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Safety and efficacy of a feed additive consisting of expressed mandarin oil from the fruit peels of *Citrus reticulata* Blanco for use in all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of expressed mandarin oil from the fruit peels of *Citrus reticulata* Blanco, when used as a sensory additive (flavouring) in feed and water for drinking for all animal species. The FEEDAP Panel concluded that the essential oil under assessment is safe up to the maximum proposed use levels in complete feed of 15 mg/kg for poultry, 33 mg/kg for pigs, 30 mg/kg for ruminants, 40 mg/kg for horses, and 15 mg/kg for fish and rabbits. The presence of perillaldehyde was identified as a source of potential concern. However, in target species fed citrus by-products as part of daily feed the use of the expressed mandarin oil in feed was not expected to increase the exposure to perillaldehyde to a relevant extent (< 4%). For dogs and cats and ornamental fish not normally exposed to citrus by-products, no conclusion can be drawn. The FEEDAP Panel considered that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. No concerns for consumer safety were identified following the use of the additive up to the maximum proposed use level in feed. The essential oil under assessment should be considered as irritant to skin, eyes and the respiratory tract, and as a skin sensitiser. The use of the additive in animal feed under the proposed conditions of use was not expected to pose a risk for the environment. Expressed mandarin oil was recognised to flavour food. Since its function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

Keywords: sensory additives, flavouring compounds, *Citrus reticulata* Blanco, expressed mandarin oil, d-limonene, perillaldehyde, polymethoxylated flavones

Requestor: European Commission

Question number: EFSA-Q-2021-00143

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Erratum: A wrong conversion was applied to derive the no observed adverse effect (NOAEL) values for nobiletin and tangeretin from the 90-day toxicity study by Nakajima et al. (2020). The wrong values of 60 and 34 mg/kg bw per day have been replaced with the correct values of 38 and 16 mg/kg bw per day for nobiletin and tangeritin, respectivley. The calculated values for maximum safe intake/feed concentration in Table 6 were amended accordingly. In addition, the wording "companion animals" was wrongly used in the conclusions and changed into "dogs, cats". The corrections have no impact on the *outcome of this scientific output. To avoid confusion, the original version of the output has been removed from the EFSA Journal, but is available on request*.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. In addition, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 20 preparations (namely, buchu leaves oil, amyris oil, olibanum extract (water based, wb), olibanum tincture, lime oil, neroli bigarade oil, petitgrain bigarade oil, petitgrain bigarade absolute, bitter orange extract of the whole fruit, lemon oil expressed, lemon oil distilled, orange oil, orange terpenes, mandarin oil, mandarin terpenes, grapefruit oil expressed, grapefruit extract (sb), grapefruit extract, quebracho extract (wb), cashew oil), belonging to botanically defined group (BDG) 8 - *Sapindales*, when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for nine preparations.[3,4] These preparations are excluded from the present assessment. In addition, during the course of the assessment, the application was split and the present opinion covers only one out of the 20 initial preparations under application: expressed mandarin oil from *Citrus reticulata* Blanco for all animal species.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 19 March 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of expressed mandarin oil from *C. reticulata* Blanco, when used under the proposed conditions of use (see Section 3.2.4).

The remaining ten preparations belonging to botanically defined group (BDG) 8 - *Sapindales* under application are assessed in separate opinions.

1.2. Additional information

Mandarin, Tangerine terpenes (CoE142) from *C. reticulata* Blanco is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined). It has not been assessed as a feed additive in the EU.

There is no specific EU authorisation for any *C. reticulata* Blanco preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[5] flavouring preparations produced from food, may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer'.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] On 13/3/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1050 Brussels, Belgium.
[3] On 27 February 2019, EFSA was informed about the withdrawal of the application on amyris oil, neroli bigarade oil, petitgrain bigarade absolute, mandarin terpenes, grapefruit oil expressed, grapefruit extract (sb), grapefruit extract, cashew oil.
[4] On 2 April 2021, EFSA was informed by the applicant about the withdrawal of the application on olibanum tincture.
[5] 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 Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

'Mandarin oil' is described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020). It is defined as the essential oil obtained without heating, by suitable mechanical treatment, from the peel of the fresh fruit of *Citrus reticulata* Blanco.

Many of the individual components of expressed mandarin oil have been already assessed as chemically defined flavourings for use in feed and food by the EFSA FEEDAP Panel, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) and the EFSA Panel on Food Additives and Flavourings (FAF). The list of flavouring compounds together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[6] and the corresponding EFSA opinion is given in Table 1.

Table 1: Flavouring compounds already assessed by EFSA as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA opinion. They are currently authorised for food[7] and feed[8] uses unless otherwise indicated

CG	Chemical group	Product – EU register name (common name)	FLAVIS No	EFSA opinion,* Year
01	Straight-chain primary aliphatic alcohols/ aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes	Octan-1-ol	02.006	2013
		Octanal	05.009	
		Decanal	05.010	
		Dodecanal	05.011	
03	a, ß-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/ acids, acetals and esters	Neral	05.170	2016a
		trans-3,7-Dimethylocta-2,6-dienal (geranial)	05.188	
04	Non-conjugated and accumulated unsaturated straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids, acetals and esters	Citronellol	02.011	2016b
		Citronellal	05.021	
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		4-Terpinenol	02.072	
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Sabinene hydrate	02.085	JECFA
		Carvone[(a)]	07.012	2014, SC
13	Furanones and tetrahydrofurfuryl derivatives	Linalool oxide[(b)]	13.140	2012b
25	Phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group	Thymol	04.006	2012c
27	Anthranilate derivatives	Methyl-*N*-methyl anthranilate	09.781	2011

[6] 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 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8.

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

[8] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf

CG	Chemical group	Product – EU register name (common name)	FLAVIS No	EFSA opinion,* Year
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	1-Isopropyl-4-methylbenzene (*p*-cymene)	01.002	2015
		Terpinolene	01.005	
		α-Phellandrene	01.006	
		1-Isopropenyl-4-methylbenzene	01.010	
		α-Terpinene	01.019	
		γ-Terpinene	01.020	
		d-Limonene	01.045	
		l-Limonene	01.046	
		Pin-2(10)-ene (β-pinene)	01.003	2016c
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		3,7-Dimethyl-1,3,6-octatriene (β-ocimene)[(c)]	01.018	
		δ-3-Carene	01.029	
		δ-Cadinene[(a),(d)]	01.021	2011, CEF
		β-Cubebene[(a),(d)]	01.030	
		3,7,10-Humulatriene[(a),(d)]	01.043	
		β-Phellandrene[(a),(d)]	01.055	
		1,1,7-trimethyltricyclo [2.2.1.0.(2.6)]heptane (tricyclene)[(a),(d)]	01.060	
		α-Farnesene[(a)]	01.040	2015a, CEF
		4(10)-Thujene (Sabinene)[(a)]	01.059	2015b, CEF

*: FEEDAP opinion unless otherwise indicated.
(a): Evaluated for use in food. According to Regulation (EC) 1565/2000, flavourings evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) before 2000 are not required to be re-evaluated by EFSA.
(b): A mixture of cis- and trans-linalool oxide (5-ring) was evaluated [13.140].
(c): β-Ocimene [01.018]: as a mixture of (*E*)- and (*Z*)-isomers, containing 50-70% (*E*)-isomer and 17-17% (*Z*)-isomer, was evaluated.
(d): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). No longer authorised for use as flavours in food.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[9] in support of the authorisation request for the use of expressed mandarin oil from *C. reticulata* Blanco as a feed additive.

The FEEDAP Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as previous risk

[9] FEED dossier reference: FAD-2010-0322.

assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports, and experts' knowledge, to deliver the present output.

Many of the components of the essential oil under assessment have been already evaluated by the EFSA FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to refer to the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 8.[10]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in the additives. The Executive Summary of the EURL report can be found in Annex A.[11]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of expressed mandarin oil from *C. reticulata* Blanco is in line with the principles laid down in Regulation (EC) No 429/2008[12] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019c).

3. Assessment

The additive under assessment, expressed mandarin oil, is obtained from the fruit peel of *Citrus reticulata* Blanco. It is intended for use as a sensory additive (functional group: flavouring compounds) in feed and in water for drinking for all animal species.

