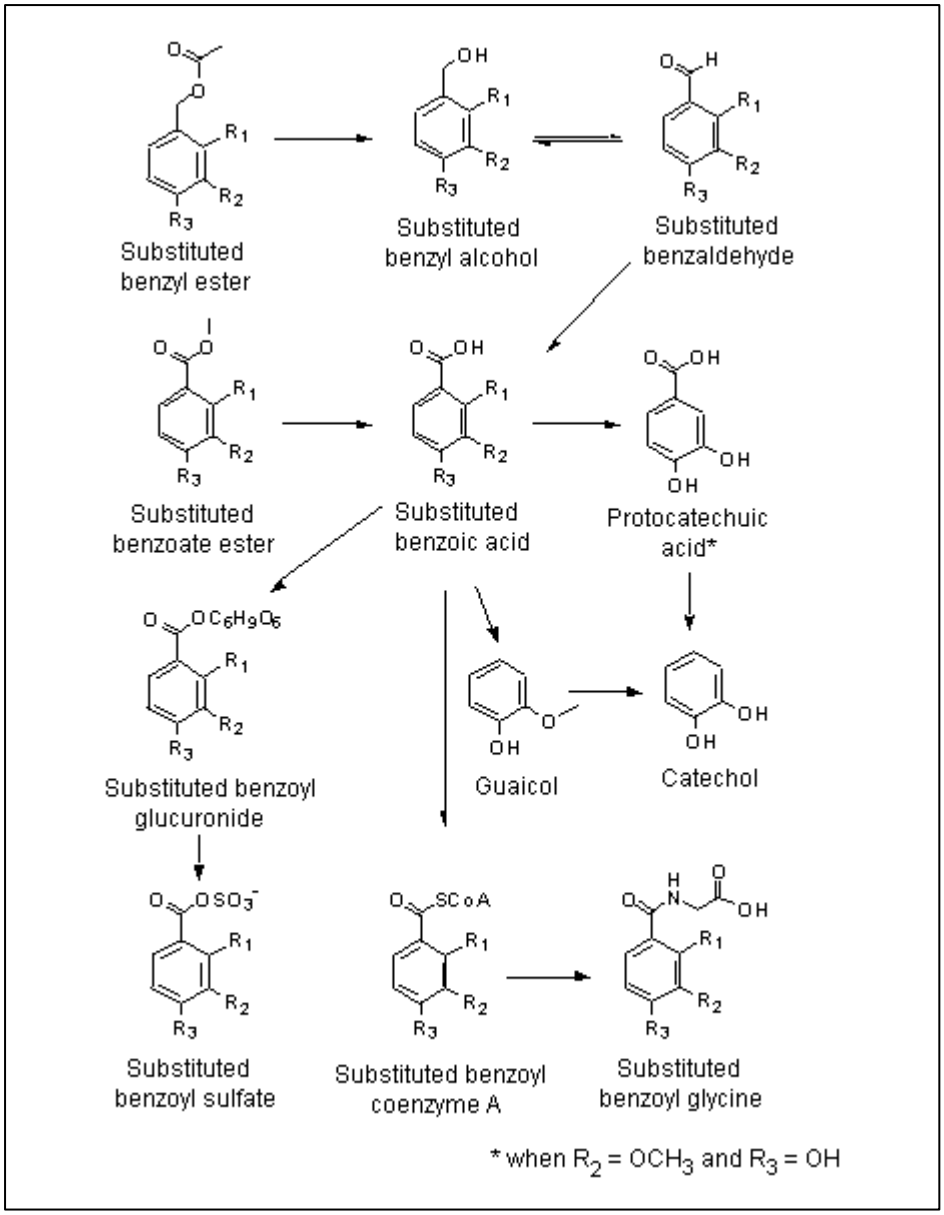


Figure 3. Metabolism of hydroxy-and alkoxy-substituted benzyl derivates (WHO Series 48)

The Applicant predicted that the Candidate Substance follows the same metabolic pathway as hydroxy-and alkoxy-substituted benzyl derivatives evaluated by JECFA both at its 57th meeting and the Flavouring Group Evaluation 52, 2002 (FGE.52). Hydroxyl-and alkoxy-substituted benzyl derivatives are rapidly absorbed by the gut and oxidized in the liver to form benzoic acid derivatives, and, to a lesser extent, reduced to their benzyl alcohol derivatives. The resulting hydroxyl-and alkoxy-benzoic acid derivatives form sulphate, glucuronide, or glycine conjugates depending

mainly on ring substitution. Methoxy-substituted benzoic acid derivatives tend to form sulphate or glucuronic acid conjugates (WHO Series 48 2014; JECFA 2002, EFSA 2008).

