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Safety and efficacy of a feed additive consisting of an essential oil from the leaves of *Citrus* × *aurantium* L. (petitgrain bigarade oil) 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 an essential oil from the leaves of *Citrus* x *aurantium* L. (petitgrain bigarade oil), when used as a sensory additive (flavouring) in feed and water for drinking for all animal species. The FEEDAP Panel concluded that the essential oil under assessment is safe up to the maximum proposed use level of 125 mg/kg complete feed for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 10 mg/kg for chicken for fattening, 14 mg/kg for laying hen, 13 mg/kg for turkey for fattening, 17 mg/kg for piglet, 20 mg/kg for pig for fattening, 25 mg/kg for lactating sow, 43 mg/kg for veal calf (milk replacer), 38 mg/kg for cattle for fattening, sheep, goat and horse, 24 mg/kg for dairy cow, 15 mg/kg for rabbit, 42 mg/kg for salmon, 44 mg/kg for dog and 8 mg/kg for cat. The FEEDAP Panel considered that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded. No concerns for consumer safety were identified following the use of the additive up to the highest safe level in feed. The essential oil under assessment should be considered as irritant to skin, eyes and the respiratory tract, and as a skin sensitiser. The use of the additive in animal feed under the proposed conditions was not expected to pose a risk for the environment. Petitgrain bigarade oil was recognised to flavour food. Since its function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

Keywords: sensory additives, flavouring compounds, *Citrus* × *aurantium* L., petitgrain bigarade oil, linalyl acetate, linalool, component-based approach

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Table of contents

Abstract 1
1. Introduction 4
1.1. Background and Terms of Reference 4
1.2. Additional information 4
2. Data and methodologies 6
2.1. Data 6
2.2. Methodologies 7
3. Assessment 7
3.1. Origin and extraction 7
3.2. Characterisation 7
3.2.1. Characterisation of petitgrain bigarade oil 7
3.2.2. Impurities 9
3.2.3. Shelf-life 10
3.2.4. Conditions of use 10
3.3. Safety 10
3.3.1. Safety for the target species 11
3.3.1.1. Conclusions on safety for the target species 14
3.3.2. Safety for the consumer 15
3.3.3. Safety for user 15
3.3.4. Safety for the environment 15
3.4. Efficacy 15
4. Conclusions 15
5. Documentation as provided to EFSA/Chronology 16
References 16
Abbreviations 19
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 20

1. Introduction

1.1. Background and Terms of Reference

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

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 20 preparations (buchu leaves oil, amyris oil, olibanum extract (water based, wb), olibanum tincture, lime oil, neroli bigarade oil, petitgrain bigarade oil, petitgrain bigarade absolute, bitter orange extract of the whole fruit, lemon oil expressed, lemon oil distilled, orange oil, orange terpenes, mandarin oil, mandarin terpenes, grapefruit oil expressed, grapefruit extract (sb), grapefruit extract, quebracho extract (wb), cashew oil), belonging to botanically defined group (BDG) 8 - *Sapindales*, when used as feed additives for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for ten preparations.[3,4] These preparations are excluded from the present assessment. In addition, during the course of the assessment, the application was split and the present opinion covers only one out of the 20 initial preparations under application: an essential oil from the leaves of *Citrus* × *aurantium* L.[5] (petitgrain bigarade oil) for all animal species.

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

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

The remaining 10 preparations belonging to botanically defined group (BDG) 8 - *Sapindales* under application are assessed in separate opinions.

1.2. Additional information

Petitgrain bigarade oil from *Citrus aurantium* L. is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined). It has not been assessed as a feed additive in the EU.

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

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[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, olibanum tincture, 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 extract (wb).

[5] Accepted name: *Citrus* × *aurantium*, synonym *Citrus aurantium* L. subspecies amara L.

