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Safety and efficacy of feed additives consisting of expressed sweet orange peel oil and its fractions from *Citrus sinensis* (L.) Osbeck 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 sweet orange oil and its fractions obtained from the fruit peels of *Citrus sinensis* (L.) Osbeck, when used as sensory additives (flavourings) in feed and water for drinking for all animal species. 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 orange oil and its fractions was not expected to increase the exposure to perillaldehyde to a relevant extent (< 10%). For dogs, cats, ornamental fish and ornamental birds not normally exposed to citrus by-products, no conclusion can be drawn. For the other species, the FEEDAP Panel concluded that the additives under assessment are safe at the maximum proposed use levels in complete feed. 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 additives up to the maximum proposed use level in feed. The additives under assessment should be considered as irritants to skin and eyes, and as skin and respiratory sensitisers. The use of the additives under the proposed conditions of use in animal feed was not expected to pose a risk for the environment. Expressed orange oil and its fractions were recognised to flavour food. Since their function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

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Keywords: sensory additives, flavouring compounds, *Citrus sinensis* (L.) Osbeck, expressed orange oil, d-limonene, perillaldehyde, polymethoxylated flavones

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 7 years after the entry into force of this Regulation.

The European Commission received a request from the 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 (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} During the course of the assessment, this application was split and the present opinion covers only 2 out of the 20 initial preparations under application: orange oil (including cold pressed orange oil and orange oil terpeneless) and orange terpenes from *Citrus sinensis* (L.) Pers.⁵ for all animal species. During the assessment, the applicant clarified that the two types of additives fall into the definition 'orange oil', i.e. orange oil cold pressed and orange oil terpeneless, obtained after distillation of cold pressed orange oil.⁶ The three preparations from *C. sinensis* will be assessed individually.

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 orange oil cold pressed and orange terpenes from *C. sinensis*, when used under the proposed conditions of use (see Sections 3.2.1.3, 3.3.1.3 and 3.4.1.3).

The remaining nine preparations belonging to BDG 8 – *Sapindales* under application are assessed in separate opinions.

1.2. Additional information

The three preparations under assessment, namely orange oil cold pressed, orange oil terpeneless and orange terpenes from *C. sinensis* (L.) Osbeck (= *C. aurantium* L. var. *dulcis*) are currently authorised as feed additives according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined). They have not been assessed as feed additives in the EU.

'Sweet orange oil' (*Aurantii dulcis aetheroleum*) is described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020). It is defined as the essential oil obtained without heating, by

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, cashew oil, neroli bigarade oil, petitgrain bigarade absolute, mandarin terpenes, grapefruit oil expressed, grapefruit extract (sb), grapefruit extract.

⁴ On 2 April 2021, EFSA was informed by the applicant about the withdrawal of the application on olibanum tincture.

⁵ Accepted name: *Citrus sinensis* (L.) Osbeck, synonym *Citrus sinensis* (L.) Pers., *C. aurantium* L. var. *dulcis*.

⁶ On 23 November 2020, EFSA and EC received clarification on the number of preparations obtained from *C. sinensis*: orange oil cold pressed, orange oil terpeneless and orange terpenes.

suitable mechanical treatment from the fresh peel of the fruit of *Citrus × sinensis* (L.) Osbeck. A suitable antioxidant may be added.

Many of the individual components of the essential oils have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC), 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 currently authorised for food⁷ and feed⁸ uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000⁹ 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

CG	Chemical group	Product (EU register 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
		Decan-1-ol	02.024	
		Hexanal	05.008	
		Octanal	05.009	
		Decanal	05.010	
		Dodecanal	05.011	
		Nonanal	05.025	
		Undecanal	05.034	
		Decanoic acid	08.011	
		Dodecanoic acid	08.012	
		Oleic acid	08.013	
		Hexadecanoic acid	08.014	
		Tetradecanoic acid	08.016	
		Octyl acetate	09.007	
Hexadecanal ^(a)	05.152	2008, AFC		
03	α , β -Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/ aldehydes/ acids, acetals and esters	(Z)-Nerol	02.058	2016a
		Neral	05.170	
		<i>trans</i> -3,7-Dimethylocta-2,6-dienal (geranial)	05.188	
		Geranyl acetate	09.011	
		Neryl acetate	09.213	2019a
		2,4-Decadienal	05.081	
		<i>trans</i> -2-Decenal	05.191	
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	
		Neral	05.170	
		Octadeca-9,12-dienoic acid ^(a)	08.041	JECFA

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

⁹ 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.

CG	Chemical group	Product (EU register name)	FLAVIS no	EFSA opinion,* year	
05	Saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols	Nonan-2-ol ^(a)	02.087	JECFA	
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		
		2-(4-Methylphenyl)propan-2-ol	02.042		
		4-Terpinenol	02.072		
		(l)- α -Bisabolol ^(a)	02.129	2011a, CEF	
		(-)- α -Elemol ^(a)	02.149		
07	Primary alicyclic saturated and unsaturated alcohols/ aldehydes/acids/acetals/ esters with esters containing alicyclic alcohols	<i>p</i> -Mentha-1,8-dien-7-ol	02.060	2017, CEF	
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Nootkatone	07.089	2016c	
		l-Carvone	07.147		
		Carveol ^(a)	02.062	JECFA	
		Dihydrocarvone	07.128	JECFA	
		Carvone ^(a)	07.012	2014, SC	
27	Anthranilate derivatives	Methyl <i>N</i> -methyl anthranilate	09.781	2011	
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	1-Isopropyl-4-methylbenzene (<i>p</i> -cymene)	01.002	2015	
		Terpinolene	01.005		
		α -Phellandrene	01.006		
		γ -Terpinene	01.020		
		d-Limonene	01.045		
		Pin-2(10)-ene (β -pinene)	01.003		2016d
		Pin-2(3)-ene (α -pinene)	01.004		
		β -Caryophyllene	01.007		
		Myrcene	01.008		
		Camphene	01.009		
		Valencene	01.017		
		3,7-Dimethyl-1,3,6-octatriene (β -ocimene) ^(b)	01.018		
		δ -3-Carene	01.029		
		δ -Cadinene ^{(a),(c)}	01.021	2011b, CEF	
		β -Cubebene ^{(a),(c)}	01.030		
		Germacrene-1(10),4(14),5-triene (δ -Germacrene) ^{(a),(c)}	01.042		
		3,7,10-Humulatriene ^{(a),(c)}	01.043		
		α -Muurolene ^{(a),(c)}	01.052	2015a, CEF	
		β -Phellandrene ^{(a),(c)}	01.055		
		4(10)-Thujene (sabinene) ^(a)	01.059		
<i>cis</i> -3,7-Dimethyl-1,3,6-octatriene (<i>cis</i> - β -Ocimene) ^(a)	01.064				

CG	Chemical group	Product (EU register name)	FLAVIS no	EFSA opinion,* year
		α -Farnesene ^{(a),(d)}	01.040	2015b, CEF
32	Epoxides	β -Caryophyllene epoxide	16.043	2014, CEF

(*): FEEDAP opinion unless otherwise indicated.

(a): Evaluated for use in food only. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.

(b): EFSA evaluated β -ocimene [01.018] as a mixture of (E)- and (Z)-isomers, containing 50–70% (E)-isomer and 15–17% (Z)-isomer (EFSA CEF Panel, 2015b).

(c): 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.

(d): JECFA and EFSA evaluated α -farnesene [01.040], as a mixture of 38–50% α -isomer, 29–40% β -isomer (EFSA CEF Panel, 2015b).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier¹⁰ in support of the authorisation request for the use of expressed orange oil and its fractions from *C. sinensis* as feed additives.

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 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 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 8.¹¹

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.¹²

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of expressed orange oil and its fractions from *C. sinensis* and its fractions obtained after distillation is in line with the principles laid down in Regulation (EC) No 429/2008¹³ 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, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 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, 2019b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018) 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

¹⁰ FEED dossier reference: FAD-2010-0322.

¹¹ Technical dossier/Supplementary information/Letter dated 29/4/2021.

¹² The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0322-bdg08.pdf>

¹³ 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.

(EFSA Scientific Committee, 2019c) and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP, 2021a).¹⁴

3. Assessment

The additives under assessment are expressed sweet orange peel oil (herein referred to as expressed orange oil) from fruit peels of *C. sinensis* (L.) Osbeck (synonyms: *Citrus sinensis* (L.) Pers., *Citrus aurantium* (L.) Dulcis) and its fractions obtained after distillation. They are obtained using different preparation techniques and are intended for use as sensory additives (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 common commercially important citrus fruits found today are thought to originate from hybrids derived from three ancestral species now represented by the cultivars described as the mandarin, pomelo, and citron. Thus, orange is considered a hybrid of the pomelo (*Citrus maxima*) and the true mandarin (*Citrus reticulata*). Although the name *Citrus sinensis* is retained in this opinion to distinguish between sweet and sour orange cultivars, both sweet and sour oranges share a common heritage and belong to the same hybrid cluster described as *Citrus* × *aurantium*.

C. sinensis is native to Asia but, after domestication, is now found growing in most warm areas of the world. The sweet orange represents the largest citrus cultivar groups grown around the world, accounting for more than 60% of the total annual production of Citrus species. Cultivars include the Valencia orange, the navel orange and the blood orange.

Application is made for three different preparations distinguished by their method of production:

- Expressed or cold pressed oil.
- Distilled oil.
- Folded oils (also described by the applicant as 'orange oil terpeneless').

Both distilled and folded oils use the expressed oil as the starting material.

Expressed oil

Extraction by mechanical pressing 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 'expression' or 'cold pressing'. In the mechanised process, the surface of the orange peel 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.

Distilled oil

The expressed oil is steam distilled and the distillate is collected by condensation and then separated from water by decantation. It differs in composition from the expressed oil only by the essential removal of any non-volatile material extracted by cold pressing.

Folded oils

Folded oils are obtained by the fractional distillation of the expressed oil to reduce the content of volatiles (terpenes) and consequently increase the concentration of any non-volatiles extracted by cold pressing. Folded oils may be misleadingly described as 'terpeneless' (term used by the applicant and in the European Union Register of Feed Additives), since most folded oils still retain a large volatile fraction.

The three additives are considered in the next sections, the expressed orange oil in section 3.2, the distilled orange oil in section 3.3 and the folded orange oils in Section 3.4.

¹⁴ <https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf>

3.2. Expressed orange oil

This application concerns the essential oil derived by cold expression from fruit peel of *C. sinensis* mainly originating from Brazil.

3.2.1. Characterisation of expressed orange oil

Expressed orange oil is an orange clear mobile liquid with a characteristic aroma. In five batches of the additive (originating from Brazil), the optical rotation at 20°C ranged between +95.5° and +98.3°, the refractive index was 1.47 (specification: 1.4710–1.4760) and the density (20°C) 845 kg/m³.¹⁵ Expressed orange oil is identified with the single Chemical Abstracts Service (CAS) number 8028-48-6, the European Inventory of Existing Commercial Chemical Substances (EINECS) number 232-433-8, the Flavor Extract Manufacturers Association (FEMA) number 2825 and the Council of Europe (CoE) 143.