3.1. Origin and extraction

The taxonomy and systematics of the *Citrus* genus, belonging to the Rutaceae family, are complex and the exact number of natural species is unclear. Almost all of the commercially important citrus fruits found today are hybrids derived from three ancestral species, one of which is now represented by the cultivars described as the mandarin or mandarin orange (*Citrus reticulata* Blanco). Tangerines and satsumas are a group of hybrids of the mandarin orange and are considered to belong to the same species.

Mandarins are thought to have originated in a region covering south China, Vietnam and Japan and then to have spread to other parts of Asia. After domestication, the many varieties of *Citrus reticulata* can now be found growing in most parts of the world with moderate to tropic climate.

Expressed mandarin oil is obtained by cold expression from the fruit peel of *Citrus reticulata* Blanco. Expression is the most commonly used method to obtain essential oils from the peel of citrus fruits, and since it does not require heat, it is often referred to as 'cold pressing'. In the mechanised process the surface of the mandarin fruit is first scarified to encourage cells containing the essential oil to break open and release their contents. Water is then sprayed over the fruit to collect the released oil and the aqueous suspension filtered to remove cell debris. Centrifugation is then used to separate the oil/water mix and to remove any fine particles.

[10] Technical dossier/Supplementary information/Letter dated 29/4/2021.
[11] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0322-bdg08.pdf
[12] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3.2. Characterisation

3.2.1. Characterisation of expressed mandarin oil

Expressed mandarin oil is a green to green-brown clear mobile liquid (green mandarin oil) or an orange to red amber clear mobile liquid (red mandarin oil), with a characteristic aroma. In ten batches of the additive (five of green mandarin oil and five of red mandarin oil, all originating from Italy except one of green mandarin oil originating from Brazil), the optical rotation at 20°C ranged between + 65.8° and + 74.7° (specification: + 61.5° to + 81.5°), refractive index between 1.470 and 1.480 (specification: 1.470–1.480) and the density at 20°C ranged between 0.845 and 0.854 kg/L (specification: 0.844–0.859).[13] Expressed mandarin oil is identified with the single Chemical Abstracts Service (CAS) number 8008-31-9, the Flavor Extract Manufacturers Association (FEMA) 2657 and the Council of Europe (CoE) number 142.

Volatile components

The product specifications are based on the standards developed by the International Organisation for Standardization (ISO) 3528:2012 for essential oil of mandarin, Italian origin of *C. reticulata*, which were adapted to reflect the concentrations of the main volatile components, analysed by gas chromatography with flame ionisation detection (GC-FID) and expressed as % of gas chromatographic peak area (% GC area). These components are d-limonene (65–80%, the phytochemical marker), γ-terpinene (13–22%), pin-2(3)-ene (hereinafter referred as to α-pinene, 1.0–3.5%), myrcene (1.0–2.0%), pin-2(10)-ene (hereinafter referred as to β-pinene, 1.0–2.0%) and methyl *N*-methyl anthranilate (0.15–0.7%). Analysis of ten batches of the additive by GC-FID showed compliance with these specifications.[14] When analysed by gas chromatography–mass spectrometry (GC–MS) these six compounds account for about 96.1% on average (range 91.7–97.0%) of the % GC area (Table 2).

Table 2: Volatile constituents of the essential oil from the fruit peels of *Citrus reticulata* Blanco as defined by the ISO standard (3528:2012): specifications and batch to batch variation based on the analysis of 10 batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent			% GC area[(b)]		
EU register name	**CAS no**	**FLAVIS no**	**Specification**	**Mean[(a)]**	**Range**
d-Limonene	5989-27-5	01.045	65–80	65.63	56.4–68.6
γ-Terpinene	99-85-4	01.020	13–22	21.85	20.5–24.7
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	1.0–3.5	3.60	2.60–4.51
Myrcene	125-35-3	01.008	1.0–2.0	2.11	1.54–2.32
β-Pinene (pin-2(10)-ene)	127-91-3	01.003	1.0–2.0	1.84	1.33–2.31
Methyl *N*-methyl anthranilate	85-91-6	09.781	0.15–0.7	1.04	0.51–1.59
Total				96.1	91.7–97.0

EU: European Union; CAS no: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Mean calculated on ten batches.
(b): Differences in the values determined by GC with different detectors are due to the fact that GC-MS method underestimates d-limonene, the major component, and consequently the other components are higher, as they are expressed as percentage of the corresponding chromatographic peak area (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

The applicant provided the full characterisation of the volatile constituents in ten batches obtained by GC-MS.[15] In total, up to 88 constituents were detected, 47 of which were identified and accounted on average for 99.7% (99.6–99.9%) of the % GC area. Besides the six compounds indicated in the product specifications, ten other compounds were detected at individual levels > 0.1% (on average) and are listed in Table 3. These 16 compounds > 0.1% together, account on average for 99.1% (97.9–

[13] Technical dossier/Supplementary information August 2019/Annex_II_ SIn_Reply_mandarin_oil_CoA.
[14] Technical dossier/Supplementary information August 2019/SIn reply_BDG08_mandarin_oil/GC-FID analysis: d-limonene (65.6–75.2%, γ-terpinene (17.3–21.7%), α-pinene (2.21–3.19%), myrcene (1.65–1.81%), β-pinene (1.25–1.73%) and methyl N-methyl anthranilate (0.20–0.66%.
[15] Technical dossier/Supplementary information August 2019/Annex_III_SIn _Reply_mandarin_oil_expressed_chromatograms.

99.5%) of the % GC area. The remaining 31 compounds (ranging between 0.002% and 0.1%) and accounting for 0.64% are listed in the footnote.[16]

Table 3: Other volatile constituents of the essential oil from the fruit peels of *Citrus reticulata* Blanco accounting on average for > 0.1% of the composition (based on the analysis of 10 batches) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent			% GC area	
EU register name	**CAS no**	**FLAVIS no**	**Mean[(a)]**	**Range**
p-Cymene (1-isopropyl-4-methylbenzene)	99-87-6	01.002	0.61	0.50–0.80
Terpinolene	586-62-9	01.005	0.60	0.35–1.49
Sabinene (4(10)-thujene)	3387-41-5	01.059	0.55	0.30–0.88
β-Phellandrene	555-10-2	01.055	0.32	0.13–0.43
α-Terpinene	99-86-5	01.019	0.30	0.17–0.76
Octanal	124-13-0	05.009	0.16	0.08–0.22
Linalool	78-70-6	02.013	0.15	0.08–0.26
α-Sinensal	17909-77-2	05.130	0.13	0.001–0.84
α-Terpineol	98-55-5	02.014	0.13	0.05–0.53
α-Farnesene	502-61-4	01.040	0.11	0.002–0.69
Total			3.05	2.45–6.13

EU: European Union; CAS no: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Mean calculated on 10 batches.

Expressed mandarin oil also contains *p*-mentha-1,8-dien-7-al (hereinafter referred as to perillaldehyde) at an average concentration of 0.018% (range: 0.008–0.063%), a substance for which EFSA has identified previously a concern for genotoxicity (EFSA CEF Panel, 2015c).

Non-volatile components

The non-volatile residue (residue on evaporation) of expressed mandarin oil accounts for 1.6–4.0% of the oil according to the European Pharmacopoeia (PhEur, 2020). The applicant performed a literature search to identify the relative percentage and the composition of the non-volatile fraction in expressed mandarin oil.[17] Non-volatile predominantly constituents include polymethoxylated flavones (PMF), e.g. tangeretin (0.2–0.5%), nobiletin (0.07–0.25%) and heptamethoxyflavone (0.05–0.15%), (ranges based on Verzera et al., 1997; Feger et al., 2003; Schipilliti et al., 2010; Dugo and Russo, 2010), and carotenoid esters (0.03–0.42%, as reported by Giuffrida et al., 2006 and Castro et al., 2018), mainly β-cryptoxanthin and its laurate, myristate and palmitate esters (Giuffrida et al., 2006; Zerlotti Mercadante et al., 2017).