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

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

CG	Chemical group	Product – EU register name (common name)	FLAVIS No	EFSA opinion*, Year
01	Straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes	Heptan-1ol	02.021	2013
		Octanal	05.009	
		Methyl geranate	09.643	2011a, CEF
03	α,ß-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/ aldehydes/acids, acetals and esters with esters containing α,β-unsaturated alcohol and acetal containing α, β-unsaturated alcohols or aldehydes	Geraniol	02.012	2016a
		(*Z*)-Nerol	02.058	
		Neral	05.170	
		trans-3,7-Dimethylocta-2,6-dienal (geranial)	05.188	
		Geranyl formate	09.076	
		Geranyl acetate	09.011	
		Neryl formate	09.212	
		Neryl acetate	09.213	
04	Non-conjugated and accumulated unsaturated straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters	Citronellyl acetate	09.012	2016b
05	Saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols	6-Methyhept-5-en-2-one	07.015	2015a
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	
		Nerolidol	02.018	
		2-(4-Methylphenyl)propan-2-ol	02.042	
		4-Terpinenol	02.072	
		Linalyl acetate	09.013	
		Linalyl propionate	09.130	
		α-Terpinyl acetate[(a)]	09.015	JECFA
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Sabinene hydrate[(a)]	02.085	JECFA
		Carvone[(a)]	07.012	2014, SC
13	Furanones and tetrahydrofurfuryl derivatives	Linalool oxide[(b)]	13.140	2012b

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

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

CG	Chemical group	Product – EU register name (common name)	FLAVIS No	EFSA opinion*, Year
14	Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms	3-Methyl-2(3-methylbut-2-enyl)furan (rose furan)	13.148	2015a, CEF 2021a,b, FAF
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012c
27	Anthranilate derivatives	Methyl anthranilate	09.715	2011
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	1-Isopropyl-4-methylbenzene (*p*-cymene)	01.002	2015b
		Terpinolene	01.005	
		α-Phellandrene	01.006	
		α-Terpinene	01.019	
		γ-Terpinene	01.020	
		d-Limonene	01.045	
		Pin-2(10)-ene (β-pinene)	01.003	2016c
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		3,7-Dimethyl-1,3,6-octatriene (β-ocimene)[(c)]	01.018	
		δ-3-Carene	01.029	
		δ-Cadinene[(a),(d)]	01.021	2011b, CEF
		3,7,10-Humulatriene[(a),(d)]	01.043	
		4(10)-Thujene (sabinene)[(a)]	01.059	2015b, CEF
		cis-3,7-Dimethyl-1,3,6-octatriene (*cis*-β-ocimene)[(a)]	01.064	
32	Epoxides	β-Caryophyllene epoxide[(a)]	16.043	2014, CEF

*: FEEDAP opinion unless otherwise indicated.
(a): Evaluated for use in food. According to Regulation (EC) 1565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(b): Linalool oxide [13.140]: A mixture of cis- and trans-linalool oxide (5-ring) was evaluated.
(c): β-Ocimene [01.018], as a mixture of (E)- and (Z)-isomers, containing 50-70% (E)-isomer and 17-17% (Z)-isomer was evaluated.
(d): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[9] in support of the authorisation request for the use of petitgrain bigarade oil from *Citrus* × *aurantium* L. as a feed additive.

The FEEDAP Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as previous risk 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.[10]

[9] FEED dossier reference: FAD-2010-0322.
[10] Technical dossier/Supplementary information/Letter dated 29/4/2021.

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

2.2. Methodologies

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

3. Assessment

The additive under assessment, petitgrain bigarade oil, is an essential oil obtained by steam distillation from the leaves of *Citrus* × *aurantium* L. It is intended for use as a sensory additive (functional group: flavouring compounds) in feed and 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 the common commercially important citrus fruits found today are hybrids derived from three ancestral species now represented by the cultivars described as the mandarin orange, pomelo, and citron. *Citrus* × *aurantium* is considered to have arisen from a cross between the pomelo (*Citrus maxima*) and the mandarin (*Citrus reticulata*). The group *Citrus* × *aurantium* now includes numerous varieties and cultivars as a result of natural and deliberate back-crossing to other parents. These include the orange, bitter orange, grapefruit and clementine. Many varietal names and sub-species have been used to distinguish between members of this hybrid complex but none have current taxonomic standing. Instead, the applicant uses the traditional description 'petitgrain bigarade oil', in which petitgrain refers to the small green fruit from which the oil was originally extracted and bigarade an anglicised version of the French for the bitter orange.

The additive is extracted from the leaves by steam distillation. The volatile constituents are condensed and then separated from the aqueous phase by decantation. Residual traces of water may be removed with sodium sulfate if necessary.