Volatile components

The product specifications are based on standards developed by the International Organization for Standardization (ISO) 3140:2019 for essential oil of sweet orange expressed (*C. sinensis* (L.)), which were adapted to reflect the concentrations of the main volatile components of the essential oil, 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 (93–97%, the phytochemical marker), myrcene (1.5–3.5%), 4(10)-thujene (hereinafter referred to as sabinene, 0.1–1.0%), pin-2(3)-ene (hereinafter referred to as α -pinene, 0.4–0.8%), linalool (0.1–0.7%), decanal (0.1–0.7%) and octanal (0.1–0.6%). Analysis of five batches of the additive by GC-FID showed compliance with these specifications: d-limonene (94.4–94.8%), myrcene (1.8–1.9%), α -pinene (0.5%), linalool (0.3–0.4%), sabinene (0.3–0.4%), decanal (0.2–0.3%) and octanal (0.2–0.3%).¹⁶ When analysed by gas chromatography–mass spectrometry (GC–MS) these seven compounds account for about 97.4% on average (range 97.0–97.8%) of the % GC area (Table 2).

Table 2: Major volatile constituents of the expressed essential oil obtained from the fruit peels of *Citrus sinensis* (L.) Osbeck as defined by the ISO standard (3140:2019): specifications and batch to batch variation based on the analysis of five 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 EU register name	CAS no	FLAVIS no	% GC area ^(b)	
			Mean ^(a)	Range
d-Limonene	5989-27-5	01.045	92.4	91.6–93.4
Myrcene	123-35-3	01.008	2.29	1.88–2.59
α -Pinene (pin-2(3)-ene)	80-56-8	01.004	0.72	0.64–0.87
Linalool	78-70-6	02.013	0.68	0.58–0.74
Sabinene (4(10)-thujene)	3387-41-5	01.059	0.53	0.37–0.69
Decanal	112-31-2	05.010	0.43	0.38–0.46
Octanal	124-13-0	05.009	0.36	0.31–0.45
Total			97.4	97.0–97.8

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.

(a): Mean calculated on five 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 of five batches obtained by GC–MS.¹⁷ In total, up to 88 constituents were detected, 55 of which were identified and accounted on average for 99.7% (99.50–99.97%) of the GC area. Besides the seven compounds indicated in the

¹⁵ Technical dossier/Supplementary information May 2020/Annex_II_SIn_Reply_orange_oil_cold_pressed_CoA.

¹⁶ Technical dossier/Supplementary information May 2020/SIn_reply_BDG08_orange_oil_cold_pressed/GC-FID analysis.

¹⁷ Technical dossier/Supplementary information May 2020/Annex_III_SIn_Reply_orange_oil_cold_pressed_chromatograms.

product specifications, 13 other compounds were detected at individual levels > 0.05% and are listed in Table 3. These 20 compounds together account on average for 99.0% (98.7–99.2%) of the product. The remaining volatile 35 compounds (ranging between 0.002% and 0.05%) and accounting together for < 1% are listed in the footnote.¹⁸

Table 3: Other volatile constituents of the expressed essential oil obtained from the fruit peels of *Citrus sinensis* (L.) Osbeck accounting for > 0.05% of the composition (based on the analysis of five 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 EU register name	CAS no	FLAVIS no	% of GC area	
			Mean ^(a)	Range
β-Phellandrene	555-10-2	01.055	0.49	0.34–0.53
δ-3-Carene	13466-78-9	01.029	0.17	0.14–0.19
Geranial (<i>trans</i> -3,7-dimethylocta-2,6-dienal)	141-27-5	05.188	0.16	0.11–0.18
Valencene	4630-07-3	01.017	0.14	0.10–0.24
α-Terpineol	98-55-5	02.014	0.11	0.08–0.13
Neral	106-26-3	05.170	0.09	0.06–0.11
Citronellal	106-23-0	05.021	0.09	0.06–0.10
Dodecanal	112-54-9	05.011	0.06	0.06–0.07
α-Copaene	3856-25-5	–	0.06	0.03–0.08
Nonanal	124-19-6	05.025	0.06	0.05–0.07
α-Phellandrene	99-83-2	01.006	0.06	0.03–0.08
δ-Cadinene	29350-73-0	01.021	0.06	0.05–0.07
Germacre-1(10),4(14),5-triene	23986-74-5	01.042	0.06	0.04–0.07
Total			1.59	1.43–1.82

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.

(a): Mean calculated on five batches.

Among the volatile components, some substances of concern were detected. Three substances, for which EFSA requested additional data to clarify the possible genotoxicity (EFSA CEF Panel, 2012, 2013), namely α-sinensal (average: 0.037%, range: 0.028–0.035%), β-sinensal (on average: 0.037%, range: 0.030–0.044%) and *p*-mentha-1,8(10)-dien-9-ol (average: 0.023%, range: 0.017–0.032%) were detected and quantified in all batches. *p*-Mentha-1,8-dien-7-al (hereinafter referred as to perillaldehyde), a substance for which EFSA identified a concern for genotoxicity (EFSA CEF Panel, 2015c), was also detected in all batches (average: 0.042%, range: 0.032–0.047%).

Non-volatile components

The non-volatile residue (residue on evaporation) of expressed orange oil accounts for 1.0–5.0% of the oil according to the European Pharmacopoeia (PhEur, 2020). The applicant performed a literature search to document the relative percentage and the composition of the non-volatile fraction in sweet orange peel oil.¹⁹ Non-volatile constituents include predominantly polymethoxylated flavones (PMF), predominantly heptamethoxyflavone (0.3–2.0 g/L), nobiletin (0.25–1.10 g/L), sinensetin (0.04–0.3 g/L), tangeretin (0.36–1.0 g/L), tetra-*O*-methoxyscutellarein (0.16–0.6 g/L) and 3,5,6,7,3',4'-hexamethoxyflavone (0–0.2 g/L) (ranges based on Dugo and Russo, 2010), and fatty acids, including caproic, lauric, myristic, palmitic, stearic, oleic and linoleic acids. Waxes and sterols are also expected to be part of the non-volatile fraction in citrus oils (Dugo et al., 2009; Gonzáles-Mas et al., 2019), although little attention has been placed on

¹⁸ Additional volatile constituents: constituents (n = 24) between < 0.05 and ≥ 0.01%: octan-1-ol, β-copaene, *trans*-3,7-dimethyl-1,3,6-octatriene, nootkatone, *p*-mentha-1,8-dien-7-al (perillaldehyde), β-cubebene, β-sinensal, terpinolene, β-caryophyllene, α-sinensal, geranyl acetate, β-pinene, *trans*-limonene epoxide, l-carvone, citronellol, *p*-mentha-1,8(10)-dien-9-ol, undecanal, α-farnesene, octyl acetate, *cis*-limonene epoxide, β-farnesene, *trans*-sabinene hydrate, *cis*-3,7-dimethyl-1,3,6-octatriene, 3,7,10-humulatriene; constituents (n = 11) between < 0.01 and ≥ 0.002%: β-elemene, γ-terpinene, isoterpinolene, 1-isopropyl-4-methylbenzene, *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, *trans*-2-decenal, camphene, sativene, γ-muurolole, hexanal and *cis*-para-2,8-menthadien-1-ol.

¹⁹ Technical dossier/Supplementary information July 2020/Request of clarification_Annex_IV_non volatile search and Supplementary information November 2020.

the composition and quantity of these components (Dugo and Di Giacomo, 2002), probably due to low toxicological concern. Wax content in cold pressed oils is commonly reduced by winterisation (de-waxing).

The literature search performed by the applicant regarding possible substances of concern and chemical composition of the plant species *C. sinensis* and its preparations did not identify furocoumarins and synephrine as substances of concern for *C. sinensis*.²⁰ Analysis of five batches²¹ showed that eight furocoumarins (phellopterin, 8-geranoxypsoralen, psoralen, 8-methoxypsoralen (xanthotoxin), 5,8-dimethoxypsoralen (isopimpinellin), imperatorin, oxypeucedanin and bergamottin (5-geranoxypsoralen)) and *p*-synephrine were below the limit of detection of the high-performance liquid chromatography (HPLC) method, confirming the findings of the literature search.

3.2.1.1. Impurities

Data on chemical and microbial impurities were provided in three batches of expressed orange oil.²² The concentrations of heavy metals and mycotoxins (aflatoxins B1, B2, G1 and G2) were below the corresponding limits of quantification (LOQs). In a multiresidue analysis, pesticides were not detected with the exception of bifenthrin (0.51–0.90 mg/kg), chlorfenapyr (< 0.02 mg/kg), cypermethrin (0.30–0.63 mg/kg), chlorpyrifos-ethyl (2.0–4.8 mg/kg), malathion (0.13–1.4 mg/kg), phosmet (< 0.2–0.39 mg/kg) and propargite (0.11–0.30 mg/kg) in some batches. In the same 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) PCDD/F + PCB TEQ ranged between 1.67 and 1.90 pg/g wet weight. None of the data on chemical impurities raised concerns.

Analysis of microbial contamination of three batches of orange oil expressed indicated that *Salmonella* spp. was not detected in 25 g, *Enterobacteriaceae*, total viable count, yeasts, moulds were < 10 colony-forming units (CFU)/g.

3.2.1.2. Shelf-life

The typical shelf-life of expressed orange 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).²³ However, no data were provided to support this statement.

3.2.1.3. Conditions of use

Orange oil expressed is intended to be added to feed for all animal species without withdrawal. The maximum proposed use level in complete feed is 80 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 144 mg/kg for piglets, 172 mg/kg for pigs for fattening, 200 mg/kg for sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 230 mg/kg for horses, 50 mg/kg for rabbits, fish and ornamental fish, and 125 mg/kg for dogs and cats.

The proposed use level in water for drinking is 20 mg/kg for all species.

3.2.2. Safety

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

Many of the major volatile components, accounting for about 99% of the % GC areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food⁷ and feed²⁴ uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Six compounds, δ -cadinene [01.021], β -cubebene [01.030], δ -germacrene [01.042], 3,7,10-humulatriene [01.043], β -phellandrene [01.055] and α -muurulene [01.052] were evaluated in

²⁰ Technical dossier/Supplementary information May 2020/Literature search_orange_oils.

²¹ Technical dossier/Supplementary information May 2020/Annex VI_SIn reply_orange_oil_cold_pressed_SOC_COA. Limit of detection (LOD): 8-geranoxypsoralen, xanthoxin, psoralen, isopimpinellin and imperatorin 0.5 mg/kg; phellopterin 1.0 mg/kg; bergamottin and *p*-synephrine 5.0 mg/kg; oxypeucedanin 10 mg/kg.