The presence of 8-methoxypsoralen (xanthotoxin), a furocoumarin, has been reported in the EFSA Compendium (EFSA, 2012) as chemical of concern for the essential oil of the fruits of *C. reticulata*.[18] Analysis of the ten batches[19] showed that 8-methoxypsoralen was below the limit of detection (LOD) in all sample, when determined by high-performance liquid chromatography (HPLC) with UV detection (0.5 mg/kg). The literature search provided by the applicant showed that only the furocoumarins bergamottin (0.001%) and 5-methoxypsoralen (bergapten, 0.0003%) were reported to occur in very low amounts (analytical values according to the evaluation of furocoumarins in cosmetic products (SCCP, 2005 as reported by Tisserand and Young, 2014). The phylogenetic analysis by Dugrand-Judek et al. (2015) on *Citrus* species confirmed that mandarins have only a low capacity to synthesise

[16] Additional constituents: constituents (n = 3) between < 0.1 and ≥ 0.05%: δ-3-carene, α-phellandrene, thymol; constituents (n = 16) between < 0.05 and > 0.01%: 4-terpinenol, decanal, cis-sabinene hydrate, β-caryophyllene, *cis*-linalool oxide, camphene, trans-sabinene hydrate, nonanal, octan-1-ol, citronellal, *p*-mentha-1,8-dien-7-al (perillaldehyde), *trans*-3,7-dimethylocta-2,6-dienal (geranial), fenchone, citronellol, cis-limonene epoxide, β-ocimene; constituents (n = 12) < 0.01%: *trans*-limonene epoxide, l-carvone, α-copaene, neral, δ-cadinene, 1,1,7-trimethyltricyclo[2.2.1.0.(2.6)]heptane, 3,7,10-humulatriene, l-camphor, α-selinene, β-cubebene, α-fenchene, 1-isopropenyl-4-methylbenzene.
[17] Technical dossier/Supplementary information July 2020/Request of clarification_Annex_IV_non volatile search.
[18] Technical dossier/Supplementary information August 2019/Literature search_mandarin_oil.
[19] Technical dossier/Supplementary information August 2019/Annex VI_Sin reply_mandarin_oil_SOC_COA.

coumarin/furocoumarins, whereas the other ancestral taxa (pummelos, citrons and papedas) and their descendant species synthesise them in high amounts.

3.2.2. Impurities

Data on chemical and microbial impurities were provided in at least three batches of green mandarin oil and three batches of red mandarin oil.[20] The concentrations of heavy metals were below the corresponding limit of quantification (LOQ) in all the batches. In the same batches, aflatoxins B1, B2, G1 and G2 were below the LOQ and pesticides were not detected in a multiresidue analysis with the exception of chlorpyrifos-ethyl in two batches of green mandarin oil (0.12 and 0.21 mg/kg) and chlorpyrifos-methyl in one batch of green mandarin oil (0.15 mg/kg). In six batches, polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and dioxin-like polychlorinated biphenyls (PCBs) were below the corresponding LOQ and the calculated upper bond for the sum of WHO (2005) PCCD/F+PCB TEQ ranged between 1.62 and 1.83 pg/g wet weight. None of the data on chemical impurities raised concerns.

Analyses of microbial contamination (six batches) indicated that *Salmonella* spp. was not detected in 25 g, and total viable counts and numbers of Enterobacteriaceae, yeasts, moulds were < 10 colony forming unit (CFU)/g.

3.2.3. Shelf-life

The typical shelf-life of expressed mandarin oil is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light).[21]

3.2.4. Conditions of use

Expressed mandarin oil is intended to be added to feed for all animal species without a withdrawal time. The maximum proposed use level in complete feed is 15 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 33 mg/kg for piglets, pigs for fattening and sows, 30 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goat, 40 mg/kg for horses, 15 mg/kg for rabbits, fish, dogs, cats and ornamental fish.

No use level has been proposed by the applicant for the use in water for drinking.

3.3. Safety

The assessment of safety is based on the maximum use levels proposed by the applicant.

Many of the major volatile components of expressed mandarin oil, accounting for about 99% of the % GC areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food[7] and feed[8] uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Four compounds, δ-cadinene [01.021], β-cubebene [01.030], 3,7,10-humulatriene [01.043], β-phellandrene [01.055] and tricyclene [01.060] have been evaluated in FGE25.Rev2 by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011). In the absence of such data, the EFSA CEF Panel was unable to complete its assessment. As a result, these compounds are not authorised for use as flavours in food. In the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach or read-across from structurally related substances. For α-sinensal [05.130] the request of additional genotoxicity data was not addressed (EFSA CEF Panel, 2012), and the compound is no longer authorised.

Several volatile components accounting for < 0.5% of the % GC area (*cis*-sabinene hydrate, fenchone, l-camphor, α-selinene, α-copaene, α-fenchene, α-sinensal, *cis*- and *trans*-limonene epoxide) have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and 8 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected

[20] Technical dossier/Supplementary information August 2019/Annex VII_Sin reply_mandarin-oil_impurities_COA. Limit of quantification (LOQ) in mg/kg for heavy metals and arsenic: 0.005 for mercury, 0.01 for cadmium, 0.05 for lead and 0.1 for arsenic LOQ for individual pesticides: 0.1 mg/kg; LOQ for mycotoxins: < 1 μg/kg for aflatoxins B1, B2, G1 and G2.

[21] Technical dossier/Section II.

to be rapidly absorbed from the gastro-intestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2016c,d).

The compounds were screened with the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox and no alert was identified for *in vitro* mutagenicity, for genotoxic and non-genotoxic carcinogenicity and for other toxicity endpoints or discounted based on read-across.[22] The genotoxicity of (+)-limonene epoxide, investigated in the Ames test and the SOS Chromotest, gave negative results (Basler et al., 1989 as referenced in EFSA CEF Panel, 2014). When V79 Chinese hamster cells were incubated with (+)-limonene epoxide, no increase in sister chromatid exchange was observed (von der Hude et al., 1991, as referenced in EFSA CEF Panel, 2014).

Other components like fatty acids, whose presence in mandarin oils has been reported in the literature (see Section 3.2) are ubiquitous in natural feed and foods and not further addressed.

Furocoumarins, occur in mandarins only in trace amounts as shown by phylogenetic analysis (Dugrand-Judek et al., 2015; see Section 3.2). Based on the safety evaluation of furocoumarins documented in the EFSA opinion on expressed lemon oil and its fractions and on lime oil (EFSA FEEDAP Panel, 2021) and considering their concentration in mandarin oils, furocoumarins are also not further considered.

The following sections focus on those compounds not previously assessed or not structurally related to flavourings previously assessed, perillaldehyde and polymethoxylated flavones (PMF), based on the evidence provided by the applicant in the form of several literature searches.[23]

3.3.1. Absorption, distribution, metabolism and excretion

Volatile components

Perillaldehyde is rapidly metabolised, largely by oxidation of the side chain to a carboxylic acid, which is excreted unchanged or as its conjugates (WHO, 2003). Perillaldehyde is also an intermediate metabolite arising from the oxidation of the methyl side chain of limonene to perillic acid and dihydroperillic acid, which are further conjugated with glucuronic acid and excreted as perillyl-glucuronide and dihydroperillyl-glucuronide (EFSA FEEDAP Panel, 2015).

Non-volatile components

The fraction of non-volatile compounds was not analysed by the applicant. According to the literature search performed by the applicant, this fraction contains between 0.1 and 0.9% of the PMF tangeretin (pentamethoxy-flavone), nobilitin (hexamethoxy-flavone) and heptamethoxy-flavone.

Absorption, distribution, metabolism and excretion (ADME) data in experimental animals are available in the literature.

After oral administration of 50 mg/kg body weight (bw) tangeretin to rats, maximum plasma concentrations of 0.9 μg/mL were achieved. The half-life was 5.6 h and the absorption rate 27%. Tangeretin was detected in all vital organs and was excreted in urine and faeces mainly as metabolites (not identified) (Hung et al., 2018). In another study (Nielsen et al., 2000), the metabolites of tangeretin in urine and faeces of rats after repeated administration of 100 mg/kg bw for 14 days were identified as demethylated or hydroxylated derivatives of the parent compound and metabolic changes were found primarily to occur in the 4′ position of the B-ring. The total urinary excretion of tangeretin metabolites with intact flavan nucleus was about 11% of the administered daily dose. About 75% of all metabolites were excreted in faeces, and 7% as intact tangeretin.