3.2. Characterisation

3.2.1. Characterisation of petitgrain bigarade oil

The essential oil under assessment is a pale yellow clear mobile liquid with a characteristic aroma. In four batches of the additive (all originating from Egypt), the refractive index was 1.46. Petitgrain bigarade oil is identified with the single Chemical Abstracts Service (CAS) number 8014-17-3, the

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

[12] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

European Inventory of Existing Chemical Substances (EINECS) number 283-881-6, the Flavor Extract Manufacturers Association (FEMA) number 2855, and the Council of Europe (CoE) number 136.

The product specifications are based on the standards developed by the International Organisation for Standardization (ISO) 8901:2003 for 'oil of bitter orange petitgrain, cultivated (*Citrus aurantium* L.)',[13] which were adapted to reflect the concentrations of the main volatile components, analysed by gas chromatography with flame ionisation detection (GC-FID) and expressed as % of gas chromatographic peak area (% GC area). These components are linalyl acetate (40–72%, selected as the phytochemical marker), linalool (10–32%, selected as the phytochemical marker), α-terpineol (1–7%), d-limonene (1–6%), geranyl acetate (1.5–5.5%) and geraniol (1–4%). Analysis of four batches of the additive by GC-FID showed compliance with these specifications[14] for all compounds, except geraniol.[15] When analysed by gas chromatography-mass spectrometry (GC–MS) these six compounds account for about 88.7% on average (range 88.4–89.2%) of the % GC area (Table 2).

Table 2: Major constituents of the essential oil from the leaves of *Citrus × aurantium* L. as defined by the ISO standard (8901:2003): specifications and batch to batch variation based on the analysis of four 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	CAS no	FLAVIS no	% GC area		
EU register name			Specification	Mean[(a)]	Range
Linalyl acetate	115-95-7	09.013	40–72	53.25	52.3–54.1
Linalool	78-70-6	02.013	10–32	24.55	23.9–25.4
Geranyl acetate	105-87-3	09.011	1.5–5.5	4.65	4.39–4.83
α-Terpineol	10482-56-1	02.014	1–7	4.14	3.90–4.38
d-Limonene	5989-27-5	01.045	1–6	1.98	1.63–2.37
Geraniol	106-24-1	02.012	1–4	0.19	0.18–0.19
Total				88.7	88.4–89.2

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

The applicant provided the full characterisation of the four batches obtained by GC–MS.[16] In total, up to 100–130 peaks were detected in the chromatogram, 56 of which were identified and accounted on average for 99.1% (99.0–99.4%) of the % GC area. Besides the six compounds indicated in the product specifications, 22 other compounds were detected at individual levels > 0.1% and are listed in Table 3. These 28 compounds together account on average for 98.1% (98.0–98.3%) of % GC area. The remaining 28 compounds (ranging between 0.002% and 0.1%) and accounting for 1.02% are listed in the footnote.[17]

13 Technical dossier/Supplementary information March 2020/Annex_IV_SIn _Reply_petitgran bigarade oil_ISO_8901_2003.
14 Technical dossier/Supplementary information March 2020/SIn reply_ petigrain bigarade_oil/GC-FID analysis: linalyl acetate (53.2–54.2%), linalool (26.0–26.8%), geranyl acetate (3.41–3.56%), α-terpineol (3.40–3.76%), d-limonene (1.57–2.28%) and geraniol (0.11–0.22%.
15 According to the applicant, the use of an apolar column during GC-FID analysis probably resulted in an underestimation of geraniol which peak might have been partly included in the linalyl acetate peak.
16 Technical dossier/Supplementary information March 2020/Annex_III_SIn _Reply_petitgran bigarade oil_chromatograms.
17 Additional constituents: constituents (n = 11) between < 0.1 and ≥ 0.05%: α-terpinyl acetate, *cis*-8-hydroxylinalool, 4(10)-thujene (sabinene), 6-methylhept-5-en-2-one, methyl geranate, δ-cadinene, 2-(4-methylphenyl)propan-2-ol (*p*-cimen-8-ol), terpinolene, linalyl propionate, nerolidol, 3,7,10-humulatriene; constituents (n = 6) between < 0.05 and > 0.01%: spathulenol, *cis*-linalool oxide (pyranoid), *trans*-linalool oxide (pyranoid), β-elemene, α-thujene, 8-hydroxycarvotanacetone; constituents (n = 11) ≤ 0.01%:, α-terpinene, bicyclogermacrene, α-phellandrene, γ-terpinene, *cis*-sabinene hydrate, rose furan, carvone, camphene, 1,8-cineole, octanal and heptan-1-ol.

