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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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......................................................................................................................... 7
    2.1. Data........................................................................................................................................... 7
    2.2. Methodologies............................................................................................................................. 7

3. Assessment.............................................................................................................................................. 8
    3.1. Origin and extraction ............................................................................................................... 8
    3.2. Expressed orange oil ................................................................................................................. 9

3.2.1. Characterisation of expressed orange oil ........................................................................... 9
    3.2.1.1. Impurities ......................................................................................................................... 11
    3.2.1.2. Shelf-life ........................................................................................................................ 11
    3.2.1.3. Conditions of use ......................................................................................................... 11

3.2.2. Safety ........................................................................................................................................ 11
    3.2.2.1. Safety for the target species ............................................................................................... 14
    3.2.2.2. Safety for the consumer ..................................................................................................... 20
    3.2.2.3. Safety for the user ............................................................................................................. 21
    3.2.2.4. Safety for the environment .............................................................................................. 21
    3.2.2.5. Safety for the target species ............................................................................................... 21

3.3. Distilled orange oil......................................................................................................................... 21
    3.3.1. Characterisation of the distilled fraction ........................................................................... 21
    3.3.1.1. Impurities ......................................................................................................................... 22
    3.3.1.2. Shelf-life ........................................................................................................................ 22
    3.3.1.3. Conditions of use ......................................................................................................... 22

3.3.2. Safety ........................................................................................................................................ 23
    3.3.2.1. Safety for the target species ............................................................................................... 23
    3.3.2.2. Safety for the consumer ..................................................................................................... 24
    3.3.2.3. Safety for the user ............................................................................................................. 24
    3.3.2.4. Safety for the environment .............................................................................................. 24
    3.3.2.5. Safety for the target species ............................................................................................... 24

3.4. Folded orange oils......................................................................................................................... 24
    3.4.1. Characterisation of the folded oils ................................................................................... 24
    3.4.1.1. Impurities ......................................................................................................................... 27
    3.4.1.2. Shelf-life ........................................................................................................................ 27
    3.4.1.3. Conditions of use ......................................................................................................... 27

3.4.2. Safety ........................................................................................................................................ 28
    3.4.2.1. Safety for the target species ............................................................................................... 28
    3.4.2.2. Safety for the consumer ..................................................................................................... 29
    3.4.2.3. Safety for the user ............................................................................................................. 30
    3.4.2.4. Safety for the consumer ..................................................................................................... 30
    3.4.2.5. Safety for the environment .............................................................................................. 30

3.5. Efficacy .......................................................................................................................................... 31

4. Conclusions........................................................................................................................................ 31

5. Documentation provided to EFSA/Chronology .............................................................................. 32

References.............................................................................................................................................. 32

Abbreviations............................................................................................................................................. 36

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, petitgrain 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.............. 38
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 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 (nearly 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

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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, 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/esters, acetals/esters       | Octan-1-ol                                                                               | 02.006    | 2013                |
|     | with esters containing saturated alcohols and acetals containing saturated      | Decan-1-ol                                                                               | 02.024    |                     |
|     | aldehydes                                                                      | 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           | (Z)-Nerol                                                                                | 02.058    | 2016a               |
|     | aliphatic primary alcohols/aldehydes/esters, acids, acetals and esters          | Neral                                                                                    | 05.170    |                     |
|     |                                                                                   | *trans*-3,7-Dimethylocta-2,6-dienal (geranial)                                           | 05.188    |                     |
|     |                                                                                   | Geranyl acetate                                                                          | 09.011    |                     |
|     |                                                                                   | Neryl acetate                                                                            | 09.213    |                     |
|     |                                                                                   | 2,4-Decadienal                                                                           | 05.081    | 2019a               |
|     |                                                                                   | *trans*-2-Decenal                                                                         | 05.191    |                     |
| 04  | Non-conjugated and accumulated unsaturated straight-chain and branched-chain    | Citronelloi                                                                             | 02.011    | 2016b               |
|     | aliphatic primary alcohols, aldehydes, acids, acetals and esters                | Citronellal                                                                             | 05.021    |                     |
|     |                                                                                   | Neral                                                                                    | 05.170    |                     |
|     |                                                                                   | Octadeca-9,12-dienoic acid(a)                                                            | 08.041    | JECFA               |