The metabolites of nobilitin and tangeretin were isolated in urine of rats receiving a diet containing 1% (w/w) of a nobiletin/tangeretin mixture (1:3 w/w), during 4 consecutive weeks (Manthey et al., 2011). Eight demethylated metabolites of nobiletin and two demethylated metabolytes of tangeretin were detected in the pooled urine collected in the last 3 days of every week of the assay. In parallel, the authors gave by gavage 50 mg/kg bw of tangeretin or nobiletin to rats and analysed the serum by

[22] Technical dossier/Supplementary information August 2019/Annex X_SIn_reply_Mandarin_oil_QSAR. Structural alerts for α-sinensal were due to the presence of aldehydes and to the presence of the epoxide for *cis*- and *trans*-limonene epoxide. In both cases, predictions of Ames mutagenicity was made by 'read-across' analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers as well as empirical profilers. Ames test (with and without S9) read across predictions were found negative for all categories of analogues. On this basis, the alerts raised for sinensal and *cis*- and *trans*-limonene epoxide were discounted.

[23] Technical dossier/Supplementary information/July 2020 and Supplementary information November 2020.

liquid chromatography–mass spectrometry (LC–MS) 24 h after administration. In addition to the parent compounds, two metabolites of tangeretin and eight metabolites of nobilitin were detected with identical oral doses, nearly a ten-fold higher absorption of nobilitin occurred compared to tangeretin. For both compounds, maximum levels of glucuronidated metabolites occurred in the blood serum at later time points ($\sim$ 5–8 h) compared to the earlier T(max) values for nobilitin and tangeretin. In most cases, the glucuronides occurred at substantially higher concentrations than the aglycone metabolites. Low levels of nobiletin and tangeretin and their metabolites were detectable in rat blood serum even at 24 h after treatment.

The tissue distribution of nobiletin was investigated in SD-rats after intragastric intubation of 67.1 μmol/kg bw (Murakami et al., 2002). Concentrations in tissues were measured at 1, 4, and 24 h after administration. Nobiletin showed a tendency to be retained from 1 to 4 h in the intestinal membrane, in liver (from 5.5 to 4.3 nmol/g) and kidney (from 2.0 to 4.2 nmol/g). Twenty-four hours after administration, nobiletin or its conjugate metabolites were no longer detected in these organs (LOD not given). The tissue retention of nobiletin caused a prolonged excretion phase. Nobiletin was completely excreted in urine, mainly as conjugate demethylated derivatives. The spectrum of metabolites of nobiletin isolated from the urine and serum after treatment with glucuronidase/sulfatase consisted of mono- (DMN) and di-demethylated nobiletin (DDMN) metabolites (three types of DMN, including 3′- DMN and two DDMN types were identified in urine, as well as 3′-DMN in serum by LC–MS analysis).

The bioavailability, metabolism and excretion of nobiletin after a single oral dose (100 mg/kg by gavage) or repeated-dose administration (15 days) was evaluated in obese and lean rats (Zhang et al., 2020). After the single oral dose, the liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis of urine and faeces showed an extensive metabolism of nobiletin and three DMN and two DDMN metabolites were identified in both samples collected from 0 to 48 h after dosing. In the repeated-dose protocol, blood samples were collected at 6 h after oral administration of nobiletin and at day 1, 3, 6, 9, 12 and 15 and faeces 12 h after oral administration at day 2, 5, 8, 11 and 14. The profile of plasma metabolites had a similar pattern as the urinary metabolites, being 4′-DMN the predominant demethylated metabolite during the 15-day consecutive dosing study. The metabolite profiles for lean and obese rats did not have a significant difference at the selected time points. The demethylation of nobiletin by gut microbiota occurred at several positions in the molecule as shown by the formation of 3′-DMN, 4′-DMN, 3′,4′-DDMN and 4′,5-DDMN at similar concentrations in faeces during the 2 weeks of administration. The absolute oral bioavailability of nobiletin was similar in both lean and obese rats (mean 20%). By comparing the demethylated metabolite profiles in the urine and faeces, the authors attributed an appreciable role of gut microbiota in the biotransformation of nobiletin. The consecutive dosing of nobiletin might lead to a higher extent of demethylated metabolites in the plasma and in faeces.

Also *in vitro* studies were performed to identify the enzymes responsible for the phase I metabolic reactions of tangeretin and nobiletin. *In vitro* incubation of tangeretin with recombinant cytochrome P450 (CYP450) 1A2, 3A4, 2C9 or 2D6 enzymes, derived from human liver microsomes expressed in *Escherichia coli* resulted in the formation of 4′-hydroxy-5,6,7,8-tetramethoxyflavone and 5,6-dihydroxy-4′,7,8-trimethoxyflavone. CYP 1A2 was shown to be the principal enzyme responsible for demethylation, mainly occurring in the B ring, in position 4′ (Breinholt et al., 2003). In a similar experiment, nobiletin was mono-demethylated in position 4′, 6 and 7 by incubation with human liver microsomes. Out of the 12 recombinant human CYPs tested, CYP1A2 and CYP3A4 were shown to be the key enzymes mediating the oxidative demethylation of nobiletin in the B-ring and A-ring, respectively (Koga et al., 2011).

The ADME studies of PMF show that the compounds are absorbed and transformed to phase I and phase II metabolites, that are excreted both in urine and faeces. The formation of glucuronides requires demethylation reactions which cause a delay in glucuronidation and excretion and prolongation of the persistence in blood and organs.

3.3.2. Toxicology

3.3.2.1. Genotoxicity

For fully defined mixtures, the EFSA Scientific Committee (EFSA SC) recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential (EFSA Scientific Committee, 2019b).

Volatile components

Expressed mandarin oil contains perillaldehyde (average: 0.018%, range: 0.008–0.063%), a substance for which EFSA identified a concern for genotoxicity (EFSA CEF Panel, 2015c), which was confirmed by JECFA (WHO, 2018).

Non-volatile components

A mixture of PMF, containing mainly the derivatives present in the additive (i.e. nobiletin 32.5%, tangeretin 14.0% and heptamethoxyflavone 25% and other components[24]) was tested in five *Salmonella* Typhimurium strains (TA98, TA100, TA102, TA1535 and TA1537) at five concentrations ranging between 0.5 ng and 5 mg/plate in the presence and absence of the metabolic activation system (S9-mix from liver of rats pre-treated with Aroclor 1254). No mutagenic response was observed in any of the strains at any dose in the presence or absence of S9 activation. The same mixture was also tested in an *in vitro* mutagenicity assay using L5178Y $tk^{+/-}$ mouse lymphoma cells at five doses ranging between 0.0005 and 0.5 mg/mL with or without S9 mix. A dose-dependent statistically significant increase of mutations was observed at 0.05 and 0.1 mg/mL in the absence but not presence of S9 (Delaney et al., 2002). The FEEDAP Panel noted that the increase in mutation frequency does not exceed the Global Evaluation Factor (a predefined induced mutant frequency) and, based on the Organisation for Economic Co-operation and Development (OECD) technical guidance (TG) 490 (2016), considered the increase in mutation frequency not biologically relevant.

A peel extract of Ponkan cultivar 'Ohta ponkan' (*C. reticulata*) containing nobiletin and tangeretin in concentrations of 50.3 and 18.7 mg/g, respectively, was tested in two *in vitro* and one *in vivo* genotoxicity assays (Nakajima et al., 2020). In the standard Ames test according to the OECD TG 471, six concentrations between 156 and 5,000 μg/plate (equivalent to 7.8–250 μg nobiletin/plate and 2.9–93 μg tangeretin/plate) were tested in four *Salmonella* Typhimurium strains (TA98, TA100, TA1535 and TA1537) and in *E. coli* WP2uvrA in the presence and absence of metabolic activation. No mutagenic response was observed at any concentration in any strain. In Chinese hamster lung (CHL) cells, a dose-dependent increase of chromosomal aberrations (CA) was observed at treatment with the same extract in the presence and absence of S9. However, the FEEDAP Panel noted that the chromosomal aberration test with CHL cells often results in false positive outcomes because of the abnormal level of p53. No micronuclei induction was achieved in bone marrow cells from male mice administered 500–2,000 mg/kg bw of the extract over two days by oral gavage. Taking into consideration the limited relevance of the *in vitro* CA test in CHL cells and the negative results obtained in the *in vivo* study, the FEEDAP Panel concluded that the extract did not induce chromosome damage.