Table 3: Constituents of the essential oil from the leaves of *Citrus* × *aurantium* L. accounting for > 0.1% of the composition (based on the analysis of four 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	CAS no	FLAVIS no	% GC area	
EU register name			Mean[(a)]	Range
Neryl acetate	141-12-8	09.213	2.43	2.27–2.54
β-Pinene (pin-2(10)-ene)	18172-67-3	01.003	0.92	0.87–0.99
δ-3-Carene	13466-78-9	01.290	0.91	0.78–1.08
Nerol	106-25-2	02.058	0.67	0.62–0.76
Myrcene	123-35-3	01.008	0.47	0.33–0.70
β-*trans*-Ocimene[(b)]	13877-91-3	–	0.38	0.12–0.88
cis-Linalool oxide[(c)]	5989-33-3	–	0.35	0.17–0.51
Methyl anthranilate	134-20-3	09.715	0.35	0.28–0.42
trans-Linalool oxide[(c)]	34995-77-2	–	0.32	0.15–0.45
8-Hydroxylinalyl acetate	–	–	0.30	0.17–0.42
trans-8-hydroxylinalool	138874-68-7	–	0.30	0.13–0.43
β-Caryophyllene	87-44-5	01.007	0.28	0.17–0.45
Geranyl formate	105-86-2	09.076	0.28	0.20–0.36
Geranial (*trans*-3,7-dimethylocta-2,6-dienal)	141-27-5	05.188	0.28	0.23–0.34
β-Caryophyllene epoxide	1139-30-6	16.043	0.22	0.13–0.29
p-Cymene (1-isopropyl-4-methylbenzene)	99-87-6	01.002	0.17	0.14–0.18
Neral	106-26-3	05.170	0.16	0.12–0.22
Neryl formate	2142-94-1	09.212	0.13	0.06–0.18
Citronellyl acetate	150-84-5	09.012	0.13	0.11–0.14
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	0.10	0.10–0.11
cis-β-Ocimene (*cis*-3,7-dimethyl-1,3,6-octatriene	3338-55-4	01.064	0.10	0.00–0.23
4-Terpinenol	562-74-3	02.072	0.10	0.09–0.10
Total			9.37	8.87–9.83

CAS no: Chemical Abstracts Service number; FLAVIS number: EU Flavour Information System numbers.
(a): Mean calculated on four batches.
(b): EFSA evaluated β-ocimene [01.018], as a mixture of (*E*)- and (*Z*)-isomers, containing 50–70% (*E*)-isomer and 17-17% (*Z*)-isomer.
(c): Furanoid structure (5-ring), linalool oxide (*cis* and *trans*) identified with FLAVIS number [13.140].

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *Citrus* × *aurantium* L. and its preparations.[18] The occurrence of furocoumarins such as 5-methoxypsoralen (5-MOP, synonym: bergapten) in the aerial parts of the plant has been reported in the EFSA Compendium (EFSA, 2012). Analysis of the four batches tested[19] showed that 5-MOP was below the limit of detection of the high performance liquid chromatography (HPLC) method in all samples. Psoralen and coumarin were reported to be not present in leaves (Lim, 2012).

3.2.2. 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 polychlorinated biphenyls (PCBs), organochloride pesticides, organophosphorous pesticides, aflatoxin B1, B2, G1, G2 and ochratoxin A. However, no data have been provided. Since petitgrain bigarade oil is produced by steam distillation, the likelihood of any measurable carry-over of heavy metals is low except for mercury.

[18] Technical dossier/Supplementary information March 2020/Literature search_petitgrain_bigarade _oil.
[19] Technical dossier/Supplementary information March 2020/Annex VI_Sin petitgrain_bigarade_oil_SOC_COA, limit of detection (LOD) 5 mg/kg.

3.2.3. Shelf-life

The typical shelf-life of the additive 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).[20]

3.2.4. Conditions of use

Petitgrain bigarade oil is 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 the different target species are reported in Table 4. No use level has been proposed by the applicant for the use in water for drinking.