²² Technical dossier/Supplementary information May 2020/Annex VII_SIn reply_orange_oil_cold_pressed_impurities. 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 μ g/kg for aflatoxins B1, B2, G1 and G2. LOQ for PCDD/F ranging between 0.392 and 0.784 pg/g wet weight, LOQ for dl-PCBs: ranging between 4.9 and 98 pg/g wet weight. LOQ for ndl-PCBs: 0.196 pg/g wet weight.

²³ Technical dossier/Section II.

²⁴ European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: https://ec.europa.eu/food/system/files/2021-09/animal-feed_additives_eu-register_1831-03_annex2.pdf

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 foods (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional toxicity data (EFSA CEF Panel, 2011b). In the absence of such toxicological data, the CEF Panel was unable to complete its assessment. As a result, these compounds are no longer authorised for use as flavours in food. In the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach (EFSA FEEDAP Panel, 2017b) or read-across from structurally related substances.

Among the volatile components, α -copaene (accounting for > 0.06% of the GC area) and several minor components accounting for < 0.05% of the GC area (β -copaene, *trans*-sabinene hydrate, β -elemene, isoterpinolene, sativene and γ -muurolene) have not been previously assessed for use as flavouring compounds. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastro-intestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2016d).

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

The following sections focus on substances of concern or on compounds not previously assessed or not structurally related to flavourings previously assessed, e.g. perillaldehyde, *p*-mentha-1,8(10)-dien-9-ol, α -sinensal and β -sinensal, and polymethoxylated flavones (PMF), based on the evidence provided by the applicant in the form of several literature searches,²⁵ and Quantitative Structure-Activity Relationship (QSAR) analysis for substances raising potential genotoxicity concern.²⁶ A more extensive safety evaluation of PMF is available in the EFSA opinion on expressed mandarin oil (EFSA FEEDAP Panel, 2021b).

3.2.2.1. 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 constituents

The fraction of non-volatile compounds was not analysed by the applicant. According to the literature search performed by the applicant, this fraction contains PMF, predominantly heptamethoxyflavone (0.3–2.0 g/L), nobiletin (0.25–1.10 g/L), sinensetin (0.04–0.3 g/L), tangeretin (0.36–1.0 g/L), tetra-O-methoxyscutellarein (0.16–0.6 g/L) and 3,5,6,7,3',4'-hexamethoxyflavone (0–0.2 g/L).

Absorption, distribution, metabolism and excretion (ADME) data in experimental animals and humans available in the literature were provided by the applicant (Nielsen et al., 2000; Murakami et al., 2002; Breinholt et al., 2003; Koga et al., 2011; Manthey et al., 2011; Hung et al., 2018).²⁷ They were reviewed by the FEEDAP Panel in the opinion on expressed mandarin oil (EFSA FEEDAP Panel, 2021b). Briefly, the ADME studies of PMF shows 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.

3.2.2.2. Toxicology

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). Therefore, the potential genotoxicity of identified constituents is first considered. Then, genotoxicity studies performed with the essential oils obtained from *C. sinensis* are described.

²⁵ Technical dossier/Supplementary information May 2020, Supplementary information July 2020 and Supplementary information November 2020.

²⁶ Technical dossier/Supplementary information May 2020/Annex IX_SIn reply orange cold pressed QSAR.

²⁷ Technical dossier/Supplementary information November 2020.

Volatile components

Expressed orange oil contains perillaldehyde (average: 0.042%, range: 0.032–0.047%), a substance for which EFSA identified a concern for genotoxicity (EFSA CEF Panel, 2015c), which was confirmed by JECFA (WHO, 2018).

The oil also contains *p*-mentha-1,8(10)-dien-9-ol [02.122] (0.017–0.032%), α -sinensal (0.028–0.035%) and β -sinensal (0.03–0.044%), for which EFSA requested further *in vivo* data to clarify the possible genotoxicity observed *in vitro* (see Section 3.2.1, EFSA CEF Panel, 2012, 2013). Following the EFSA request, the industry decided to no longer support these compounds which were withdrawn from the market. In the absence of data, the EFSA CEF Panel was unable to complete its assessment and uncertainty remains on their potential genotoxicity.

These compounds together with other compounds accounting for < 0.05% and not previously assessed as flavourings (*trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, *cis*-limonene-epoxide and *trans*-limonene epoxide) were screened with the Organisation for Economic Co-operation and Development (OECD) 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.²⁸

The genotoxicity of d-limonene epoxide (*cis/trans*-isomer not specified), 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 d-limonene epoxide, no increase in sister chromatid exchange was observed (von der Hude et al., 1991, as referenced in EFSA CEF Panel, 2014).

Non-volatile components

Genotoxicity data on PMF available in the literature were provided by the applicant (Delaney et al., 2002; Nakajima et al., 2020).²⁷ They were reviewed by the FEEDAP Panel in the EFSA opinion on expressed mandarin oil (EFSA FEEDAP Panel, 2021b). Briefly, a mixture of PMF (containing nobiletin 32.5%, tangeretin 14.0% and heptamethoxyflavone 25% and other components²⁹) was not mutagenic in an Ames test and in an *in vitro* mutagenicity assay using L5178Y *tk*^{+/-} mouse lymphoma cells (Delaney et al., 2002). 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 not mutagenic in the Ames test *in vitro* and did not induce chromosomal damage *in vivo* (Nakajima et al., 2020).

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

Genotoxicity of essential oils obtained from *Citrus sinensis* (L.)

The applicant also provided a structured database literature search,³⁰ which identified two references on the genotoxicity of essential oils extracted from the peel of the *C. sinensis* and were considered relevant (Cohen et al., 2019; Karlović et al., 2004).

The evaluation of the safety of 54 Citrus-derived natural flavour complexes (NFCs) for their intended use as flavouring ingredients was carried out by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) in 2015 and reported by Cohen et al. (2019). The test item, 'Orange peel sweet oil' (cold-expressed from the peel of the fruit FEMA No. 2825) showed a similar composition (d-limonene 95%, myrcene 2%) as the cold expressed orange oil under assessment. *In vitro* and *in vivo* genotoxicity studies on Sweet orange oil are summarised in the report, concluding that, overall, Citrus oils including sweet orange oil and the major individual Citrus constituents gave negative results for genotoxicity.

The potential to induce gene mutations of orange oil (botanical origin not specified) was evaluated in *Salmonella* Typhimurium strains TA98 and TA100 applying the plate incorporation method in the

²⁸ Technical dossier/Supplementary information May 2020/Annex IX_SIn_reply_orange_oil_cold pressed_QSAR. Structural alerts for *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol and *p*-mentha-1,8(10)-dien-9-ol were due to the presence of vinyl/allyl alcohols, for α -sinensal and β -sinensal to the presence of aldehydes and for *cis*- and *trans*-limonene epoxide to the presence of the epoxide. In all cases, predictions of Ames mutagenicity were 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 *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, *p*-mentha-1,8(10)-dien-9-ol, α -sinensal, β -sinensal, *cis*- and *trans*-limonene epoxide were discounted.

²⁹ Other components of the mixture: trimethylscutellarein (9.1%), sinensetin (3.9%), 5-demethyl-nobiletin (2.8%), hexa-O-methylquercetagenin (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%).

³⁰ Technical dossier/Supplementary information May 2020/Literature search_orange oils.

presence and absence of metabolic activation. Five concentrations were tested using 10 μL , 30 μL , 50 μL , 100 μL and 200 μL /plate. Significant toxicity was induced at 50 μL /plate and above. No changes in the number of revertant colonies were observed at 10 and 30 μL /plate. However, data on the number of revertant colonies were not shown in the study report (Karlović et al., 2004).

Repeated-dose toxicity studies

Volatile components

No information is available on the subchronic toxicity of 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 in rats at doses of 54, 180, or 540 mg/kg body weight (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 has been frequently reported to 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, 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. 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.

3.2.2.3. 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 (> 99.5%), the 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 orange oil as a feed additive. Feeding animals citrus by-products is a common practice with no report of adverse effects (Bampidis and Robinson, 2006; Feedipedia³¹).

Volatile components

Based on considerations related to structural and metabolic similarities, the components accounting for > 0.05% were allocated to seven assessment groups, corresponding to the chemical groups (CGs) 1, 3, 4, 6, 8, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), the application of sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 is 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.

³¹ <https://www.feedipedia.org/node/680>

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 assessed 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 of subchronic studies, from which NOAELs 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] in CG 4 (EFSA FEEDAP Panel, 2016b), terpineol³² [02.230] and linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), l-carvone [07.147] in CG 8 (EFSA FEEDAP Panel, 2016c), myrcene [01.008], d-limonene [01.045], *p*-cymene [01.002] and β -caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016d).

Considering the structural and metabolic similarities, read-across was applied using the NOAEL of 120 mg/kg bw per day for octyl acetate [09.007] to decanal [05.010], octanal [05.009], nonanal [05.025], dodecanal [05.011], octan-1-ol [02.006], undecanal [05.034] and hexanal [05.008], and selected as the reference point for CG 1.

Read-across was also applied using the NOAEL of 345 mg/kg bw per day for citral [05.020] to neral [05.170], geranial [05.188] and geranyl acetate [09.011] in CG 3, and the NOAEL of citronellol [02.011] to citronellal [05.021] in CG 4.

For α -terpineol [02.014] in CG 6, 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].

Considering the structural and metabolic similarities, the NOAELs for the representative compounds of CG 31, myrcene [01.008], d-limonene [01.045] and β -caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (α -farnesene [01.040], β -farnesene [01.041], *cis*- β -ocimene [01.064] and *trans*- β -ocimene), group III (β -phellandrene [01.055], α -phellandrene [01.006], terpinolene [01.005], isoterpinolene and γ -terpinene [01.020]) and group V (α -pinene [01.004], sabinene [01.059], valencene [01.017], δ -3-carene [01.029], α -copaene, δ -cadinene [01.021], β -copaene, β -cubebene [01.030], β -pinene [01.003] and camphene [01.009] and to *trans*-sabinene hydrate in CG 8) (EFSA CEF Panel, 2015a,b).

For the remaining compounds, namely *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, nootkatone [07.089], β -elemene, γ -muurulene, sativene, germacra-1(10),4(14),5-triene [01.042] and 3,7,10-humulatriene [01.043], 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, respectively, for Cramer Class I, II and III compounds). 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 Scientific Committee, 2019a). A MOET > 100 allowed for interspecies- and intra-individual variability (as in the default 10 \times 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.³³

The approach to the safety assessment of expressed orange oil for the target species is summarised in Table 4. The calculations were based on chickens for fattening, the species with the highest ratio of feed intake/body weight, representing the worst-case scenario at the use level of 80 mg/kg.

³² Terpineol is a mixture of four isomers: α -terpineol [02.014], a mixture of (*R*)-(+)- α -terpineol and (*S*)-(-)- α -terpineol, β -terpineol, γ -terpineol and 4-terpinenol [02.072].