On these bases, the FEEDAP Panel concludes that PMF do not raise concern for genotoxicity.

3.3.2.2. Repeated-dose toxicity studies

Volatile components

No studies on subchronic toxicity or carcinogenicity testing are available for perillaldehyde.

Non-volatile components

Nakajima et al., 2020 evaluated the peel extract of Ponkan cultivar 'Ohta ponkan' (*C. reticulata* Blanco) that is rich in nobiletin and tangeretin in a 90-day study at doses of 54, 180 or 540 mg/kg bw per day. The amounts of nobiletin and tangeretin in the test item were 69.7 mg/g extract and 29.5 mg/g extract, respectively. Hyaline droplet nephropathy, which specifically occurs in adult male rats, was observed in males of the 540 mg/kg bw per day group and was not considered a relevant endpoint. No other adverse effects were observed in this study. The no observed adverse effect level (NOAEL) was considered to be 540 mg/kg bw per day for female rats and less than 540 mg/kg bw per day for male rat, equivalent to 38 and 16 mg/kg bw per day for nobiletin and tangeretin, respectively.

Conclusions on toxicology

Perillaldehyde is genotoxic. No studies on the endpoints of subchronic toxicity or carcinogenicity are available for perillaldehyde.

[24] Other components of the mixture: trimethylscutellarein (9.1%), sinensetin (3.9%), 5-demethyl-nobiletin (2.8%), hexa-*O*-methylquercetagetin (3.3%), 5-demethyl-tetramethylscutellarein (0.7%), 5-hydroxy-3,30,40,6,7,8-hexamethoxyflavone (0.7%), and a small quantity of unidentified flavonoid compounds (3.9%).

Polymethoxylated flavones do not raise concern for genotoxicity. The FEEDAP Panel identified NOAEL values of 38 and 16 mg/kg bw per day for nobiletin and tangeretin, respectively.

3.3.3. Safety for the target species

Tolerance studies and/or toxicological studies made with the essential oil under application were not submitted.

In the absence of these data, the approach to the safety assessment of a mixture whose individual components are known is based on the safety assessment of each individual component (component-based approach). This approach requires that the mixture is sufficiently characterised. The individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component.

As the additive under assessment is sufficiently characterised (> 99.6%), the EFSA FEEDAP Panel applied a component-based approach to assess the safety for target species of the volatile constituents of the essential oil, except perillaldehyde. For substances for which a concern for genotoxicity has been identified (perillaldehyde), the assessment of the safety for target species is based on the comparison between the intake via the consumption of citrus by-products as feed material and that via the use of expressed mandarin oil as a feed additive. Feeding animals citrus by-products is a common practice with no report of adverse effects (Bampidis and Robinson, 2006; Feedipedia[25]).

Volatile components

Based on considerations related to structural and metabolic similarities, the components were allocated to 10 assessment groups, corresponding to the chemical groups (CGs) 1, 3, 4, 6, 8, 13, 25, 27, 32 and 31, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 are applied (EFSA CEF Panel, 2015a,b). The allocation of the components to the (sub-)assessment groups is shown in Table 4.

For each component in the assessment group, exposure in target animals was estimated considering the use levels in feed, the percentage of the component in the oil and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b). Default values on body weight are used to express exposure in terms of mg/kg bw per day. The intake levels of the individual components calculated for chickens for fattening, the species with the highest ratio of feed intake/body weight per day, are shown in Table 4.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in the assessment group toxicological data were available to derive NOAEL values. Structural and metabolic similarity among the components in the assessment groups were evaluated to explore the application of read-across allowing extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL or, if sufficient evidence were available for members of a (sub-)assessment group, to derive a (sub-)assessment group NOAEL.

Toxicological data for subchronic studies, from which NOAEL values could be derived, were available for octyl acetate [09.007] in CG 1 (EFSA FEEDAP Panel, 2013), citral [05.020] in CG 3 (EFSA FEEDAP Panel, 2016a), citronellol [02.011] and related citronellyl derivatives in CG 4 (EFSA FEEDAP Panel, 2016b), terpineol [02.230] and linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), thymol [04.006] in CG 25 (EFSA FEEDAP Panel, 2012c), methyl *N*-methyl anthranilate [09.781] in CG 27 (EFSA FEEDAP Panel, 2011), myrcene [01.008], d-limonene [01.045], p-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016c).

Considering the structural and metabolic similarities in CG 1, the NOAEL of 120 mg/kg bw per day for octyl acetate [09.007] was selected as the reference point for the group and extrapolated to octanal [05.009], octan-1-ol [02.006], nonanal [05.025] and decanal [05.010].

Read-across was also applied using the NOAEL of 345 mg/kg bw per day for citral [05.020] to extrapolate to geranial [05.188] and neral [05.170] in CG 3.

For the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.072] and terpinen-4-ol [02.072], the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [02.230] and d-limonene [01.045].

[25] https://www.feedipedia.org/node/680

Considering the structural and metabolic similarities, the NOAELs for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045], *p*-cymene [01.002] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (α-farnesene [01.140] and *trans*-β-ocimene), group III (γ-terpinene [01.020], terpinolene [01.005], α-terpinene [01.019], β-phellandrene [01.055] and α-phellandrene [01.006]), group IVe (1-isopropenyl-4-methylbenzene [01.010]) and group V (β-pinene [01.003], α-pinene [01.004], sabinene [01.059], δ-3-carene [01.029], camphene [01.009], α-copaene, δ-cadinene [01.021], α-selinene, β-cubebene and tricyclene [01.060]) (EFSA CEF Panel, 2015a,b). The same NOAEL value for sabinene [01.059] is applied to sabinene hydrate [02.085] and *cis*-sabinene hydrate in CG 8.

For the remaining compounds, namely α-sinensal [05.130], fenchone, l-camphor, *cis*-linalool oxide, α-fenchene, 3,7,10-humulatriene [01.043], *cis*– and *trans*-limonene epoxide, toxicity studies and NOAEL values performed with the compounds under assessment were not available and read-across was not possible. Therefore, the threshold of toxicological concern (TTC) approach was applied (EFSA FEEDAP Panel, 2017b).

As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from *in vivo* toxicity study or read across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e. 3, 0.91 and 0.15 mg/kg bw per day for Cramer Class I, II and III compounds, respectively). Reference points selected for each compound are shown in Table 4.

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances (EFSA SC, 2019). A MOET > 100 allowed for interspecies- and intra-individual variability (as in the default 10 × 10 uncertainty factor). The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible.[26]

The approach to the safety assessment of expressed mandarin oil for the target species is summarised in Table 4. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 15 mg/kg.

Table 4: Compositional data, intake values (calculated for chickens for fattening at 15 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of expressed mandarin oil classified according to assessment groups

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS no**	**Max conc. in the oil**	**Max feed conc.**	**Intake[(a)]**	**Cramer class**	**NOAEL[(b)]**	**MOE**	**MOET**
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 1								
Octanal	05.009	0.22	0.0330	0.0030	I	120	40,506	
CG 3								
α-Sinensal	05.130	0.84	0.125	0.0112	I	*3*	267	
CG 6								
Linalool	02.013	0.53	0.080	0.0071	I	**117**	35029	
α-Terpineol	02.097	0.26	0.039	0.0035	I	**250**	33677	
MOET CG 6				0.0093				17,170
CG 8								
Fenchone	n.a.	0.03	0.005	0.0004	II	*0.91*	2,253	