Table 4: Conditions of use for the essential oil from the leaves of *Citrus* × *aurantium* L.: maximum proposed use levels in complete feed for the different target species

Animal category	Maximum use level (mg/kg complete feed)
Chicken for fattening	10.5
Laying hen	16
Turkey for fattening	14.5
Piglet	19
Pig for fattening	23
Sow lactating	28
Veal calf (milk replacer)	45
Cattle for fattening	42.5
Dairy cow	27.5
Sheep/goat	42.5
Horse	42.5
Rabbit	17
Salmon	48.5
Dog	51
Cat	42.5
Ornamental fish	125

3.3. Safety

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

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

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

For 3-methyl-2(3-methylbut-2-enyl)furan (rose furan) [13.148], the EFSA CEF Panel had requested additional genotoxicity data to complete the assessment (EFSA CEF Panel, 2015a). Based on the data submitted on 2-pentylfuran [13.059] and 2-acetylfuran [13.054], the EFSA Panel on Food Additives and Flavourings (EFSA FAF Panel) ruled out the genotoxicity concern and concluded that there is no safety concern at the estimated levels of intake as flavouring substances based on the maximised survey-derived intake (MSDI) approach (EFSA FAF Panel, 2021a,b).

[20] Technical dossier/Section II.

Several volatile components accounting for < 0.5% of the % GC area (8-hydroxylinalyl acetate, *trans*-8-hydroxylinalool, *cis*-8-hydroxylinalool, *cis*-sabinene hydrate, *cis*- and *trans*-linalool oxide (pyranoid structures), spathulenol, 8-hydroxycarvotanacetone, β-elemene, α-thujene and bicyclogermacrene) have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CGs 6, 31 and 8 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2012a, 2016c,d). These compounds were screened with the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox and no alerts were identified for *in vitro* mutagenicity, for genotoxic and non-genotoxic carcinogenicity and for other endpoints.[21]

3.3.1. 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%), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the essential oil.

Based on considerations related to structural and metabolic similarities, the components were allocated to 12 assessment groups, corresponding to the CGs 1, 3, 4, 5, 6, 8, 13, 14, 16, 27, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For CG 31 ('aliphatic and aromatic hydrocarbons'), sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 are applied (EFSA CEF Panel, 2015b,c). The allocation of the components to the (sub-) assessment groups is shown in Table 5.

For each component in the assessment group, exposure of 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, 2017a–c). Default values on body weight are used to express exposure in terms of mg/kg body weight (bw) per day. The intake levels of the individual components calculated for chicken for fattening, the species with the highest ratio of feed intake/body weight per day, are shown in Table 5.

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 no observed adverse effect level (NOAEL) values. Structural and metabolic similarity among the components in the assessment groups were evaluated to explore the application of read-across allowing extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL or, if sufficient evidence were available for members of a (sub-)assessment group, to derive a (sub-)assessment group NOAEL.

Toxicological data for subchronic studies, from which NOAEL values could be derived, were available for octyl acetate [09.007] in CG 1 (EFSA FEEDAP Panel, 2013), citral [05.020] in CG 3 (EFSA FEEDAP Panel, 2016a), citronellol [02.011] in CG 4 (EFSA FEEDAP Panel, 2016b), terpineol [02.230] and linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), carvone [07.147] in CG 8 (EFSA SC, 2014; EFSA FEEDAP Panel, 2016d), 1,8-cineole in CG 16 (EFSA FEEDAP Panel, 2012c, 2021), methyl *N*-methyl anthranilate [09.781] in CG 27 (EFSA FEEDAP Panel, 2011), myrcene [01.008], d-limonene [01.045], *p*-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015b, 2016c), and β-caryophyllene epoxide in CG 32 (EFSA CEF Panel, 2014).

[21] Technical dossier/Supplementary information March 2020/Annex IX_SIn_reply_petitgrain_bigarade_oil_QSAR. 'For 8-hydroxycarvotanacetone, predictions of Ames mutagenicity was made by "read-across" analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers as well as empirical profilers. Subcategorisation was performed in order to exclude analogues less similar to 8-hydroxycarvotanacetone. Ames test (with and without S9) read across predictions for 8-hydroxycarvotanacetone were found negative'.

The NOAEL of 120 mg/kg for octyl acetate [09.007] was selected as reference point for CG 1 compounds. Similarly, read-across was also applied using the NOAEL of 345 mg/kg bw per day for citral [05.020] to extrapolate to geraniol [02.012], geranial [05.188], geranyl acetate [09.011], geranyl formate [09.076], nerol [02.058], neral [05.170], neryl acetate [09.213] and neryl formate [09.212] in CG 3.