³³ Compounds included in the assessment groups but not reported in the table: undecanal, octyl acetate and hexanal (CG 1); *trans*-2-decenal (CG 3); *trans*-sabinene hydrate (CG 8); terpinolene, β -elemene, isoterpinolene and γ -terpinene (CG 31, III); 4-isopropenyl-4-methylbenzene (CG 31, IVe); β -copaene, β -cubebene, β -pinene, β -caryophyllene and camphene (CG 31, V).

Table 4: Compositional data, intake values (calculated for chickens for fattening at 80 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of expressed orange 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 ^(b)	NOAEL ^(c)	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 1								
Decanal	05.010	0.46	0.365	0.0327	(I)	120	3,664	
Octanal	05.009	0.45	0.358	0.0322	(I)	120	3,730	
Dodecanal	05.011	0.07	0.059	0.0053	(I)	120	22,580	
Nonanal	05.025	0.07	0.057	0.0051	(I)	120	23,534	
Octan-1-ol	02.006	0.06	0.046	0.0041	(I)	120	29,314	
MOET CG 1								1,511
CG 3								
Geranial	05.188	0.18	0.143	0.0129	(I)	345	26,837	
Neral	05.170	0.11	0.086	0.0077	(I)	345	44,895	
Geranyl acetate	09.011	0.11	0.087	0.0078	(I)	345	44,072	
MOET CG 3								12,162
CG 4								
Citronellal	05.021	0.10	0.080	0.0072	(I)	50	6,962	
Citronellol	02.011	0.03	0.024	0.0022	(I)	50	23,207	
MOET CG 4								5,355
CG 6								
Linalool	02.013	0.74	0.594	0.0534	(I)	117	2,193	
α -Terpineol	02.014	0.13	0.101	0.0090	(I)	250	27,627	
<i>trans</i> -1-methyl-4-(1-methyl vinyl)cyclohex-2-en-1-ol	–	0.01	0.006	0.0006	I	3	5,222	
<i>cis</i> - <i>para</i> -2,8-Menthadien-1-ol	–	0.01	0.006	0.0006	II	0.91	1,584	
MOET CG 6								760
CG 8								
Nootkatone	07.089	0.08	0.065	0.0058	II	0.91	156	
l-Carvone	07.147	0.03	0.027	0.0024	(I)	60	24,572	
MOET CG 8								155
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	2.59	2.072	0.1860	(I)	44	237	
β -Ocimene <i>trans</i>	–	0.07	0.052	0.0047	(I)	44	9,426	
α -Farnesene	01.040	0.02	0.017	0.0015	(I)	44	29,174	
β -Farnesene	01.041	0.02	0.014	0.0013	(I)	44	34,037	
β -Ocimene, <i>cis</i>	01.064	0.002	0.014	0.0012	(I)	44	36,039	
MOET CG 31, II								226
CG 31, III (Cyclohexene hydrocarbons)								
d-Limonene	01.045	93.4	74.72	6.7078	(I)	250	37	
β -Phellandrene	01.055	0.53	0.421	0.0378	(I)	250	6,618	
α -Phellandrene	01.006	0.08	0.065	0.0058	(I)	250	42,975	
MOET CG 31, III								37

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max feed conc.	Intake ^(a)	Cramer class ^(b)	NOAEL ^(c)	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 31, V (Bi-, tricyclic, non aromatic hydrocarbons)								
α -Pinene	01.004	0.87	0.699	0.0628	(I)	222	3,537	
Sabinene	01.059	0.69	0.555	0.0498	(I)	222	4,454	
Valencene	01.017	0.24	0.195	0.0175	(I)	222	12,669	
δ -3-Carene	01.029	0.19	0.155	0.0139	(I)	222	15,934	
α -Copaene	–	0.08	0.066	0.0060	(I)	222	37,243	
δ -Cadinene	01.021	0.07	0.055	0.0050	(I)	222	44,799	
γ -Muurolene	–	0.01	0.008	0.0007	I	3	4,177	
Sativene	–	0.004	0.003	0.0003	I	3	10,443	
MOET CG 31, V								968
CG 31, VI (macrocyclic non aromatic hydrocarbons)								
δ -Germacrene	01.042	0.07	0.057	0.0051	I	3	588	
3,7,10-Humulatriene	01.043	0.02	0.013	0.0011	I	3	2,611	
MOET CG 31, VI								480
CG 32 (epoxides)								
<i>cis</i> -Limonene epoxide	–	0.04	0.030	0.0027	I	3	1,129	
<i>trans</i> -Limonene epoxide	–	0.03	0.025	0.0022	I	3	1,347	
MOET CG 32								614

(a): Intake calculations for the individual components are based on the use level of 80 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): When a NOAEL value is available or read-across is applied, the allocation to the Cramer class is put into parentheses.

(c): 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.

As shown in Table 4, for all the assessment groups, the MOET was ≥ 100 except for d-limonene and the assessment group CG 31, III, for which the MOE was 37.

From the lowest MOET of 37 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 target animal categories at the proposed use level

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use level (mg/kg feed) ^(a)	Lowest MOET
Chicken for fattening	2	158	80	37
Laying hen	2	106	80	55
Turkey for fattening	3	176	80	50
Piglet	20	880	144	37
Pig for fattening	60	2,200	172	37
Sow lactating	175	5,280	200	39
Veal calf (milk replacer)	100	1,890	130	95
Cattle for fattening	400	8,000	130	90

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use level (mg/kg feed) ^(a)	Lowest MOET
Dairy cow	650	20,000	130	58
Sheep/goat	60	1,200	130	90
Horse	400	8,000	230	51
Rabbit	2	100	50	94
Salmon	0.12	2.1	50	260
Dog	15	250	125	110
Cat	3	60	125	94 ^(b)
Ornamental fish	0.012	0.54	50	935

(a): Complete feed containing 88% DM, milk replacer 94.5% DM.

(b): The MOET for cats is increased to 500 because of the reduced capacity of glucuronidation.

At the proposed use levels, the MOET was below the value of 100 for all species except for fish and dogs. For cats, a higher magnitude of the MOET is needed (> 500) considering their unusually low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021).

In the assessment of d-limonene as a feed flavouring, the FEEDAP Panel noted that 'd-limonene is a common constituent of feed material and forage (Cifuni et al., 2005). Feeding animals citrus by-products is a common practice (Bampidis and Robinson, 2006). Taking into consideration d-limonene content in citrus peel (25 g/kg (IARC, 1993)) and peel content of citrus by-products (650 g/kg dry matter (DM)), as well as the inclusion levels of citrus by-products in animal diets (100 g/kg in dogs, 120 g/kg in poultry, 150–200 g/kg in pigs and 450–600 g/kg in cattle) obtained from various sources (Malafaia et al., 2002; O'Sullivan et al., 2003; Bampidis and Robinson, 2006; Nazok et al., 2010; Brambillasca et al., 2013; Crosswhite et al., 2013), the content of d-limonene in feed was calculated to be 1,625 mg/kg DM in dog feed, 1,950 mg/kg DM in poultry feed, 2,440–3,250 mg/kg in pig feed and 7,310–9,750 mg/kg DM in feed for ruminants. These concentrations are 65- to 390-fold higher than the high use level in feed (25 mg/kg) proposed by the applicant as feed flavouring. Target animal exposure to d-limonene was calculated to be 0.21 g/day in poultry, 0.41 g/day in dogs, 6.4–17.2 g/day in pigs and 78–146 g/day in cattle'. The FEEDAP Panel concluded that, d-limonene is considered safe for all animal species at the proposed maximum use level (25 mg/kg complete feed) (EFSA FEEDAP Panel, 2015). Only for cats, conclusion was based on the NOAEL of 250 mg/kg bw per day. To further support this approach, the applicant provided additional evidence from literature, which shows that limonene is ubiquitous in feed material and forage, such as citrus-by products.³⁴

Based on the same comparative approach, the above concentrations of d-limonene in feed are 10- to 81-fold higher than the high use level in d-limonene resulting from the use of expressed orange oil at the proposed use levels in feed. For cats, the maximum safe use levels in feed of 25 mg/kg complete feed was calculated in order to ensure a MOET \geq 500 for cats, considering their unusually low capacity for glucuronidation (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 orange oil when used as a feed additive for all animal species except cats up to the maximum proposed use levels in complete feed of 80 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 144 mg/kg for piglets, 172 mg/kg for pigs for fattening, 200 mg/kg for sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 230 mg/kg for horses, 50 mg/kg for rabbits, fish and ornamental fish, and 125 mg/kg for dogs. For cats, the maximum safe concentration is 25 mg/kg complete feed.

When used in water for drinking, the intake of the additive via water would be two or three times higher than the intake via feed for poultry, pigs and rabbits (EFSA FEEDAP Panel, 2010). The applicant proposed a maximum use level of 20 mg/kg for the use in water for drinking. The 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.

Volatile components: Perillaldehyde

Low concentrations of perillaldehyde were detected in all batches of the additive under assessment (average: 0.041%, range: 0.032–0.047%). The use of expressed orange oil at the proposed use levels

³⁴ Technical dossier/Supplementary information May 2020/Appendix_II_Limonene in feed.

in feed for the different target species (ranging from 80 to 230 mg/kg complete feed, see Section 3.2.2), would result in a daily intake of perillaldehyde up to 3.4 µg/kg bw for poultry and pigs, 2.1 µg/kg bw for ruminants, 2.5 µg/kg bw for horse, 1.3 µg/kg bw for rabbit and 0.5 µg/kg bw for fish.³⁵

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).³⁶ 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 horses, 10 g DM/kg bw for rabbits and 3.6 g DM/kg bw for fish.

Based on the literature data provided by the applicant³⁷ 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-product³⁸ (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.³⁹ 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 10-fold higher than those resulting from the high use level of expressed orange oil in feed as proposed by the applicant (80–230 mg expressed orange oil/kg feed).

Non-volatile components: Polymethoxylated flavones

The main non-volatile constituents in expressed orange oil are PMF, mainly tangeretin and nobiletin. Based on the data reported in the literature on the maximum occurrence of PMF in orange oil (approximately 0.2% heptamethoxyflavone, 0.1% tangeretin, 0.1% nobiletin, 0.06% tetra-O-methoxyscutellarein, 0.03% sinensetin and 0.02% 3,5,6,7,3',4'-hexamethoxyflavone, total PMF 0.5%, 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 80 mg/kg in poultry to 230 mg/kg in horses, see Section 3.2.2) was calculated to range between 0.4 and 1.15 mg PMF/kg complete feed.

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 per day was selected as a group NOAEL for PMF. Applying an uncertainty factor (UF) of 100 to the NOAEL, the safe daily dose of PMF for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), 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 orange 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 different target animal categories

Animal category	Default values		Maximum safe intake/feed 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

³⁵ 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%).

³⁶ Technical dossier/Supplementary information July 2020/SiIn FAD-2010-322-request of clarification.