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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](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<sup>(a)</sup> | 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               |
|    |                                                                                 | 2-(4-Methylphenyl)propan-2-ol | 02.042 |                     |
|    |                                                                                 | 4-Terpinolinol | 02.072 |                     |
|    |                                                                                 | (1)-<i>α</i>-Bisabolol<sup>(a)</sup> | 02.129 | 2011a, CEF          |
|    |                                                                                 | (1)-<i>α</i>-Elemol<sup>(a)</sup> | 02.149 |                     |
| 07 | Primary alicyclic saturated and unsaturated alcohols/ aldehydes/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<sup>(a)</sup> | 02.062 | JECFA               |
|    |                                                                                 | Dihydrocarvone | 07.128 | JECFA               |
|    |                                                                                 | Carvone<sup>(a)</sup> | 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 |                     |
|    |                                                                                 | <i>α</i>-Phellandrene | 01.006 |                     |
|    |                                                                                 | γ-Terpinene | 01.020 |                     |
|    |                                                                                 | d-Limonene | 01.045 |                     |
|    |                                                                                 | Pin-2(10)-ene (<i>β</i>-pinene) | 01.003 |                     |
|    |                                                                                 | Pin-2(3)-ene (<i>α</i>-pinene) | 01.004 |                     |
|    |                                                                                 | <i>β</i>-Caryophyllene | 01.007 |                     |
|    |                                                                                 | Myrcene | 01.008 |                     |
|    |                                                                                 | Camphene | 01.009 |                     |
|    |                                                                                 | Valencene | 01.017 |                     |
|    |                                                                                 | 3,7-Dimethyl-1,3,6-octatriene (<i>β</i>-ocimene)<sup>(b)</sup> | 01.018 |                     |
|    |                                                                                 | δ-3-Carene | 01.029 |                     |
|    |                                                                                 | δ-Cadinene<sup>(a),(c)</sup> | 01.021 | 2011b, CEF          |
|    |                                                                                 | β-Cubebene<sup>(a),(c)</sup> | 01.030 |                     |
|    |                                                                                 | Germacra-1(10),4(14),5-triene (<i>β</i>-Germacrene)<sup>(a),(c)</sup> | 01.042 |                     |
|    |                                                                                 | 3,7,10-Humulatriene<sup>(a),(c)</sup> | 01.043 |                     |
|    |                                                                                 | <i>α</i>-Muurulene<sup>(a),(c)</sup> | 01.052 |                     |
|    |                                                                                 | <i>β</i>-Phellandrene<sup>(a),(c)</sup> | 01.055 |                     |
|    |                                                                                 | 4(10)-Thujene (sabinene)<sup>(a)</sup> | 01.059 | 2015a, CEF          |
|    |                                                                                 | <i>cis</i>-3,7-Dimethyl-1,3,6-octatriene (<i>cis</i>-<i>β</i>-Ocimene)<sup>(a)</sup> | 01.064 |                     |
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 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. 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.

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 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. 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.

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 429/2008 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 and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

(*) 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-caryophyllene [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 γ-farnesene [01.040], as a mixture of 38–50% γ-isomer, 29–40% β-isomer (EFSA CEF Panel, 2015b).
(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).

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.