Considering the structural and metabolic similarities, the NOAEL of 117 mg/kg bw per day for linalool was extrapolated to linalyl acetate [09.013], linalyl propionate [09.130], nerolidol [02.018], 8-hydroxylinalyl acetate and *trans*-8-hydroxylinalool in CG 6. For the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.072] and terpinen-4-ol [02.072], the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [02.230] and d-limonene [01.045].

Similarly, 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 (*cis*-β-ocimene [01.064] and trans-β-ocimene), group III (terpinolene [01.005], β-elemene, α-terpinene [01.019], phellandrene [01.006] and γ-terpinene [01.020]) and group V (β-pinene [01.003], δ-3-carene [01.029] and α-pinene [01.004]) (EFSA CEF Panel, 2015b,c). The FEEDAP Panel applied the same NOAEL to *trans*-sabinene hydrate [02.085] in CG 8, as it is structurally related to sabinene in group V. The NOAEL for *p*-cymene [01.002] in group IVe was applied to 2-(4-methylphenyl)propan-2-ol (*p*-cimen-8-ol) in CG 8.

For the remaining compounds, 6-methylhept-5-en-2-one [07.015] in CG 5, spathulenol in CG 6, 8-hydroxycarvotanacetone in CG 8, *cis*-linalool oxide and trans-linalool oxide in CG 13, rosefuran [13.148] in CG 14, bicyclogermacrene and 3,7,10-humulatriene [01.043] and in CG 31, toxicity studies and NOAEL values performed with the compounds under assessment were not available and read-across was not possible. Therefore, the 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 5.

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 × 10 uncertainty factor). The compounds resulting individually in a MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible. They are listed in the footnote.[22]

The approach to the safety assessment of petitgrain bigarade oil expressed for the target species is summarised in Table 5. The calculations were done for chicken for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 10.5 mg/kg.

Table 5: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of petitgrain bigarade oil classified according to assessment groups

Essential oil composition			**Exposure**		**Hazard characterisation**		**Risk characterisation**	
Assessment group	**FLAVIS no**	**Max conc. in the oil**	**Max feed conc.**	**Intake[(a)]**	**Cramer class**	**NOAEL[(b)]**	**MOE**	**MOET**
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 3								
Geranyl acetate	09.011	4.83	0.507	0.0455	I	345	7,578	
Neryl acetate	09.213	2.54	0.267	0.0239	I	345	14,410	
Nerol	02.058	0.76	0.080	0.0072	I	345	48,095	