³⁷ Technical dossier/Supplementary information/November 2020.

³⁸ 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.

³⁹ Occurrence of perillaldehyde in citrus by-products calculated considering the composition of citrus by-products as 60% oranges, 20% lemon and lime, 30% mandarin (Mahato et al., 2019): $0.0006\% \times 0.6 + 0.0002 \times 0.3 + 0.0002 \times 0.1 = 0.0004\%$.

Animal category	Default values		Maximum safe intake/feed concentration	
	Body weight (kg)	Feed intake (g DM/day)	Intake (mg/day)	Concentration in feed (mg/kg feed) ^(a)
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
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.

As shown in Table 6, the calculated safe PMF concentrations in feed for the different animal categories are well above the concentration expected in feed from the use of the additive at the highest proposed use levels (ranging between 0.4 and 1.15 mg PMF/kg complete feed). The FEEDAP Panel concludes that the presence of PMF in expressed orange oil does not raise concern for the target species.

Conclusions on safety for the target species

The FEEDAP Panel concludes that expressed orange oil from the fruit peels of *C. sinensis* is safe up to the maximum proposed use levels in complete feed of 80 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 144 mg/kg for piglets, 172 mg/kg for pigs for fattening, 200 mg/kg for sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 200 mg/kg for horses, 50 mg/kg for rabbits and fish.

These target species are fed citrus by-products as part of their daily feed. For these species, the use of expressed orange oil in feed is not expected to increase the exposure to perillaldehyde to a relevant extent (< 10%). 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 of 20 mg/kg is safe for all animal species except for dogs and cats, provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.2.2.4. Safety for the consumer

Sweet orange peel oil obtained by cold expression and its fractions are 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 flavour ingredients (Burdock, 2009) cites intake values of 3.1 mg/kg bw per day for expressed orange oil, 0.05 mg/kg bw per day for orange oil terpeneless and 0.38 mg/kg bw per day for orange oil distilled.

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, see Section 1.2).

No data on residues in products of animal origin were made available for any of the constituents of the additive. However, the Panel recognises that the constituents of expressed orange oil are expected to be extensively metabolised and excreted in the target species (see Section 3.2.2.1). 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 sweet orange peel oil preparations in food (Burdock, 2009), it is unlikely that consumption of products from animals given expressed orange 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 orange oil up to the highest safe use level in feed.

3.2.2.5. Safety for the user

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

The applicant produced a safety data sheet⁴⁰ for orange oil where hazards for users have been identified. The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser.

3.2.2.6. Safety for the environment

C. sinensis is a native species to Europe where it is widely grown both for commercial and decorative purposes. Use of the essential oil under the proposed conditions of use in animal production is not expected to pose a risk for the environment.

3.3. Distilled orange oil

This application concerns the volatile fraction (i.e. the distillate) from the distillation of orange oil expressed (cold pressed), which is obtained from the fruit peel of *C. sinensis*.

3.3.1. Characterisation of the distilled fraction

Distilled orange oil is a colourless clear mobile liquid with a characteristic odour. In five batches of the additive, the relative density (at 20°C) ranged between 837 and 842 kg/m³ (specification: 837–847 kg/m³) and the refractive index (at 20°C) was 1.472–1.473 (specification: 1.471–1.474).⁴¹

The product specifications for distilled orange oil are based on the standards developed by ISO (3140:2019) for essential oil of sweet orange expressed (*C. sinensis* (L.)), which were adapted to reflect the concentrations of the main components of the essential oil, analysed by GC-FID and expressed as % GC area. These components are d-limonene (93–97.5%, the phytochemical marker), myrcene (1.5–3.5%), sabinene (0.2–1.0%), α -pinene (0.3–0.8%), linalool (0.05–0.5%) and octanal (0.05–0.4%). Analysis of five batches of the additive by GC-FID showed compliance with these specifications: d-limonene (95.6–96.3%), myrcene (1.8–2.2%), α -pinene (0.5–0.6%), sabinene (0.3–0.4%), linalool (0.1–0.3%) and octanal (0.1–0.3%).⁴² When analysed by GC-MS, these six compounds account for about 97.4% on average (range 97.0–97.8%) of the % of the GC area (Table 7).

Table 7: Major constituents of distilled fraction of expressed orange oil obtained from the fruit peels of *Citrus sinensis* (L.) Osbeck as defined by the ISO standard (3140:2019) specifications and batch to batch variation based on the analysis of five 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 EU register name	CAS no	FLAVIS no	% of GC area ^(b)	
			Mean ^(a)	Range
d-Limonene	5989-27-5	01.045	92.6	91.3–94.6
Myrcene	123-35-3	01.008	3.14	2.50–3.58
α -Pinene (pin-2(3)-ene)	80-56-8	01.004	1.09	0.77–1.29
Sabinene (4(10)-thujene)	3387-41-5	01.059	0.70	0.54–0.88
Linalool	78-70-6	02.013	0.40	0.21–0.74
Octanal	124-13-0	05.009	0.28	0.15–0.41
Total			98.2	96.9–98.9

⁴⁰ Technical dossier/Supplementary Information May 2020/Annex_X_SIn_reply_orange_oil_cold_pressed_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315), skin sensitisation (H317, category 1B).

⁴¹ Technical dossier/supplementary information May 2020/Annex_II_SIn_reply_orange_terpenes_COA_batches.

⁴² Technical dossier/Supplementary information May 2020. SIn_reply_BDG08_orange_oil_terpenes/GC-FID analysis.

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.

(a): Mean calculated on five 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 five batches obtained by GC-MS.⁴³ In total, up to 52 constituents were detected, 39 of which were identified and accounted on average for 99.83% (99.77–99.90) of the product (as the GC area). Besides the six compounds indicated in the product specifications, three other compounds were detected at levels > 0.05% and are listed in Table 8. The remaining 30 compounds (ranging between 0.001% and 0.05%) are listed in the footnote.⁴⁴

Table 8: Other volatile constituents of the distilled fraction of expressed orange oil obtained from the fruit peels of *Citrus sinensis* (L.) Osbeck accounting for > 0.05% of the composition (based on the analysis of five 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 EU register name	CAS no	FLAVIS no	% of GC area	
			Mean ^(a)	Range
β-Phellandrene	555-10-2	01.055	0.85	0.14–2.32
δ-3-Carene	13466-78-9	01.029	0.21	0.14–0.24
Octan-1-ol	111-87-5	02.006	0.13	0.04–0.28

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.

(a): Mean calculated on five batches.

The HPLC analysis of five batches⁴⁵ of distilled orange oil showed that furocoumarins (phellopterin, 8-geranoxypsoralen, psoralen, 5-methoxypsoralen (bergapten), 8-methoxypsoralen (xanthotoxin), dimethoxypsoralen (isopimpinellin), imperatorin, oxypeucedanin and bergamottin (5-geranoxypsoralen) and synephrine were below the limit of detection (LOD).

3.3.1.1. Impurities

The applicant makes reference to the 'periodic testing' of some representative flavourings premixtures for heavy metals (mercury, cadmium and lead), arsenic, fluoride, dioxins and PCBs, organochloride pesticides, organophosphorus pesticides, aflatoxin B1, B2, G1, G2 and ochratoxin A. However, no data has been provided on the presence of these impurities. Since distilled orange oil is produced by steam distillation, the likelihood of any measurable carry-over of heavy metals is low except for mercury.

3.3.1.2. Shelf-life

The typical shelf-life of the distilled orange 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).²³ However, no data supporting this statement was provided.

3.3.1.3. Conditions of use

Distilled orange oil is intended to be added to feed for all animal species without withdrawal. The maximum proposed use level in complete feed is 80 mg/kg for chickens for fattening, laying hens and

⁴³ Technical dossier/Supplementary information May 2020/Annex III_SIn_Reply_orange_terpenes_chromatograms.

⁴⁴ Additional constituents: *cis*-limonene oxide, α -phellandrene, pin-2(10)-ene, *trans*-carveol, γ -terpinene, nonanal, terpinolene, *trans*- β -ocimene, 1-isopropyl-4-methylbenzene, carvone, *trans*-limonene oxide, *cis*-carveol, decanal, *cis*- β -ocimene, *trans*-sabinene hydrate, *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, geranial, citronellal, neral, isoterpinolene, nonan-2-ol, camphene, citronellol, *p*-mentha-1,8-dien-7-ol, *p*-mentha-1,8-dien-7-al, 4-terpinenol, decan-1-ol, nerol, hexanal and α -terpineol.

⁴⁵ Technical dossier/Supplementary information May 2020/Annex VI_SIn_reply_orange_terpenes_SOC_COA. Limit of detection (LOD): 8-geranoxypsoralen, xanthoxin, psoralen, isopimpinellin and imperatorin 0.5 mg/kg; phellopterin 1.0 mg/kg; bergamottin and *p*-synephrine 5.0 mg/kg; oxypeucedanin 10 mg/kg.

turkeys for fattening, 200 mg/kg for piglets, pigs for fattening and sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 225 mg/kg for horses, 80 mg/kg for rabbits, fish, dogs and cats, ornamental fish and ornamental birds.

No specific proposal was made for the use in water for drinking.

3.3.2. Safety

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

Many of the major volatile components, accounting for about 99.5% of the % GC areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food⁷ and feed⁸ uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Additional considerations on the volatile components not assessed by EFSA have been addressed in Section 3.2.2.

The ADME and the considerations relevant to perillaldehyde has been already addressed in Sections 3.2.2.1 and 3.2.2.2.

3.3.2.1. Safety for the target species

Considering the similarity in the composition of the volatile fraction of expressed orange oil and its distilled fraction, the same conclusions reached on the safety for the target species for expressed orange oil (see Section 3.2.2.3) apply to distilled orange oil, except for perillaldehyde for which a separate assessment is presented.

With respect to the exposure to the volatiles present in the additive (except perillaldehyde) no safety concern was identified for expressed distilled orange oil when used as a feed additive for all animal species (except cats) up to the maximum proposed use levels in complete feed of 80 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 200 mg/kg for piglets, pigs for fattening and sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 225 mg/kg for horses, 80 mg/kg for rabbits, fish, ornamental fish and ornamental birds, and dogs. For cats, the maximum safe concentration is 25 mg/kg complete feed.

No specific proposals have been made by the applicant for the use level in water for drinking. The 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.

Perillaldehyde

Low concentrations of perillaldehyde were detected in two batches of the additive under assessment (0.002 and 0.005%). The use of distilled orange oil at the proposed use levels in feed for the different target species (ranging from 80 to 230 mg/kg complete feed, see Section 3.3.1.3), would result in a daily intake of perillaldehyde up to 0.36 µg/kg bw for poultry and pigs, 0.23 µg/kg bw for ruminants, 0.26 µg/kg bw for horses, 0.14 µg/kg bw for rabbit and 0.05 µg/kg bw for fish.³⁵

These concentrations are at least 100-fold lower than those resulting from the intake of perillaldehyde from citrus by-products, when used in diets at concentrations of 10% for poultry and 20% for the other species (daily intake 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 rabbit and 14 µg/kg bw for fish, see section 3.2.2.3, *Volatile components: perillaldehyde*).