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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 | 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

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\(^{15}\) Technical dossier/Supplementary information May 2020/Annex_IIL_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 | 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 (trans-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-Terpinol                 |                  | 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|
| Germacr-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-ol (hereinafter referred to as 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-methoxycutellarein (0.16–0.6 g/L) and 3,5,6,7,3′,4′-hexamethoxyflavone (0.0–2.0 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-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, ocyl 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, isoloterpinolene, 1-isopropyl-4-methylenbenzene, trans-1-methyl-4-(1-methylyvinyl)cyclohex-2-en-1-ol, trans-2-decenc, camphe ne, sativene, γ-murolene, 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*. Analysis of five batches showed that eight furocoumarins (phellopterin, 8-geranoxypsoralen, psoralen, 8-methoxypsoralen (xanthotoxin), 5,8-dimethoxypsoralen (isopimpellin), 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. 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) PCCD/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). 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 food and feed 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-cubenene [01.030], δ-germacrene [01.042], 3,7,10-humulatriene [01.043], β-phellandrene [01.055] and α-muurulene [01.052] were evaluated in

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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, xanthotoxin, 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 µg/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

www.efsa.europa.eu/efsajournal 11 EFSA Journal 2021;19(11):6891
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 flavourings in food. In the absence of such toxicological 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 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, and Quantitative Structure-Activity Relationship (QSAR) analysis for substances raising potential genotoxicity concern. 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 heptamethoxylavone (0.3–2.0 g/L), nobiletin (0.25–1.0 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). 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%) and α-sinensal (0.028–0.035%) and β-sinensal (0.03-0.044%), for which EFSA requested further in vitro 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 LS178Y 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 flavourings 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 proflers as well as empirical profliers. 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-methylquercetagetin (3.3%), 5-demethyl-tetramethylyscutellarein (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 \( \mu \)L, 30 \( \mu \)L, 50 \( \mu \)L, 100 \( \mu \)L and 200 \( \mu \)L/plate. Significant toxicity was induced at 50 \( \mu \)L/plate and above. No changes in the number of revertant colonies were observed at 10 and 30 \( \mu \)L/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), terpineol [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], \( \beta \)-cymene [01.002] and \( \beta \)-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 \( \alpha \)-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 \( \beta \)-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within sub-assessment group II (\( \alpha \)-farnesene [01.040], \( \beta \)-farnesene [01.041], cis-\( \beta \)-ocimene [01.064] and trans-\( \beta \)-ocimene), group III (\( \alpha \)-phellandrene [01.055], \( \alpha \)-phellandrene [01.006], terpinolene [01.005], isoterpinolene and \( \gamma \)-terpinene [01.020]) and group V (\( \alpha \)-pinene [01.004], sabinen [01.059], valencene [01.017], \( \delta \)-3-carene [01.029], \( \alpha \)-copaene, \( \delta \)-cadinene [01.021], \( \beta \)-copaene, \( \beta \)-cubebene [01.030], \( \beta \)-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)cy clohex-2-en-1-ol, nootkatone [07.089], \( \beta \)-elemene, \( \gamma \)-muurulene, sativene, germacr-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 \textit{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 MOEs 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.

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.

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32 Terpineol is a mixture of four isomers: \( \alpha \)-terpineol [02.014], a mixture of (R)-(\( \cdot \))\( \alpha \)-terpineol and (S)-(\( \cdot \))\( \alpha \)-terpineol, \( \beta \)-terpineol, \( \gamma \)-terpineol and 4-terpineol [02.072].

33 Compounds included in the assessment groups but not reported in the table: undecanal, octyl acetate and hexanal (CG 1); trans-2-decanal (CG 3); trans-sabinene hydrate (CG 8); terpinolene, \( \beta \)-elemene, isoterpinolene and \( \gamma \)-terpinene (CG 31, III); \( \delta \)-isopropenyl-4-methylbenzene (CG 31, IVe); \( \beta \)-copaene, \( \beta \)-cubebene, \( \beta \)-pinene, \( \beta \)-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<sup>(a)</sup> mg/kg | Cramer class<sup>(b)</sup> | NOAEL<sup>(c)</sup> mg/kg bw per day | MOE | MOET |
| 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 |
|                           | trans-1-methyl-4-(1-methyl vinyl)cyclohex-2-en-1-ol | – | 0.01 | 0.006 | 0.0006 | I | 3 | 5,222 |
|                           | cis-para-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-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, 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 |           |
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                                | 37          |
| Turkey for fattening        | 3                | 176                    | 80                                | 37          |
| Piglet                      | 20               | 880                    | 144                               | 37          |
| Pig for fattening           | 60               | 2,200                  | 172                               | 37          |
| Sow lactating               | 175              | 5,280                  | 200                               | 37          |
| Veal calf (milk replacer)   | 100              | 1,890                  | 130                               | 95          |
| Cattle for fattening        | 400              | 8,000                  | 130                               | 90          |

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(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.
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.