3. Discussion

3.1 Overall conclusions of the applicant on safety

The Applicant evaluated the Candidate Substance in accordance with the EFSA Guidance (EFSA 2010). A group-based evaluation can be conducted by assigning the candidate substance to EFSA FGE.52 (flavouring group evaluation). The applicant stated that:" The threshold of toxicological concern for the Candidate Substance (2-hydroxy-4-methoxybenzaldehyde (CAS 673-22-3) is 1800 µg/person/day. The Candidate Substance is metabolized to innocuous products, so the safety assessment was carried out through the A side of the procedure".

It was concluded by the Applicant that estimated human exposure to the Candidate Substance, based on the variety of foods to which it will be added, is 36-120 µg/ person/ day, which is well below the threshold of toxicological concern for Cramer Class I substances (1800 µg/ person/day), to which the Candidate Substance belongs. The Applicant estimated exposure using the Maximized Survey-Derived Daily Intake (MSDI) method based on annual production of 200 kg will be 28.5 µg/person/day which corresponds to 0.00048 mg/kg/day. The Applicant considered that the Candidate Substance will undergo rapid oxidation and all the metabolites are expected to be excreted in the urine. No genotoxicity alerts were noted since it produced negative results when tested with *in vitro* bacterial and mammalian genotoxicity testing methods. The Candidate Substance therefore does not present a risk for genotoxicity. From these results, the Applicant stated that: "it was determined that additional toxicology studies were not necessary to support the use of the Candidate Substance as a flavouring ingredient."

The Applicant stated that overall, the Candidate Substance is not expected to be a safety concern and no additional toxicology data was necessary to support this conclusion.

3.2 AEJEG conclusions

The AEJEG has considered the application using the key information presented above.

The AEJEG were satisfied with the information on the manufacturing process, following a request for further information, although the applicant did not supply step-by-step details for this.

The AEJEG were satisfied with the clarifications related to the exposure assessments in terms of the absence of data from other possible sources of exposure coming from the EU; updated acute and chronic exposure in adults, infants and toddlers (1 – 3 years); the exposure data for the food categories 13.3, 13.4, and 13.5 presented, and the missing values for the exposure estimates.

With regards to the toxicological information provided, the EFSA evaluation (2021) was used by the Applicant to support the safety of the Candidate Substance. The AEJEG was satisfied with the overall conclusions. on the EFSA evaluation.

The AEJEG overall concluded that the Candidate Substance is not expected to present a safety concern from the uses proposed.

4. Conclusions

The Additives and Enzymes Joint Expert Group (AEJEG) were asked to review the dossier, including all the subsequent additional information requested to the Applicant, and to advise and support the FSA and FSS in evaluating the dossier.

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) also reviewed the AEJEG advice and agreed with the conclusions of the AEJEG.

The FSA and FSS agreed with the conclusions from the AEJEG on the safety of use of a new flavouring 2-hydroxy-4-methoxybenzaldehyde for use as a flavouring

substance in or on foods, in that the candidate substance is safe under the proposed conditions of use and at the anticipated levels of intake.

The AEJEG advised the FSA and FSS that sufficient information had been provided to allow for a full evaluation of the approval of The Candidate Substance (2-hydroxy-4-methoxybenzaldehyde; CAS 673-22- 3). On advice from the AEJEG, the FSA and FSS were satisfied with the information on the manufacturing process, following a request for further information, and although the applicant did not supply a detailed step-by-step manufacturing process no further concerns were raised by the AEJEG.