Conclusions on safety for the target species

The FEEDAP Panel concludes that distilled orange oil is safe up to the maximum proposed use level in complete feed of 80 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 200 mg/kg for piglets, pigs for fattening and sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 225 mg/kg for horses, 80 mg/kg for rabbits and fish.

These target species are fed citrus by-products as part of daily feed. For these species, the use of distilled orange oil in feed is not expected to increase the exposure to perillaldehyde to a relevant extent (< 1%). For dogs, cats, ornamental fish and ornamental birds, not normally exposed to citrus by-products, no conclusion can be drawn.

The Panel considers that the use level in water for drinking is safe for all animal species except dogs, cats and ornamental birds, provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.3.2.2. Safety for the consumer

Considering the similarity in the composition of the volatile fraction of expressed orange oil and its distilled fraction, the same considerations on the volatile constituents apply to the assessment of the safety for the consumer (see Section 3.2.2.4).

Consequently, no safety concern would be expected for the consumer from the use of the distilled orange oil up to the highest safe use level in feed.

3.3.2.3. 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 sheet⁴⁶ for distilled orange oil where hazards for users have been identified. The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser.

3.3.2.4. Safety for the environment

C. sinensis is a native species to Europe where it is widely grown both for commercial and decorative purposes. Use of the essential oil under the proposed conditions of use in animal production is not expected to pose a risk for the environment.

3.4. Folded orange oils

The applicant provided data on three folded oils produced by the fractional distillation of an expressed oil.⁴⁷ In each case, the percentage remaining after drying at 100 [REDACTED] of three representative batches of each folded oil was determined.⁴⁸ It can be assumed that the volatiles were removed during drying and that the dry matter values obtained provide a measure of the non-volatile components of the folded oils.

Folded oil A (described by the applicant as orange oil terpeneless *4). Fractional distillation of [REDACTED] expressed oil [REDACTED] with a mean dry matter content of 10.5% (range 9.6–11.9%). Based on these values, [REDACTED]

[REDACTED] an enrichment factor for the non-volatiles of about 4.

Folded oil B (described by the applicant as orange oil terpeneless *10). Fractional distillation of [REDACTED] expressed oil [REDACTED] with a mean dry matter content of 20.9% (range 20.2–21.5%). Based on these values, [REDACTED]

[REDACTED] an enrichment factor for the non-volatiles of about 10.

Folded oil C (described by the applicant only as a folded oil). [REDACTED]

3.4.1. Characterisation of the folded oils

Folded oils are clear orange mobile liquids with a characteristic odour. In five batches of each type of oil, the relative density at 20°C was in the range 856–857 kg/m³ (specification: 852–862 kg/m³) for folded oil **A**, 872–876 kg/m³ (specification: 867–889 kg/m³) for folded oil **B** and [REDACTED] for folded oil **C**; the refractive index at 20°C was in the range 1.475–1.477 (specification: 1.473–1.479) for folded oil **A**, 1.479–1.480 for folded oil **B** (specification: 1.475–1.485) and [REDACTED] for folded oil **C**.⁴⁹ The optical rotation at 20°C was in the range [REDACTED] for folded oil **C**. Folded orange oils are identified with the CAS number 8028-48-6,⁵⁰ and the FEMA number 2822.

⁴⁶ Technical dossier/Supplementary Information May 2020/Annex_XI_SIn_reply_orange_oil_terpeneless_MSDS_4 and Annex_XII_SIn_reply_orange_oil_terpeneless_MSDS_10. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1).

⁴⁷ Technical dossier/Supplementary Information May 2020/SIn_reply_BDG08_orange_oil_terpeneless.

⁴⁸ Technical dossier/Supplementary information June 2021/Annexes_IIa-IIc_Dry_matter_orange_oil_terpeneless_1 × 4, Annexes_IIa-IIc_Dry_matter_orange_oil_terpeneless_1 × 10 and [REDACTED]

⁴⁹ Technical dossier/Supplementary information May 2020/Annex_II_SIn_Reply_orange_oil_terpeneless_batches_CoA and [REDACTED]

⁵⁰ Technical dossier/Supplementary information May 2020/Annex_XI_SIn_Reply_orange_oil_terpeneless_MSDS_4 and Annex_XII_SIn_reply_orange_oil_terpeneless_MSDS_10.

Volatile components

The applicant proposed different specifications for the three folded oils based on the concentrations of the main volatile components analysed by GC-FID and expressed as % of GC peak area of the total volatile fraction (area % area).

The volatile fraction of folded oil **A** is specified to contain d-limonene (89–96%, the phytochemical marker), decanal (0.5–2.0%), linalool (0.7–1.7%), myrcene (0.1–1.0%) and geranial (0.1–1.0%); the volatile fraction of folded oil **B** is specified to contain d-limonene (79–89%, the phytochemical marker), decanal (3.0–5.0%), linalool (2.0–5.0%), myrcene (0.1–1.0%) and geranial (0.5–1.8%); and the volatile fraction of folded oil **C** is specified to contain d-limonene (85–95%, the phytochemical marker) and linalool (0.5–4.0%). For each folded oil, compliance with the respective specifications for the phytochemical marker (d-limonene) was shown by GC-FID analysis in the samples submitted to the EURL.⁵¹

When analysed by GC-MS in five batches of each oil,⁵² these five compounds account for about 92.3% on average (range 89.7–93.6%) of the volatile fractions of folded oil **A**, for 79.9% (range 76.8–82.0%) of the volatile fraction of folded oil **B**, and for [REDACTED] of the volatile fraction of folded oil **C** (Table 9).

Table 9: Major constituents of the volatile fraction of the folded oils obtained from the fruit peel of *Citrus sinensis* (L.) Osbeck: batch to batch variation based on the analysis of five batches for each type. 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	CAS no	FLAVIS no	(% of GC area) ^(b)					
			A		B		C	
			Mean ^(a)	Range	Mean ^(a)	Range	[REDACTED]	[REDACTED]
d-Limonene	5989-27-5	01.045	87.2	83.3–89.0	68.4	64.6–71.6	[REDACTED]	[REDACTED]
Linalool	78-70-6	02.013	2.35	1.73–3.21	4.06	2.92–5.51	[REDACTED]	[REDACTED]
Decanal	112-31-2	05.010	1.53	0.87–1.92	5.56	4.75–6.44	[REDACTED]	[REDACTED]
Myrcene	123-35-3	01.008	0.76	0.48–1.10	0.36	0.21–0.58	[REDACTED]	[REDACTED]
Geranial	141-27-5	05.188	0.52	0.41–0.61	1.60	1.03–1.99	[REDACTED]	[REDACTED]
Total			92.3	89.7–93.6	79.9	76.8–82.0	[REDACTED]	[REDACTED]

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.

(a): Mean calculated on five 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 fraction in five batches of the folded oils obtained by GC-MS.⁵² In total, up to 158 constituents were detected, of which 96 were identified in the folded oil **A** and accounted on average for 99.5% (99.1–99.8%) of the % GC area. In the folded oil **B**, 103 identified compounds accounted for 99.2% (99.1–99.3%) of the % GC area. In the folded oil **C**, 32 components accounted for [REDACTED] of % GC area. Besides the five compounds indicated in the product specifications, the 15 other compounds listed in Table 3 for expressed orange oil were also detected in the three types of the folded oils (Table 10). These 20 compounds together account on average for 95.3% (93.1–96.9%), 86.5% (83.9–87.7%) and [REDACTED] of the volatile fraction of the folded oils **A**, **B** and **C**, respectively. The FEEDAP Panel notes that these percentages refer only to the volatile part as 100% and do not consider the whole additive as 100%. When related to the whole additive as 100%, these figures may be reduced of about 12% to 21% to account for the presence of non-volatiles in the folded oils.

⁵¹ Technical dossier/Supplementary information May 2020/Appendix_I_EURL_orange_oil_terpeneless.

⁵² Technical dossier/Supplementary information May 2020/Annex_III_SIn_Reply_orange_oil_terpeneless_analytical_results and [REDACTED]

Table 10: Other constituents of the volatile fraction of the folded oils obtained from the fruit peels of *C. sinensis* (L.) Osbeck accounting for > 0.1% of the volatile fraction of the additive (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 EU register name	CAS no	FLAVIS no	(% of GC area)					
			A		B		C	
			Mean ^(a)	Range	Mean ^(a)	Range	Mean ^(a)	Range
α -Pinene	80-56-8	01.004	0.02	0.001–0.06	0.01	0.002–0.01	0.01	0.001–0.06
Sabinene	3387-41-5	01.059	0.07	0.02–0.13	0.02	0.01–0.05	0.02	0.01–0.05
Octanal	124-13-0	05.009	0.08	0.05–0.12	0.06	0.03–0.10	0.06	0.03–0.10
β -Phellandrene	555-10-2	01.055	0.28	0.02–0.48	0.32	0.25–0.41	0.28	0.02–0.48
δ -3-Carene	13466-78-9	01.029	0.08	0.04–0.12	0.04	0.03–0.06	0.04	0.03–0.06
Valencene	4630-07-3	01.017	0.37	0.06–0.51	0.76	0.20–1.17	0.37	0.06–0.51
α -Terpineol	98-55-5	02.014	0.46	0.40–0.57	1.18	0.79–1.54	0.46	0.40–0.57
Neral	106-26-3	05.170	0.30	0.24–0.35	0.79	0.65–0.97	0.30	0.24–0.35
Citronellal	106-23-0	05.021	0.30	0.15–0.35	0.63	0.38–0.91	0.30	0.15–0.35
Dodecanal	112-54-9	05.011	0.24	0.14–0.34	0.68	0.33–0.88	0.24	0.14–0.34
α -Copaene	3856-25-5	–	0.24	0.18–0.31	0.61	0.49–0.73	0.24	0.18–0.31
Nonanal	124-19-6	05.025	0.16	0.09–0.22	0.29	0.21–0.41	0.16	0.09–0.22
α -Phellandrene	99-83-2	01.006	0.02	0.01–0.04	0.02	0.01–0.03	0.02	0.01–0.04
δ -Cadinene	29350-73-0	01.021	0.24	0.17–0.28	0.61	0.48–0.69	0.24	0.17–0.28
Germacrene-1(10),4(14),5-triene	23986-74-5	01.042	0.22	0.16–0.28	0.57	0.40–0.80	0.22	0.16–0.28
Total			3.02	2.65–3.40	6.59	5.67–7.87	3.02	2.65–3.40
			95.3	93.1–96.9	86.5	83.9–87.7	95.3	93.1–96.9

EU: European Union; CAS no.: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.

(a): Mean calculated on five batches.