[26] Compounds included in the assessment groups but not reported in the table: decanal, nonanal and octan-1-ol (CG 1); geranial and neral (CG 3); citronellol and citronellal (CG 4); 4-terpineol (CG 6); *cis*-sabinene hydrate, sabinene hydrate and l-carvone (CG 8); 1,8-cineole (CG 16); β-ocimene (CG 31, II); α-phellandrene (CG 31, III); 4-isopropenyl-4-methylbenzene (CG 31,IVe); β-caryophyllene, δ-carene, camphene, α-copaene, δ-cadinene, α-selinene, β-cubebene, α-fenchene and tricyclene (CG 31, V).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS no**	**Max conc. in the oil**	**Max feed conc.**	**Intake[a]**	**Cramer class**	**NOAEL[b]**	**MOE**	**MOET**
l-Camphor	n.a.	0.01	0.002	0.0001	II	*0.91*	6,143	
MOET CG 8				0.0005				1,648
CG 13								
cis-Linalool oxide	n.a.	0.08	0.011	0.0010	II	*0.91*	901	
CG 25								
Thymol	04.006	0.37	0.055	0.0049	I	36	7,285	
CG 27								
Methyl *N*-methylanthranilate	09.781	1.59	0.238	0.0214	I	**20**	936	
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	2.11	0.348	0.0312	I	**44**	1,408	
α-Farnesene	01.040	0.11	0.104	0.0093	I	44	4,722	
MOET CG 31, II				0.0406				1,085
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.045	68.6	10.29	0.9238	I	**250**	271	
γ-Terpinene	01.020	24.7	3.705	0.3326	I	250	752	
Terpinolene	01.005	1.49	0.223	0.0200	I	250	12,477	
α-Terpinene	01.019	0.76	0.115	0.0103	I	250	24,300	
β-Phellandrene	01.055	0.43	0.065	0.0058	I	250	42,876	
MOET CG 31, III				1.2925				193
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	0.80	0.120	0.0108	I	**154**	14,154	
CG 31, V (Bi-, tricyclic, non aromatic hydrocarbons)								
α-Pinene	01.004	4.51	0.677	0.0607	I	222	3,655	
β-Pinene	01.003	2.31	0.347	0.0311	I	222	7,137	
Sabinene	01.059	0.88	0.133	0.0119	I	222	18,649	
MOET CG 31, V				0.1037				2,140
CG 31, VI (macrocyclic non aromatic hydrocarbons)								
3,7,10-Humulatriene	01.043	0.03	0.004	0.0004	I	*3*	8,569	
CG 32 (epoxides)								
cis-Limonene epoxide	n.a.	0.02	0.002	0.0002	I	*3*	13,924	
trans-Limonene epoxide	n.a.	0.01	0.001	0.0001	I	*3*	24,754	
MOET CG 32				0.0012				8,911

CG: chemical group; bw: body weight.

(a): Intake calculations for the individual components are based on the use level of 15 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

(b): Values **in bold** refer to those components for which the NOAEL value was available, values *in italics* are the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class, other values (plain text) are NOAELs extrapolated by using read-across.

In its opinion on anthranilate derivatives (EFSA FEEDAP Panel, 2011), the FEEDAP Panel noted that 'Chemical formulations containing methyl anthranilate have been found to be effective bird aversion agents, acting as chemosensory repellents (Müller-Schwartze, 2009) by activating pain receptors associated with taste and smell (Kirifides et al., 2004)'. As a consequence, the FEEDAP Panel concluded that the use of methyl anthranilate and methyl *N*-methyl anthranilate as feed flavourings in avian

species is contraindicated. However, considering the low occurrence of methyl *N*-methyl anthranilate in the oil under assessment (< 2%, resulting in 0.24 mg/kg complete feed in chickens for fattening) and that methyl anthranilate concentrations ≥ 0.1% are needed to be effective (Clark, 1999), the FEEDAP Panel considers that the presence of methyl *N*-methyl anthranilate in expressed mandarin oil is not of concern for avian species.

As shown in Table 4, for all the assessment groups, the lowest MOET was ≥ 193. Therefore, no safety concern was identified for the expressed mandarin oil when used as a feed additive for chickens for fattening at the proposed use levels (15 mg/kg). From the lowest MOET of 193 for chickens for fattening, the MOET was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 5.

Table 5: Combined margin of exposure (MOET) for the assessment group 'Cyclohexene hydrocarbons' (CG 31, III) calculated for the different target animal categories at the proposed use level in feed

Animal category	Body weight (kg)	Daily feed intake (g DM/kg bw)	Feed intake (g DM/day)	Proposed use level (mg/kg feed)	Lowest MOET
Chicken for fattening	2	79	158	15	193
Laying hen	2	53	106	15	288
Turkey for fattening	3	59	176	15	258
Piglet	20	44	880	33	158
Pig for fattening	60	37	2,200	33	187
Sow lactating	175	30	5,280	33	231
Veal calf (milk replacer)	100	19	1,890	30	431
Cattle for fattening	400	20	8,000	30	381
Dairy cow	650	31	20,000	30	246
Sheep/goat	60	20	1,200	30	381
Horse	400	20	8,000	40	286
Rabbit	2	50	100	15	305
Salmon	0.12	18	2.1	15	847
Dog	15	17	250	15	897
Cat	3	20	60	15	762
Ornamental fish	0.012	5	0.054	15	3,049

DM: dry matter; bw: body weight.

At the proposed use levels for the different species, the lowest MOET is ≥ 158 (Table 5) and > 500 for cats (Court and Greenblatt, 1997; Lautz et al., 2021). Therefore, with respect to the exposure to the volatiles present in the additive (except perillaldehyde), no safety concern was identified for expressed mandarin oil, when used as a feed additive at the proposed use levels. No specific proposals have been made by the applicant for the use level in water for drinking. The FEEDAP Panel considers that the use in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed (EFSA FEEDAP Panel, 2010).

Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded.

Volatile components: Perillaldehyde

Low concentrations of perillaldehyde were detected in all batches of the additive under assessment (average: 0.018%, range: 0.008–0.063%). The use of expressed mandarin oil at the proposed use levels in feed for the different target species (ranging from 15 to 40 mg/kg complete feed, see Section 3.2.2), would result in an intake of perillaldehyde up to 0.9 μg/kg bw for poultry, 1.0 μg/kg bw for pigs, 0.7 μg/kg bw for ruminants, 0.6 μg/kg bw for horses, 0.5 μg/kg bw for rabbits and 0.2 μg/kg bw for fish.[27]

[27] Intake values calculated considering the maximum concentration of perillaldehyde in the additive (0.063%), the default values for feed intake (Table 5), the proposed use levels in feed for the different species (Table 5) and that complete feed contains 88% DM except milk replacer for veal calves (94.5%).

Perillaldehyde occurs in citrus by-products, which are used in diets at different concentrations depending on the target species (e.g. from 5% up to 30% in ruminants).[28] Taking into account an inclusion level of 10% for poultry and 20% for the other species and considering the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b), the daily intake of citrus by-products has been estimated to be 7.9 g dry matter (DM)/kg bw for poultry, 8.8 g DM/kg bw for pigs, 6.2 g DM/kg bw for ruminants, 4 g DM/kg bw for horse, 10 g DM/kg bw for rabbits and 3.6 g DM/kg bw for fish.

Based on the literature data provided by the applicant[29] on the occurrence of perillaldehyde in citrus peel (e.g. 0.0004% for mandarins and lemons, and 0.001% for oranges according to Qadir et al., 2018; Kamal et al., 2011; Bourgou et al., 2012) and considering that citrus peel represents 62.5% of citrus by-product[30] (Bampidis and Robinson, 2006), the occurrence of perillaldehyde in citrus by-products was estimated to be 0.0002% in mandarin and lemon and 0.0006% in orange by-products, 0.0004% on average in citrus by-products.[31] Based on citrus by-product intake (see above), the intake of perillaldehyde via feed was calculated to be 32 μg/kg bw for poultry, 36 μg/kg bw for pigs, 24 μg/kg bw for ruminants, 16 μg/kg bw for horses, 40 μg/kg bw for rabbits and 14 μg/kg bw for fish.

These concentrations are at least 25-fold higher than those resulting from the high use level of expressed mandarin oil in feed as proposed by the applicant (15–40 mg expressed mandarin oil/kg feed).

Non-volatile components

The main non-volatile constituents in expressed mandarin oil are PMF, mainly tangeretin and nobiletin. Based on the data reported in the literature on the maximum occurrence of PMF in mandarin oil (e.g. 0.5% tangeretin, 0.25% nobiletin and 0.10% heptamethoxyflavone, total PMF 0.85%, see section 3.2 Non-volatile constituents), the concentration of PMF in feed at the maximum proposed use levels for the different species (ranging from 15 to 40 mg/kg) was calculated to range between 0.13 and 0.34 mg/kg.