The FSA and FSS therefore conclude that the new approval of use for 2-hydroxy-4-methoxybenzaldehyde is safe under the proposed conditions and at the anticipated levels of intake, as described within this safety assessment, noting that a step-by-step detail on the manufacturing process by the Applicant was not received.

5. References

Adams et al. (2005) The FEMA GRAS assessment of hydroxy-and alkoxy: substituted benzyl derivatives used as flavour ingredients. Food and Chemical Toxicology 43 1241-1271.The FEMA GRAS assessment of hydroxy-and alkoxy: substituted benzyl derivatives used as flavour ingredients

Cramer G.M., Ford R.A. and Hall R.L. (1978). Estimation of Toxic Hazard—A Decision Tree Approach. Fd Cosmet. Toxicol. Vol. 16. pp. 255-276.Estimation of Toxic Hazard—A Decision Tree Approach.

EFSA (2008). Flavouring Group Evaluation 52 (FGE.52): Consideration of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (57th meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated by EFSA in FGE.20 (2005) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal 637, 1-69.https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.637

EFSA (2010). Guidance on the data required for the risk assessment of flavourings to be used in or on food. EFSA Journal; 8(6):1623.https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2010.1623

EFSA (2012a). Scientific Opinion on Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 301. EFSA Journal; 10(12): 2994.https://www.efsa.europa.eu/en/efsajournal/pub/2994

EFSA (2012b). Proposed template to be used in drafting scientific opinion on flavouring substances (explanatory notes for guidance included). EFSA Journal; 10(1): 218.https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2012.EN-218

EFSA, 2021: Scientific opinion on flavouring group evaluation 414 (FGE.414): 2-hydroxy-4-methoxybenzaldehyde. Authors: Younes et al, EFSA Journal 2021;19(11): 6883.https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6883

JEFCA (2001) 'Evaluation of certain food additives and contaminants: fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives.' Evaluation of certain food additives and contaminants: fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives

OECD 471. Test No. 471: Bacterial Reverse Mutation Test. https://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en

OECD 487. Test No. 487: In Vitro Mammalian Cell Micronucleus Test. https://www.oecd.org/chemicalsafety/test-no-487-in-vitro-mammalian-cell-micronucleus-test-9789264264861-en.htm

OECD Guideline 476. Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes. https://www.oecd.org/chemicalsafety/test-no-476-in-vitro-mammalian-cell-gene-mutation-tests-using-the-hprt-and-xprt-genes-9789264264809-en.htm

Rathi N., et al. (2017). 2-Hydroxy-4-Methoxybenzaldehyde, an astounding food flavouring metabolite: a review. Asian Journal of Pharmaceutical and Clinical Research. Vol 10 Issue 10.2-Hydroxy-4-Methoxybenzaldehyde, an astounding food flavouring metabolite: a review.

WHO (2014). 48th report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations); WHO 48th

6. Abbreviations

°C	Celsius
ADI	Acceptable daily intake
AEJEG	Joint Expert Group on Additives, Enzymes and other Regulated products
APET	Added Portions Exposure Technique
CAS	Chemical Abstracts Service
COT	The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment.
EAT	Exposure Assessment Team
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAIM	The Food Additives Intake Model
FCs	Food categories
FEMA	Flavour and Extract Manufactures Association
FGE.52	Flavouring Group Evaluation 52
FSA	Food Standards Agency
FSS	Food Standards Scotland
GLP	Good laboratory practice
GMOs	Genetically Modified Organisms
GRAS	Generally Recognised as Safe
Hg	Mercury
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
IUPAC	International Union of Pure and Applied Chemistry
mg/kg bw/day	Milligram per kilogram body weight per day
ML	Maximum Permitted Level
mm	Millimetre
MSDI	Maximised Survey-Derived Daily Intake
MUL	Maximum Use Level

NDSL	Canadian Domestic Substances List
ng/kg bw/day	nanograms per kilogram of body weight per day
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RFI	Request for Information
SCF	Scientific Committee on Food
TTC	Threshold of Toxicological Concerns
TG	Test guideline
TSCA	The Toxic Substances Control Act
WHO	The World Health Organization
µg	Microgram
µg/kg bw/day	Microgram per kilogram body weight per day
µm	Micrometres

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