Thirty-three out of the 35 compounds, which were detected in orange oil expressed and are listed in the footnote,⁵³ were also detected in the folded oils **A** and **B**, accounting for together for 2.58% (range 2.05–3.45%) and 6.08% (4.71–7.37%) of the % GC area, respectively. In the folded oil **C**, 10 out of the 35 compounds were detected and accounted for 1.08% of % GC area (identified with an asterisk (*) in the footnote).

For the folded oil **B**, long-chain hydrocarbons (C8–C30 and squalene) and related acids and esters⁵⁴ accounted for 4.41% (2.78–5.82%) of the % GC area. In the folded oil **A**, these long-chain hydrocarbons accounted for about 1% of the % GC area (0.34–1.72%).

The FEEDAP Panel notes that there is high qualitative and quantitative similarity among the composition of the volatile fraction in expressed orange oil and the composition of the volatile fraction in the folded oils. The same 55 compounds accounting for 99% of expressed orange oil (as % GC area) also account for 97.9% (96.5–99%), 92.6% (91.3–94.0%) and 98.8% (98.7–98.9%) of the volatiles in the folded oils A, B and C, respectively. From the quantitative point of view, the main difference among the volatiles in expressed orange oil and in the folded oils is due to the different % of d-limonene.

⁵³ Additional volatile constituents: octan-1-ol(*), β -copaene(*), *trans*-3,7-dimethyl-1,3,6-octatriene(*), nootkatone(*), *p*-mentha-1,8-dien-7-ol (perillaldehyde), β -cubebene, β -sinensal(*), terpinolene, β -caryophyllene(*), α -sinensal(*), geranyl acetate, β -pinene(*), *trans*-limonene epoxide, l-carvone, citronellol(*), *p*-mentha-1,8(10)-dien-9-ol, undecanal(*), α -farnesene, octyl acetate, *cis*-limonene epoxide, β -farnesene, *trans*-sabinene hydrate, *cis*-3,7-dimethyl-1,3,6-octatriene(*), 3,7,10-humulatriene, β -elemene, γ -terpinene, isoterpinolene, 1-isopropyl-4-methylbenzene, *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, *trans*-2-decenal, sativene, γ -muurolene and *cis*-para-2,8-menthadien-1-ol.

⁵⁴ Additional constituents: octanoic acid, octyl acetate, decan-1-ol, 2,4-decadienal, decanoic acid, dodecan-1-ol, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, hexadecanal, oleic acid, octadeca-9,12-dienoic acid, 2-methyl docosane, tricosane, 3-methyl tricosane, tetracosane, pentacosane, tridecanoic acid and squalene.

Among the volatile components, α -sinensal (average: 0.13%; range: 0.03–0.19%), β -sinensal (0.16%; 0.06–0.29%), *p*-mentha-1,8(10)-dien-9-ol (0.015%; 0.005–0.025%) and perillaldehyde (0.18%; 0.15–0.26%) were detected and quantified in all batches of folded oil **A**. The corresponding concentrations in folded oil **B** were α -sinensal 0.38% (range: 0.24–0.58%), β -sinensal 0.53% (0.18–0.90%), *p*-mentha-1,8(10)-dien-9-ol 0.028% (0.015–0.037%) and perillaldehyde (0.44%; 0.39–0.52%). In folded oil **C**, [REDACTED] was present.

Non-volatile components

The dry matter content determined in three batches of type of oil was 9.6–11.9%, 20.2–21.5% and [REDACTED] for the folded oils **A**, **B** and **C**, respectively.⁴⁸ The literature search performed by the applicant¹⁹ identified PMF, predominantly heptamethoxyflavone (0.3–2.0 g/L), nobiletin (0.25–1.10 g/L), sinensetin (0.04–0.3 g/L), tangeretin (0.36–1.0 g/L), tetra-*O*-methoxyscutellarein (0.16–0.6 g/L) and 3,5,6,7,3',4'-hexamethoxyflavone (0–0.2 g/L; ranges based on Dugo and Russo, 2010), fatty acids (including capronic, lauric, myristic, palmitic, stearic, oleic and linoleic acids), waxes and sterols as part of the non-volatile fraction in citrus oils (Dugo et al., 2009; González-Mas et al., 2019). The FEEDAP Panel notes that the percentages of non-volatile components in the residual fractions of expressed orange oil under assessment will by far exceed the levels reported in literature for citrus oils which did not undergo a concentrating process.

The literature search performed by the applicant did not identify furocoumarins and synephrine as substances of concern for *C. sinensis* (see Section 3.2.1).²⁰ Furocoumarins (phellopterin, 8-geranoxypsoralen, psoralen, 8-methoxypsoralen (xanthotoxin), dimethoxypsoralen (isopimpinellin), imperatorin, oxypeucedanin and bergamottin (5-geranoxypsoralen)) and *p*-synephrine were below the limit of detection of the HPLC method in ten batches (folded oils **A** and **B**, five batches each).⁵⁵ Fourteen furocoumarins (including also trioxalene, herniarine, citroptene, toncarine, epoxybergamotone and byakangelicol) were below the LOQ of 1 mg/kg in additional three batches analysis of orange oil terpenless folded.⁵⁶

3.4.1.1. Impurities

Data on chemical and microbial impurities were provided in three batches of the folded oil **A** and in two batches of the folded oil **B**.⁵⁷ The concentrations of heavy metals were below the corresponding LOQ. In the same batches, mycotoxins (aflatoxins B1, B2, G1 and G2) were below the LOQ and pesticides were not detected in a multiresidue analysis with the exception of bifenthrin (1.3–6.6 mg/kg), chlorfenapyr (< LOQ–0.049 mg/kg), cyflutrin (< LOQ–1.0 mg/kg), cypermethrin (1.4–5.1 mg/kg), fenpropathrin (< LOQ–0.44 mg/kg), chlorpyrifos ethyl (9.4–21.4 mg/kg), malathion (1.7–4.6 mg/kg), methidathion (< LOQ–0.18 mg/kg), phosmet (0.34–2.9 mg/kg) and propargite (0.54–5.3 mg/kg). In the same batches, the sum of polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and dioxin-like polychlorinated biphenyls (PCBs) ranged between 1.52 and 1.90 pg/g (upper bond). [REDACTED] None of the data on chemical impurities raised concerns.

Analysis of microbial contamination of the same batches of the folded oils indicated that *Salmonella* spp. was not detected in 25 g, Enterobacteriaceae, total viable count, yeasts, moulds were < 10 CFU/g.

3.4.1.2. Shelf-life

The typical shelf-life of folded orange oils 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).²³ However, no evidence supporting this statement were provided.

⁵⁵ Technical dossier/Supplementary information May 2020/Annex VI_SIn reply_orange_oil_terpenless_SOC_COA. Limit of detection (LOD): 8-geranoxypsoralen, xanthoxin, psoralen, isopimpinellin and imperatorin 0.5 mg/kg; phellopterin 1.0 mg/kg; bergamottin and *p*-synephrine 5.0 mg/kg; oxypeucedanin 10 mg/kg.

⁵⁶ Technical dossier/Supplementary information May 2020/Conf_Annex XVIII_SIn reply_orange_oil_terpenless_folded_SOC_method_COA.

⁵⁷ Technical dossier/Supplementary information May 2020/Annex VIII_Sin reply_orange_oil_terpenless_COA_batches_impurities. 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: < 0.1 μ g/kg for aflatoxins B1, B2, G1 and G2.

⁵⁸ Technical dossier/Supplementary information May 2020/Conf_Annex XX_SIn reply_orange_oil_terpenless_folded_impurities_COA.

3.4.1.3. Conditions of use

Folded oils are intended to be added to feed and water for drinking for all animal species without a withdrawal time. The maximum proposed use levels in complete feed for each type of folded oil and for the different target species are reported in Table 11. Folded oil C is not intended for use in horse, rabbit, dog, cat, ornamental fish and ornamental birds.

The proposed use level in in water for drinking is 5 mg/kg for all animal species.

Table 11: Conditions of use for the folded orange oils **A**, **B** and **C**: maximum proposed use levels in complete feed for each type of additive and for the different target species

Animal category	Maximum use level (mg/kg complete feed)		
	A	B	C
Chicken for fattening	15.5	5.5	50
Laying hen	23.5	8	50
Turkey for fattening	21	7	50
Piglet	28.5	9.5	50
Pig for fattening	34	11.5	50
Sow lactating	41.5	14	50
Veal calf (milk replacer)	66.5	23	70
Cattle for fattening	62.5	21.5	60
Dairy cow	40.5	14	60
Sheep/goat	62.5	21.5	70
Horse	62.5	21.5	–
Rabbit	25	8.5	–
Salmon	70	24.5	2
Dog	70	25.5	–
Cat	62.5	21.5	–
Ornamental fish	70	45	–
Ornamental bird	9	3	–

3.4.2. Safety

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

Many of the major volatile components, accounting for about 87-98% of the % GC areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food⁷ and feed⁸ uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

The ADME of the non-volatile individual components of the residual fraction of expressed orange oil has been already addressed in Section 3.2.2.1.

The studies relevant to the assessment of the non-volatile individual components of the residual fraction of expressed orange oil have been already described in Section 3.2.2.2.

The following section focus on the genotoxicity assessment of those compounds not previously assessed in Section 3.2.2.2, based on the QSAR analysis provided by the applicant.

3.4.2.1. 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).

Besides the components already identified in expressed orange oil and screened by QSAR (*trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, mentha-1,8(10)-dien-9-ol, α -sinensal, β -sinensal, *cis*-limonene epoxide and *trans*-limonene epoxide, see Section 3.2.2.2), four additional constituents present only in the residual fraction (folded orange oils **A** and **B**), namely germacrene D-4-ol, *p*-mentha-1,8-dien-7-yl acetate, *p*-mentha-1,8-dien-7-ol and (*S*)-isopiperitenone, were screened using

the 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.⁵⁹

3.4.2.2. Safety for the target species

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

Volatile components

In view of the similarity in the composition of the volatile fraction of the folded oils with the composition of the volatile fraction of expressed orange oil (see Section 3.3.1.1), the lower concentrations of volatiles in the folded oils and the lower proposed use levels in feed for the folded oils (see Section 3.4.1.3), the FEEDAP Panel considers that the same conclusions reached on the safety for the target species for expressed orange oil apply to the volatile fraction of the folded oils (see Section 3.2.2.3.), except for perillaldehyde for which a separate assessment is presented.

With respect to the exposure to the volatiles present in the additive (except perillaldehyde) no safety concern was identified for the folded oils when used as a feed additive for all animal species (except cats) up to the maximum proposed use levels in complete feed (see Table 11). For cats, the maximum safe concentration is 25 mg/kg complete feed.

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.