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
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 constituents: 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 | 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/SIn 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% × 0.6 = 0.0002 × 0.3 + 0.0002 × 0.1 = 0.0004%.
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, 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 sheet 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).

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%). 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<sup>(b)</sup> Mean<sup>(a)</sup> | 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 |

<sup>40</sup> 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 1).

<sup>41</sup> Technical dossier/Supplementary information May 2020/Annex II SIn reply_orange_terpenes_COA_batches.

<sup>42</sup> Technical dossier/Supplementary information May 2020. SIn reply_BDG08_orange_oil_terpenes/GC-FID analysis.
The applicant provided the full characterisation of the five batches obtained by GC–MS. 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.

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 |

The HPLC analysis of five batches of distilled orange oil showed that furocoumarins (phellopterin, 8-geranoxypsoralen, psoralen, 5-methoxypsoralen (bergapten), 8-methoxypsoralen (xanthotoxin), dimethoxypsoralen (isopimpellin), 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). 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 pigs.

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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, a-terpinol, carvone, trans-β-ocimene, 1,8-dien-7-ol, p-mentha-1,8-dien-7-ol, 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, xanthotoxin, 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.
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 food and feed 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.

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 sheet 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

*Citrus 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. In each case, the percentage remaining after drying at 100 °C of three representative batches of each folded oil was determined. 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 expressed oil with a mean dry matter content of 10.5% (range 9.6–11.9%). Based on these values, 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 expressed oil with a mean dry matter content of 20.9% (range 20.2–21.5%). Based on these values, an enrichment factor for the non-volatiles of about 10.

*Folded oil C* (described by the applicant only as a folded oil).

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 [missing data] for folded oil C; the refractive index at 20 °C was in the range 1.473–1.479 for folded oil A, 1.479–1.480 for folded oil B (specification: 1.475–1.485) and [missing data] for folded oil C. The optical rotation at 20 °C was in the range [missing data] for folded oil C. Folded orange oils are identified with the CAS number 8028-48-6, and the FEMA number 2822.
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 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 | EU register name | CAS no | FLAVIS no | A (%) of GC area (b) | B (%) of GC area (b) |
|-------------|------------------|--------|-----------|----------------------|----------------------|
|             |                  |        |           | Mean(a) | Range     | Mean(a) | Range     |
| d-Limonene  |                  | 5989-27-5 | 01.045  | 87.2 | 83.3–89.0 | 68.4 | 64.6–71.6 |
| Linalool    |                  | 78-70-6 | 02.013  | 2.35 | 1.73–3.21 | 4.06 | 2.92–5.51 |
| Decanal     |                  | 112-31-2 | 05.010  | 1.53 | 0.87–1.92 | 5.56 | 4.75–6.44 |
| Myrcene     |                  | 123-35-3 | 01.008  | 0.76 | 0.48–1.10 | 0.36 | 0.21–0.58 |
| Geranial    |                  | 141-27-5 | 05.188  | 0.52 | 0.41–0.61 | 1.60 | 1.03–1.99 |
| Total       |                  |        |          | 92.3 | 89.7–93.6 | 79.9 | 76.8–82.0 |

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 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 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_1_EURL_orange_oil_terpeneless.
52 Technical dossier/Supplementary information May 2020/Annex_III_SIn_Reply_orange_oil_terpeneless_analytical_results and
Thirty-three out of the 35 compounds, which were detected in orange oil expressed and are listed in the footnote, 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, out of the 35 compounds were detected and accounted for 0.1% 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 esters 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.