The FEEDAP Panel identified NOAELs of 38 and 16 mg/kg bw per day for nobiletin and tangeretin, respectively. The lowest NOAEL of 16 mg/kg bw was selected as a group NOAEL for PMF. Applying an uncertainty factor (UF) of 100 to the NOAEL, the safe daily dose for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), and thus the maximum safe feed concentration of PMF was calculated (Table 6).

Since glucuronidation of the hydroxylated or oxygenated metabolites of the individual constituents of expressed mandarin oil is an important metabolic pathway facilitating the excretion of these compounds, the calculation of safe concentrations in cat feed needs an additional UF of 5. This factor is due to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997; Lautz et al., 2021).

Table 6: Maximum safe concentration in feed of polymethoxylated flavones for the different target animal categories

Animal category	Default values		Maximum safe intake/concentration	
	Body weight (kg)	Feed intake (g DM/day)	Intake (mg/day)	Concentration in feed (mg/kg feed)[(a)]
Chicken for fattening	2	158	0.3	1.8
Laying hen	2	106	0.3	2.7
Turkey for fattening	3	176	0.5	2.4
Piglet	20	880	3.2	3.2
Pig for fattening	60	2,200	9.6	3.8
Sow lactating	175	5,280	28	5.0
Veal calf (milk replacer)	100	1,890	16	8.0
Cattle for fattening	400	8,000	64	7.0
Dairy cow	650	20,000	104	4.6

[28] Technical dossier/Supplementary information July 2020/SIn FAD-2010-322-request of clarification.
[29] Technical dossier/Supplementary information/November 2020.
[30] Composition of fresh citrus by-products: 62.5% citrus peel, 32.5% pulp and 5% seeds. Similar proportions are assumed in dried citrus by-products.
[31] Occurrence of perillaldehyde in citrus by-products calculated considering the composition of citrus by-products as 60% oranges, 20% lemon and lime, 30% mandarin: 0.0006% × 0.6+0.0002 × 0.3 + 0.0002 × 0.1 = 0.0004%.

Animal category	Default values		Maximum safe intake/concentration	
	Body weight (kg)	Feed intake (g DM/day)	Intake (mg/day)	Concentration in feed (mg/kg feed)[(a)]
Sheep/goat	60	1,200	9.6	7.0
Horse	400	8,000	64	7.0
Rabbit	2	100	0.3	2.8
Salmon	0.12	2.1	0.02	8.0
Dog	15	250	2.4	8.4
Cat[(b)]	3	60	0.5	1.4
Ornamental fish	0.012	0.054	0.002	31.3

DM: dry matter.
(a): Complete feed containing 88% DM, milk replacer 94.5% DM.
(b): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

The FEEDAP Panel concludes that the presence of PMF in mandarin oil does not raise concern for the target species.

3.3.3.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that expressed mandarin oil from the fruit peels of *C. reticulata* is safe up to the maximum proposed use levels in complete feed of 15 mg/kg for poultry, 33 mg/kg for pigs, 30 mg/kg for ruminants, 40 mg/kg for horses, and 15 mg/kg for fish and rabbits. These target species are fed citrus by-products as part of their daily feed. For these species, the use of expressed mandarin oil in feed is not expected to increase the exposure to perillaldehyde to a relevant extent (< 4%). For dogs, cats and ornamental fish not normally exposed to citrus by-products, no conclusion can be drawn.

The FEEDAP Panel considers that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed, except for companion animals.

Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded.

3.3.4. Safety for the consumer

Mandarin oil is added to a wide range of food for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavor ingredients (Burdock, 2009) cites values of 0.001 mg/kg bw per day for expressed mandarin oil (FEMA 2657).

The majority of the individual constituents of the essential oil under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (Table 1).

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the FEEDAP Panel recognises that the constituents of expressed mandarin oil are expected to be extensively metabolised and excreted in the target species and are not expected to accumulate in animal tissues and products (see Section 3.3.1). Therefore, a relevant increase of the uptake of these compounds by humans consuming products of animal origin is not expected.

Considering the reported human exposure due to direct use of expressed mandarin oil in food (Burdock, 2009) it is unlikely that consumption of products from animals given expressed mandarin oil at the proposed maximum use level would significantly increase human background exposure.

Consequently, no safety concern would be expected for the consumer from the use of expressed mandarin oil up to the maximum proposed use level in feed for the target animals.

3.3.5. Safety for user

No specific data were provided by the applicant regarding the safety of the additive for users.

The applicant produced a safety data sheets[32] for green and red expressed mandarin oils where hazards for users have been identified.

[32] Technical dossier/Supplementary Information August 2019/Annex_IX_SIn reply_mandarin_oil_greed_MSDS and Annex_XII_Sin reply_mandarin oil_red_MSDS. Inhalation hazard (H304, may be fatal if swallowed and enters airways), hazards for skin irritation (H315), skin sensitisation (H317b, category 1B).

3.3.6. Safety for the environment

C. reticulata Blanco is widely grown in Europe both for commercial and decorative purposes. The use of the additive in animal feed under the proposed conditions of use is not expected to pose a risk for the environment.

3.4. Efficacy

C. reticulata Blanco and its oil obtained after cold expression of the peel of almost ripe fruits are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2657.

Since mandarin oil is recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Expressed mandarin oil from the fruit peels of *C. reticulat*a Blanco is safe up to the maximum proposed use levels in complete feed of 15 mg/kg for poultry, 33 mg/kg for pigs, 30 mg/kg for ruminants, 40 mg/kg for horses, and 15 mg/kg for fish and rabbits. These target species are fed citrus by-products as part of their daily feed. For these species, the use of expressed mandarin oil in feed is not expected to increase the exposure to perillaldehyde to a relevant extent (< 4%). For dogs, cats and ornamental fish not normally exposed to citrus by-products, no conclusion can be drawn. The EFSA FEEDAP Panel considers that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed, except for companion animals. Simultaneous use in feed and water for drinking may lead to the safe maximum dose being exceeded.

No concerns for consumer safety were identified following the use of the additive up to the maximum proposed use level in feed for the target animals.

The essential oil under assessment should be considered as irritant to skin, eyes and the respiratory tract, and as a skin sensitiser.

The use of the additive in animal feed under the proposed conditions of use is not expected to pose a risk for the environment.

Since expressed mandarin oil is recognised to flavour food, and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. Documentation as provided to EFSA/Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 08 – Sapindales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG) and registered with the Question number EFSA-Q-2010-01517
14/12/2010	Reception mandate from the European Commission
26/02/2011	EFSA informed the applicant (EFSA ref. 7150727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission
24/06/2015	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals
17/06/2016	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products". Discussion on the ongoing work regarding the pilot dossiers BDG08 and BDG 09
27/04/2017	Trilateral meeting organised by the European Commission with EFSA and the applicant FEFANA on the assessment of botanical flavourings: characterisation, substances of toxicological concern present in the botanical extracts, feedback on the pilot dossiers
19/03/2018	Application validated by EFSA – Start of the scientific assessment

Date	Event
03/05/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
20/06/2018	Comments received from Member States
13/07/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: Method of analysis
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: amyris oil, cashew oil, neroli bigarade oil, petitgrain bigarade absolute, mandarin terpenes, grapefruit oil expressed, grapefruit extract (sb), grapefruit extract
01/08/2019	Reception of supplementary information from the applicant (partial submission)
24/11/2020	Reception of supplementary information from the applicant (partial submission)
12/03/2021	The application was split and a new EFSA-Q-2021-00143 was assigned to the preparation included in the present assessment.
17/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started for the preparation included in the present assessment
02/04/2021	Partial withdrawal by applicant (EC was informed) for the following additive: olibanum tincture
05/05/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment

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Abbreviations

ADME	Absorption, distribution, metabolism and excretion
AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
BDG	Botanically defined group
bw	body weight
CA	Chromosomal aberrations

CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	chemically defined group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CHL	Chinese hamster lung
CYP	cytochrome P450
DDMN	didemethylated nobiletin
DM	dry matter
DMN	demethylated nobiletin
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food Additives and Flavourings
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavor Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
FL-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
HPLC	high-performance liquid chromatography
ISO	International standard organisation
LC–MS	liquid chromatography-mass spectrometry
LC–MS/MS	liquid chromatography tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
MOET	combined margin of exposure (total)
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCBs	polychlorobiphenyls
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PMF	polymethoxylated flavones
QSAR	Quantitative Structure–Activity Relationship
SC	EFSA Scientific Committee
TEF	toxc equivalent
TG	Technical guidance
TTC	threshold of toxicological concern
UF	uncertainty factor
UV	ultraviolet
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for buchu leaves oil, olibanum extract (wb), lime oil, petigrain bigarade oil, bitter orange extract of the whole fruit, lemon oil expressed, lemon oil distilled (residual fraction), lemon oil distilled (volatile fraction), orange oil cold pressed, orange terpenless (concentrated 4 times), orange terpenless (concentrated 10 times), orange terpenless (folded), orange terpenes, mandarin oil and quebracho extract (wb) from botanically defined flavourings Group (BDG 08) – Sapindales

In the current grouped application an authorisation is sought under Articles 4(1) and 10(2) for *buchu leaves oil, olibanum extract (wb), lime oil, petigrain bigarade oil, bitter orange extract of the whole fruit, lemon oil expressed, lemon oil distilled (residual fraction), lemon oil distilled (volatile fraction), orange oil cold pressed, orange terpenless (concentrated 4 times), orange terpenless (concentrated 10 times), orange terpenless (folded), orange terpenes, mandarin oil and quebracho extract (wb)* from *botanically defined flavourings group 08 (BDG 08)1*, under the category/functional group 2(b) 'sensory additives'/flavouring compounds', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species. For each preparation the Applicant indicated the corresponding phytochemical marker(s) and the corresponding range of content. The *feed additives* are intended to be incorporated into *feedingstuffs* or drinking *water* directly or through flavouring *premixtures* with no proposed minimum or maximum levels. However, the Applicant suggested the typical maximum inclusion level of the *feed additives* of 25 mg/kg *feedingstuffs*.

For the quantification of the phytochemical markers *d-limonene* and *d,l-isomenthone* in *buchu leaves oil* and *d-limonene* in *orange terpenless (concentrated 10 times)* oil, the Applicant submitted a method using gas chromatography coupled with flame ionisation detection (GC-FID) based on the generic standard ISO 11024. The quantification is performed by using the normalisation approach for the estimation of the area percentage of individual components. The Applicant tested the method, following an experimental design proposed by the EURL, and obtained satisfactory performance characteristics.

For the quantification of the phytochemical markers *11-keto-β-boswellic acid* and *3-O-acetyl-11-keto-β-boswellic acid* in *olibanum extract (wb)*, the Applicant submitted a method using high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection at 250 nm described in the European Pharmacopeia monograph for Indian Frankincense (Olibanum indicum). The quantification of *11-keto-β-boswellic acid* and *3-O-acetyl-11-keto-β-boswellic acid* is performed by means of specific expressions and is indicated as percentage content (absolute value). The Applicant, using the HPLC-UV method, analysed 5 batches of the *feed additive* obtaining results within the proposed specifications.

For the quantification of the phytochemical marker *d-limonene* in *lime oil* the Applicant submitted a GC-FID method based on the corresponding standard ISO 3519:2005 for the characterisation of the "oil of lime distilled, Mexican type (Citrus aurantifolia [Christm.] Swingle)". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *d-limonene* in *lime oil*.

For the quantification of the phytochemical markers *linalyl acetate* and *linalool* in *petigrain bigarade oil* the Applicant submitted a GC-FID method based on the corresponding standard ISO 8901:2003 for "Oil of bitter orange petitgrain, cultivated (Citrus aurantium L.)". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *linalyl acetate* and *linalool* in *petigrain bigarade oil*.

For the quantification of the phytochemical marker *naringin* in *bitter orange extract of the whole fruit* the Applicant submitted a single-laboratory validated and further verified method based on HPLC-UV (284 nm). The method has been developed for the determination of total flavonoids (including *naringin* alone) in a mixture of citrus flavonoids. The quantification of *naringin* is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant provided validation and verification studies demonstrating the applicability of the method for

the analysis of pure *naringin*. Furthermore, *naringin* has been satisfactory quantified in the *feed additive* by the proposed method in 5 different lots of *bitter orange extract of the whole fruit*.

For the quantification of the phytochemical marker *d-limonene* in *lemon oil expressed, lemon oil distilled (residual fraction)* and *lemon oil distilled (volatile fraction)* the Applicant submitted a GC-FID method based on the corresponding standard ISO 855:2003 for "Oil of lemon (Citrus limon (L.) Burm. f.), obtained by expression". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *d-limonene* in *lemon oil expressed, lemon oil distilled (residual fraction)* and *lemon oil distilled (volatile fraction)*.

For the quantification of the phytochemical marker *d-limonene* in *orange oil cold pressed*, *orange terpenless (concentrated 4 times)* oil, *orange terpenless (folded)* oil and *orange terpenes* oil the Applicant submitted a GC-FID method based on the corresponding standard ISO 3140:2019 for "Essential oil of sweet orange expressed (Citrus sinensis (L.))". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. The Applicant presented a chromatogram and the specific analytical procedure for the analysis of *d-limonene* in *orange oil cold pressed*, *orange terpenless (concentrated 4 times)* oil, *orange terpenless (folded)* oil and *orange terpenes* oil.

For the quantification of the phytochemical marker *d-limonene* in *mandarin oil* the Applicant submitted a GC-FID method based on the corresponding standard ISO 3528:2012 for "Essential oil of mandarin, Italian type (Citrus reticulate Blanco)". The quantification is performed using the normalisation approach for the estimation of the area percentage of individual components. For *mandarin oil*, the Applicant presented a chromatogram and the specific analytical procedure for the analysis of the *d-limonene* in *mandarin oil*.

For the quantification of the phytochemical marker *tannins* in *quebracho extract (wb)* the Applicant submitted the method ISO 14088:2020 "Leather - Chemical tests - Quantitative analysis of tanning agents by filter method". The method proposed is suitable for the determination of tanning agents in all vegetable tanning products and it is based on indirect gravimetric analysis of tanning agents with fixing of the absorbent compounds in low chromed hide powder. The quantification of *tannins* in *quebracho extract (wb)* is performed by means of specific expressions and is indicated as percentage content (absolute value). Furthermore, the Applicant provided satisfactory results for the analysis of *tannins* in 3 batches of *quebracho extract (wb)*.

The accurate quantification of the *feed additives* in *premixtures* and *feedingstuffs* is not achievable experimentally and the Applicant did not provide experimental data to determine the *feed additives* in *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify the *feed additives* in *premixtures*, *feedingstuffs* and *water*.

Based on the information above, the EURL recommends for official control: (i) the GC-FID method based on the generic standard ISO 11024 for the quantification of *d-limonene* and *d,l-isomenthone* in *buchu leaves oil* and *d-limonene* in *orange terpenless (concentrated 10 times)* oil; (ii) the HPLC-UV method described in the European Pharmacopeia monograph "Indian Frankincense (Olibanum indicum)" for the quantification of *11-keto-β-boswellic acid* and *3-O-acetyl-11-keto-β-boswellic acid* in *olibanum extract (wb)*; (iii) the GC-FID method based on the standard ISO 3519:2005 for the quantification of *d-limonene* in *lime oil*; (iv) the GC-FID method based on the standard ISO 8901:2003 for the quantification of *linalyl acetate* and *linalool* in *petigrain bigarade oil*; (v) the HPLC-UV single-laboratory validated and further verified method for the quantification of *naringin* in *bitter orange extract of the whole fruit*; (vi) the GC-FID method based on the standard ISO 855:2003 for the quantification of *d-limonene* in *lemon oil expressed, lemon oil distilled (residual fraction)* and *lemon oil distilled (volatile fraction)*; (vii) the GC-FID method based on the standard ISO 3140:2019 for the quantification of *d-limonene* in *orange oil cold pressed*, *orange terpenless (concentrated 4 times)* oil, *orange terpenless (folded)* oil and *orange terpenes* oil; (viii) the GC-FID method based on the standard ISO 3528:2012 for the quantification of *d-limonene* in *mandarin oil*; and (ix) the indirect gravimetric analysis of tanning agents with fixing of the absorbent compounds in low chromed hide powder described in ISO 14088:2020 for the quantification of *tannins* in *quebracho extract (wb)*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.