[22] Compounds included in the assessment groups but not reported in the table: octanal, heptan-1-ol and methyl geranate (CG 1); geranyl formate, geranial, geraniol, neral and neryl formate (CG 3); 4-terpineol, α-terpinyl acetate, nerolidol, linalyl propionate, *p*-cimen-8-ol and *cis* 8-hydroxylinalool (CG 6); carvone and *cis*-sabinene hydrate (CG 8); 1,8-cineole (CG 16) terpinolene, β-elemene, α-terpinene, α-phellandrene and γ-terpinene (CG 31, III); *p*-cymene (CG 31,IVe); δ-cadinene, β-caryophyllene, α-pinene, β-pinene, sabinene, α-thujene and camphene (CG 31, V).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS no**	**Max conc. in the oil**	**Max feed conc.**	**Intake[(a)]**	**Cramer class**	**NOAEL[(b)]**	**MOE**	**MOET**
MOET CG 3				0.0766				4,501
CG 4								
Citronellyl acetate	09.012	0.14	0.015	0.0013	I	50	37,094	
CG 5								
6-Methylhept-5-en-2-one	07.015	0.08	0.009	0.0008	II	*0.91*	1,192	
CG 6								
Linalyl acetate	09.013	54.1	5.681	0.5100	I	117	229	
Linalool	02.013	25.4	2.667	0.2394	I	**117**	489	
α-Terpineol	02.014	4.38	0.460	0.0413	I	250	6,055	
Spathulenol	n.a.	0.04	0.005	0.0004	I	*3*	7,233	
8-Hydroxylinalyl acetate	n.a.	0.42	0.044	0.0039	I	117	29,694	
trans-8-Hydroxylinalool	n.a.	0.43	0.045	0.0041	III	117	28,666	
MOET CG 6				0.7991				148
CG 8								
8-Hydroxycarvotanacetone	n.a.	0.02	0.002	0.0002	III	*0.15*	884	
CG 13								
cis-Linalool oxide (5-ring)	n.a.	0.51	0.053	0.0048	II	*0.91*	190	
trans-Linalool oxide (5-ring)	n.a.	0.45	0.047	0.0043	II	*0.91*	214	
cis-Linalool oxide (6-ring)	n.a.	0.05	0.006	0.0005	II	*0.91*	1,788	
trans-Linalool oxide (6-ring)	n.a.	0.05	0.005	0.0005	II	*0.91*	2,011	
MOET CG 13				0.0100				91
CG 14								
Rosefuran	13.148	0.012	0.001	0.0001	II	*0.91*	8,045	
CG 27								
Methyl anthranilate	09.715	0.42	0.044	0.0040	I	**150**	37,989	
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	0.70	0.074	0.0066	I	**44**	6,668	
β-*trans*-Ocimene	n.a.	0.88	0.093	0.0083	I	44	5,286	
β-*cis*-Ocimene	01.064	0.23	0.024	0.0022	I	44	20,207	
MOET CG 31, II				0.0171				2,573
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.045	2.37	0.249	0.0223	I	**250**	11,191	
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
β-Pinene	01.003	0.99	0.104	0.0093	I	222	23,862	
δ-3-Carene	01.029	1.08	0.113	0.0102	I	222	21,807	
Bicyclogermacrene	n.a.	0.03	0.003	0.0003	I	*3*	11,788	
MOET CG 31, V				0.0197				5,794
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
3,7,10-Humulatriene	01.043	0.07	0.007	0.0007	I	*3*	4,547	
CG 32 (epoxides)								
β-Caryophyllene epoxide	16.043	0.29	0.030	0.0027	II	**109**	39,874	

FLAVIS: EU Flavour Information System; bw: body weight; NOAEL: no observed adverse effect level; MOE: margin of exposure; MOET: (total) margin of exposure.

(a): Intake calculations for the individual components are based on the use level of 10.5 mg/kg in feed for chicken for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

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

As shown in Table 5, for all the assessment groups, the MOET was ≥ 91. From the lowest MOET of 91 for chicken 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 6.

Table 6: Combined margin of exposure (MOET) for the assessment group 'Linalool oxides' (CG 13) calculated for the different target animal categories at the proposed use level and maximum safe use levels in feed calculated to ensure a MOET ≥ 100 (500 for cats)

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use level (mg/kg feed)	Lowest MOET	Maximum safe use level (mg/kg feed)[(a)]
Chicken for fattening	2	158	10.5	91	10
Laying hen	2	106	16	89	14
Turkey for fattening	3	176	14.5	88	13
Piglet	20	880	19	90	17
Pig for fattening	60	2,200	23	89	20
Sow lactating	175	5,280	28	90	25
Veal calf (milk replacer)	100	1,890	45	88	43
Cattle for fattening	400	8,000	42.5	89	38
Dairy cow	650	20,000	27.5	89	24
Sheep/goat	60	1,200	42.5	89	38
Horse	400	8,000	42.5	89	38
Rabbit	2	100	17	89	15
Salmon	0.12	2.1	48.5	86	42
Dog	15	250	51	87	44
Cat	3	60	42.5	89	8[(b)]
Ornamental fish	0.012	0.054	125	121	–

CG: chemical group; MOET: (total) margin of exposure; DM: dry matter.

(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 ornamental fish. The maximum safe use levels in feed were calculated in order to ensure a MOET ≥ 100 for the different target species and > 500 for cats, considering their unusually low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021). The calculated maximum safe levels in feed are shown in Table 6.

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 (EFSA FEEDAP Panel, 2010). Simultaneous use in feed and water for drinking may lead to the maximum safe dose being exceeded.