### Table 10: Additional 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 | A Mean (a) | A Range | B Mean (a) | B Range |
|-------------|-----------------|--------|-----------|------------|---------|------------|---------|
| α-Pinene    | 80-56-8         | 01.004 | 0.02      | 0.001–0.06 | 0.01    | 0.002–0.01 |         |
| Sabine     | 3387-41-5       | 01.059 | 0.07      | 0.02–0.13  | 0.02    | 0.01–0.05  |         |
| Octanal     | 124-13-0        | 05.009 | 0.08      | 0.05–0.12  | 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  |         |
| δ-3-Carene | 13466-78-9      | 01.029 | 0.08      | 0.04–0.12  | 0.04    | 0.03–0.06  |         |
| Valencene  | 4630-07-3       | 01.017 | 0.37      | 0.06–0.51  | 0.76    | 0.20–1.17  |         |
| α-Terpineol | 98-55-5         | 02.014 | 0.46      | 0.40–0.57  | 1.18    | 0.79–1.54  |         |
| Neral      | 106-26-3        | 05.170 | 0.30      | 0.24–0.35  | 0.79    | 0.65–0.97  |         |
| Citronellal | 106-23-0        | 05.021 | 0.30      | 0.15–0.35  | 0.63    | 0.38–0.91  |         |
| Dodecanal | 112-54-9        | 05.011 | 0.24      | 0.14–0.34  | 0.68    | 0.33–0.88  |         |
| α-Copaene | 3856-25-5       | –      | 0.24      | 0.18–0.31  | 0.61    | 0.49–0.73  |         |
| Nonanal    | 124-19-6        | 05.025 | 0.16      | 0.09–0.22  | 0.29    | 0.21–0.41  |         |
| β-Phellandrene | 99-83-2        | 01.006 | 0.02      | 0.01–0.04  | 0.02    | 0.01–0.03  |         |
| δ-Cadinen | 29350-73-0      | 01.021 | 0.24      | 0.17–0.28  | 0.61    | 0.48–0.69  |         |
| Germacre-1(10),4(14),5-triene | 23986-74-5 | 01.042 | 0.22      | 0.16–0.28  | 0.57    | 0.40–0.80  |         |
| Total      | –               | 3.02   | 2.65–3.40 | 6.59      | 5.67–7.87 | 86.5      | 83.9–87.7 |         |

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

(a): Mean calculated on five batches.

53 Additional volatile constituents: 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, 1-carvone, citronellol, p-mentha-1,8(10)-dien-9-ol, undecanone, 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-methylibenzene, trans-1-methyl-4-(1-methylibenzyl)cyclohex-2-en-1-ol, trans-2-decalen, sabinene, γ-muurolene and cis-para-2,8-methadendien-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, 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 20.4–20.5% for the folded oils A, B and C, respectively. The literature search performed by the applicant 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-Más 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). Furocoumarins (phellopterin, 8-geranoxypsoralen, psoralen, 8-methoxypsoralen (xanthotoxin), dimethoxypsoralen (isopimpellin), 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). Fourteen furocoumarins (including also trioxalene, herniarine, citroptene, toncarine, epoxybergamotine and byakangelicol) were below the LOQ of 1 mg/kg in additional three batches analysis of orange oil terpeneless folded.

3.4.1. 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. 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), cyfluthrin (< 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).

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). However, no evidence supporting this statement were provided.
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 (5)-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 sheet 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)                                                                 |
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015c. Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic hydrocarbons evaluated by EFSA in FGE.25Rev3. EFSA Journal 2015;13(4):4067, 72 pp. https://doi.org/10.2903/j.efsa.2015.4067

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Expressed orange oil and its fractions for all animal species

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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
| Acronym | Description |
|---------|-------------|
| 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 |
| EUURL   | 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 |
| FEG     | 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), 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 Pharmacopoeia 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 aurantiifolia [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 linallyl acetate and linalool in petitgrain 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.

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