Volatile components: Perillaldehyde

The concentration of perillaldehyde detected in the volatile fraction of all batches of the folded oil **A** was on average 0.18% (range 0.15–0.26%). The use of folded oil A at the proposed use level in feed (15.5–70 mg/kg) would result in a daily intake of perillaldehyde up to 3.7 µg/kg bw for poultry, pigs, ruminants, horses and rabbits, and 3.6 µg/kg bw for fish.⁶⁰

In the volatile fraction of the folded oil **B**, the concentration of perillaldehyde was 0.44% (0.39–0.52%). The use of the folded oil B at the proposed use level in feed (5.5–25.5 mg/kg) would result in a daily intake of perillaldehyde up to 2.6 µg/kg bw for poultry and ruminants, 2.5 µg/kg bw for pigs, and horses, rabbit and fish.⁶¹

The FEEDAP Panel notes that the figures for perillaldehyde intake are overestimated, as they were calculated without taking into account the non-volatile fraction of folded oils **A** and **B** but considering the volatile part of the oils as 100%.

These concentrations are about 10-fold lower than those resulting from the intake of perillaldehyde from citrus by-products, when used in diets at concentrations of 10% for poultry and 20% for the other species (daily intake 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 horse, 40 µg/kg bw for rabbits and 14 µg/kg bw for fish, see Section 3.2.2.3, *Volatile components: perillaldehyde*).

Perillaldehyde was not detected in folded oil **C**.

Non-volatile components. Polymethoxylated flavones

A maximum occurrence of 0.5% total PMF in expressed orange oil has been estimated from literature (see Section 3.2.2.3). The manufacturing process would result in an enrichment of PMF in the folded oils. Considering an enrichment factor of 4, 10 and 3.3 for the folded oils **A**, **B** and **C**, the

⁵⁹ Technical dossier/Supplementary information May 2020/Annex X_SIn_reply_orange_oil_terpeneless_QSAR. Structural alerts for germacrene D-4-ol and *p*-mentha-1,8-dien-7-ol were due to the presence of vinyl/allyl alcohols, for *p*-mentha-1,8-dien-7-yl acetate to the presence of esters and allyl acetates, and for (*S*)-isopiperitenone to the presence of ketones and α,β -unsaturated carbonyls. In all cases, predictions of Ames mutagenicity were 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 germacrene D-4-ol, *p*-mentha-1,8-dien-7-ol, *p*-mentha-1,8-dien-7-yl acetate and (*S*)-isopiperitenone were discounted.

⁶⁰ Calculated considering the concentration of perillaldehyde in the additive (0.26%), the default values for feed intake (Table 6) and the proposed use levels in feed for the different species (Table 13), and that complete feed contains 88% DM except milk replacer for veal calves (94.5%).

⁶¹ Calculated considering the concentration of perillaldehyde in the additive (0.52%), the default values for feed intake (Table 6) and the proposed use levels in feed for the different species (Table 13), and that complete feed contains 88% DM except milk replacer for veal calves (94.5%).

corresponding concentrations of 2.0, 5.0 and 1.65% total PMF have been estimated for the folded oils **A**, **B** and **C**.

At the maximum proposed use levels for the different species and the three folded oils (see Section 3.4.1.3, Table 11), the concentration of PMF in feed was calculated to range between 0.31 and 1.40 mg PMF/kg complete feed for folded oil **A**, 0.28 and 2.25 mg PMF/kg complete feed for folded oil **B**, and 0.03 and 1.16 mg PMF/kg complete feed for folded oil **C**.

These values are below the maximum safe concentrations in feed calculated for the target species based on a NOAEL of 16 mg/kg bw per day (see Table 6). The FEEDAP Panel concludes that the presence of PMF in folded oil does not raise concern for the target species.

Conclusions on safety for the target species

The FEEDAP Panel concludes that the use of the folded oils is safe up to the maximum proposed use levels in complete feed:

- Folded oil **A** oil is safe up to the maximum proposed use level in complete feed of 15.5 mg/kg for chickens for fattening, 23.5 mg/kg for laying hens, 21 mg/kg turkeys for fattening, 28.5 mg/kg for piglets, 34 mg/kg for pigs for fattening, 41.5 mg/kg for sows, 66.5 mg/kg for veal calves (milk replacer), 62.5 mg/kg for cattle for fattening, 40.5 mg/kg for dairy cows, 62.5 mg/kg for sheep, goats and horses, 25 mg/kg for rabbits and 70 mg/kg for fish.
- Folded oil **B** oil is safe up to the maximum proposed use level in complete feed of 5.5 mg/kg for chickens for fattening, 8 mg/kg for laying hens, 7 mg/kg turkeys for fattening, 9.5 mg/kg for piglets, 11.5 mg/kg for pigs for fattening, 14 mg/kg for sows, 23 mg/kg for veal calves (milk replacer), 21.5 mg/kg for cattle for fattening, 14 mg/kg for dairy cows, 21.5 mg/kg for sheep, goats and horses, 8.5 mg/kg for rabbits and 24.5 mg/kg for fish.
- Folded oil **C** oil is safe up to the maximum proposed use level in complete feed of 50 mg/kg for chickens for fattening, laying hens, turkeys for fattening, piglets, pigs for fattening, sows, 70 mg/kg for veal calves (milk replacer), 60 mg/kg for cattle for fattening and dairy cows, 70 mg/kg for sheep and goats, and 2 mg/kg for fish.

For the target species that are fed citrus by-products as part of their daily feed, the use of the folded oils **A** and **B** in feed is not expected to increase the exposure to perillaldehyde to a relevant extent (< 10%). For dogs, cats, ornamental fish and ornamental birds not normally exposed to citrus by-products, no conclusion can be drawn. Folded oil **C** does not contain perillaldehyde, however it is not intended for use in these species.

The FEEDAP Panel considers that the use level in water for drinking is safe for all animal species except dogs, cats and ornamental birds, provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.4.2.3. Safety for the consumer

Considering the similarity in the composition of expressed orange oil and its residual fractions, the same considerations on the safety for the consumer apply (see Section 3.2.2.4).

No safety concern would be expected for the consumer from the use of the folded oils up to the highest safe use level in feed.

3.4.2.4. 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 sheet⁴⁶ for folded orange oils **A** and **B**, where hazards for users have been identified. The additives under assessment should be considered as irritants to skin and eyes, and as skin and respiratory sensitisers.

3.4.2.5. Safety for the environment

C. sinensis is a native species to Europe where it is widely grown both for commercial and decorative purposes. Use of the essential oil under the proposed conditions of use in animal production is not expected to pose a risk for the environment.

3.5. Efficacy

C. sinensis (L.) Osbeck (*C. aurantium* var. *dulcis* L.) and its oils are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2821 (orange oil distilled), orange oil terpeneless (2822) and orange peel sweet oil (2825, expressed oil).

Since orange (sweet) and its oils are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Since orange oils may be produced by various processes resulting in preparations with different toxicological profiles, the following conclusions apply only to expressed orange oil from fruit peels of *C. sinensis* (L.) Osbeck and its fractions obtained after distillation.

The use of the expressed orange oil and its fractions in feed is not expected to increase the exposure to perillaldehyde of those target species that are already fed citrus by-products to a relevant extent (< 10%). For dogs, cats, ornamental fish and ornamental birds, not normally exposed to citrus by-products, no conclusion on the safety of these products can be drawn.

The FEEDAP Panel concludes that the additives under assessment are safe for the target species at the following use levels:

- **Expressed orange oil** is safe up to the maximum proposed use levels in complete feed of 80 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 144 mg/kg for piglets, 172 mg/kg for pigs for fattening, 200 mg/kg for sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 230 mg/kg for horses, 50 mg/kg for fish and rabbit. For these species, the use level in water for drinking of 20 mg/kg 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.
- **Distilled orange oil** is safe up to the maximum proposed use levels in complete feed of 80 mg/kg for chickens for fattening, laying hens and turkeys for fattening, 200 mg/kg for piglets, pigs for fattening and sows, 130 mg/kg for veal calves (milk replacer), cattle for fattening, dairy cows, sheep and goats, 225 mg/kg for horses, 80 mg/kg for fish and rabbits. For these species, 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.
- **Folded oil A** oil is safe up to the maximum proposed use levels in complete feed of 15.5 mg/kg for chickens for fattening, 23.5 mg/kg for laying hens, 21 mg/kg turkeys for fattening, 28.5 mg/kg for piglets, 34 mg/kg for pigs for fattening, 41.5 mg/kg for sows, 66.5 mg/kg for veal calves (milk replacer), 62.5 mg/kg for cattle for fattening, 40.5 mg/kg for dairy cows, 62.5 mg/kg for sheep, goats and horses, 25 mg/kg for rabbits and 70 mg/kg for fish. For these species, 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.
- **Folded oil B** oil is safe up to the maximum proposed use levels in complete feed of 5.5 mg/kg for chickens for fattening, 8 mg/kg for laying hens, 7 mg/kg turkeys for fattening, 9.5 mg/kg for piglets, 11.5 mg/kg for pigs for fattening, 14 mg/kg for sows, 23 mg/kg for veal calves (milk replacer), 21.5 mg/kg for cattle for fattening, 14 mg/kg for dairy cows, 21.5 mg/kg for sheep, goats and horses, 8.5 mg/kg for rabbits and 24.5 mg/kg for fish. For these species, 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.
- **Folded oil C** oil is safe up to the maximum proposed use levels in complete feed of 50 mg/kg for chickens for fattening, laying hens, turkeys for fattening, piglets, pigs for fattening, sows, 70 mg/kg for veal calves (milk replacer), 60 mg/kg for cattle for fattening and dairy cows, 70 mg/kg for sheep and goats, and 2 mg/kg for fish. For these species, 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.

No concerns for consumer safety were identified following the use of the additives at the maximum proposed use level in feed.

The additives under assessment should be considered as irritants to skin and eyes, and as skin and respiratory sensitisers.

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

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

5. Documentation provided to EFSA/Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Chemically 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)
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
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. <i>Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment</i>
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. <i>Issues: Method of analysis</i>
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
18/05/2020	Reception of supplementary information from the applicant (partial submission: orange oil cold pressed, orange oil terpeneless, orange oil terpenes)
24/11/2020	Reception of supplementary information from the applicant (partial submission: clarification request)
12/03/2021	The application was split and a new EFSA-Q-2021-00146 was assigned to the preparations included in the present assessment
17/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
02/04/2021	Partial withdrawal by applicant (EC was informed) for the following additive: olibanum tincture
22/06/2021	Reception of supplementary information from the applicant (partial submission: orange oil terpeneless)
29/09/2021	Opinion adopted by the FEEDAP Panel on expressed orange oil and its fractions. End of the Scientific assessment for the preparations included in the present assessment. The assessment of other preparations is still ongoing

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Abbreviations

AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food
BDG	botanically defined group
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision

CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CDG	chemically defined group
DM	dry matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour 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
LOD	limit of detection
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
PCBs	polychlorobiphenyls
PPR	EFSA Panel on Plant Protection Products and their Residues
TTC	threshold of toxicological concern
UF	uncertainty factor
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 satisfactorily 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 reticulata* 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.