3.3.1.1. Conclusions on safety for the target species

The FEEDAP Panel concludes that petitgrain bigarade oil from the leaves of *Citrus* × *aurantium* L. is safe up to the maximum proposed use level of 125 mg/kg complete feed for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 10 mg/kg for chicken for fattening, 14 mg/kg for laying hen, 13 mg/kg for turkey for fattening, 17 mg/kg for piglet, 20 mg/kg for pig for fattening, 25 mg/kg for lactating sow, 43 mg/kg for veal calf (milk replacer), 38 mg/kg for cattle for fattening, sheep, goat and horse, 24 mg/kg for dairy cow, 15 mg/kg for rabbit, 42 mg/kg for salmon, 44 mg/kg for dog and 8 mg/kg for cat.

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.

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

3.3.2. Safety for the consumer

Petitgrain bigarade oil obtained by steam distillation of the leaves and twigs of the bitter orange tree is added to a wide range of food categories for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 0.76 μg/kg bw per day for petitgrain bigarade oil.

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 (see Table 1).

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the Panel recognises that the constituents of petitgrain bigarade oil are expected to be extensively metabolised and excreted in the target species. Therefore, a relevant increase of the uptake of the individual constituents by humans consuming products of animal origin is not expected.

Considering the reported human exposure due to direct use of petitgrain bigarade oil in food (Burdock, 2009), it is unlikely that consumption of products from animals given petitgrain bigarade 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 petitgrain bigarade essential oil up to highest safe level in feed for the target animals.

3.3.3. 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 sheet[23] for petitgrain bigarade oil where skin and eye irritancy and sensitisation hazards for users have been identified.

3.3.4. Safety for the environment

Citrus × *aurantium* L. is a native species to Europe where it is widely grown both for commercial and decorative purposes. The use of the additive in animal feed under the proposed conditions is not expected to pose a risk for the environment.

3.4. Efficacy

Petitgrain bigarade oil from the leaves of *Citrus* × *aurantium* L. (petitgrain) is listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2855.

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

4. Conclusions

The essential oil from the leaves of *Citrus* × *aurantium* L. (petitgrain bigarade oil) is safe up to the maximum proposed use levels of 125 mg/kg for ornamental fish. For the other species, the calculated safe concentrations in complete feed are 10 mg/kg for chicken for fattening, 14 mg/kg for laying hen, 13 mg/kg for turkey for fattening, 17 mg/kg for piglet, 20 mg/kg for pig for fattening, 25 mg/kg for lactating sow, 43 mg/kg for veal calf (milk replacer), 38 mg/kg for cattle for fattening, sheep, goat and horse, 24 mg/kg for dairy cow, 15 mg/kg for rabbit, 42 mg/kg for salmon, 44 mg/kg for dog and 8 mg/kg for cat. The FEEDAP Panel considers that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed. Simultaneous use in feed and water for drinking may lead to the safe maximum dose being exceeded.

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

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

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

[23] Technical dossier/Supplementary Information March 2020/Annex_X_SIn reply_petitgrain_bigarade_oil_MSDS. Hazards for skin irritation (H315), skin sensitisation (H317b, category 1B), eye damage (H318).

Since petitgrain bigarade oil from *Citrus* × *aurantium* L. is recognised to flavour food, and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. Documentation as provided to EFSA/Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 08 – Sapindales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG) and registered with the Question number EFSA-Q-2010-01517
14/12/2010	Reception mandate from the European Commission
26/02/2013	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. *Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
20/06/2018	Comments received from Member States
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: amyris oil, cashew oil, olibanum tincture, neroli bigarade oil, petitgrain bigarade absolute, mandarin terpenes, grapefruit oil expressed, grapefruit extract (sb), grapefruit extract
26/03/2020	Reception of supplementary information from the applicant (partial submission)
12/03/2021	The application was split and a new EFSA-Q-2021-00134 was assigned to the preparation included in the present assessment
17/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started for the preparation included in the present assessment
02/04/2021	Partial withdrawal by applicant (EC was informed) for the following additive: olibanum extract (wb)
05/05/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment

References

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Abbreviations

BDG	botanically defined group
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	chemically defined group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
DM	dry matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EU	European Union
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavor Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
FL-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
HPLC	high-performance liquid chromatography
ISO	International standard organiszation
LOD	limit of detection
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
MOET	combined margin of exposure (total)
MSDI	maximised survey-derived intake
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PPR	EFSA Panel on Plant Protection Products and their Residues
QSAR	Quantitative Structure–Activity Relationship
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 satisfactory quantified in the *feed additive* by the proposed method in 5 different lots of *bitter orange extract of the whole fruit*.

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

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

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

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

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

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

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