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Safety and efficacy of feed additives consisting of expressed lemon oil and its fractions from *Citrus limon* (L.) Osbeck and of lime oil from *Citrus aurantiifolia* (Christm.) Swingle 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 lemon oil and distilled lemon oil from *Citrus limon* (L.) Osbeck and lime oil from *Citrus aurantiifolia* (Christm.) Swingle, when used as sensory additives in feed for all animal species. The use of the expressed lemon oil and its residual fraction and the use of lime oil in feed is not expected to increase the exposure to furocoumarins and methoxycoumarins and perillaldehyde of those target species that are already fed citrus by-products a relevant extent (< 10%). For companion animals and ornamental fish not normally exposed to citrus by-products, no conclusion can be drawn. For the other species and for the distilled fraction of expressed lemon oil, the FEEDAP Panel concludes that the additives under assessment are safe at the maximum proposed or at reduced use levels in complete feed. 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. 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 the respiratory tract and as skin sensitizers. Since expressed lemon oil and its fractions contain furocoumarins, they may cause phototoxicity. The use of the additives under the proposed conditions of use in animal feed is not expected to pose a risk for the environment. Since *C. limon* and *C. aurantiifolia* and their preparations are recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

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Keywords: sensory additives, flavouring compounds, *Citrus limon* (L.) Osbeck, *Citrus aurantiifolia* (Christm.) Swingle, furocoumarins, perillaldehyde, component-based approach

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. In addition, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

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

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

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of expressed lemon oil and distilled lemon oil from *C. limon* (L.) Burm. f. and lime oil from *C. aurantiifolia* (Christm.) Swingle, when used under the proposed conditions of use (see Section 3.2.1.2, 3.3.1.2, 3.4.1.2 and 3.5.1.2).

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

1.2. Additional information

The three preparations under assessment, namely expressed lemon oil and distilled lemon oil from *C. limon* (L.) Burm. f., and lime oil from *C. aurantiifolia* (Christm.) Swingle, 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. The FEEDAP Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) notes that two different additives fall into the definition 'lemon oil distilled' obtained by distillation of expressed lemon oil, a residual fraction and a volatile fraction.

There is no specific EU authorisation for any *C. limon* and *C. aurantiifolia* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008⁵, flavouring preparations

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

⁴ Accepted name: *Citrus limon* (L.) Osbeck, synonym *Citrus limon* (L.) Burm. f.

⁵ Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

produced from food, may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer'.

'Lemon oil' is described in a monograph of the European Pharmacopoeia 10.0 (PhEur, 2020). It is defined as the essential oil obtained by a suitable mechanical procedure without heating from the fresh peel of *Citrus limon* (L.) Burm. f. (currently accepted name: *C. limon* (L.) Osbeck). As identity-test the monograph describes thin-layer chromatography for detection of citral and several furocoumarins, namely bergamottin, 5-geranyloxy-7-methoxycoumarin, citropten, a psoralen derivative and biakangelicin.

In 2004, the Deutsche Forschungsgemeinschaft (DFG) Senate Commission on Food Safety (SKLM) issued a toxicological assessment of furocoumarins in foodstuffs (translated in English in 2006) (SKLM, 2006), which has been updated based on new exposure data in 2010 (SKLM, 2010). The SKLM concluded for food that may contain furocoumarins, a final assessment of the carcinogenic risk was not possible due to the complexity of the influencing factors, such as levels of exposure, the underlying metabolism and the influence of light. An additional risk of skin cancer arising from the intake of typical quantities of flavoured and non-flavoured furocoumarin-containing foods, which leads to an exposure well below the phototoxic dose-range, was regarded as negligible. For phototoxic effects, the SKLM (2010) did not see a significant risk associated with the consumption of typical quantities of correctly stored, processed foods that may contain furocoumarins.

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 Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) and the EFSA Panel on Food Additives and Flavourings (FAF). The list of flavouring compounds together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000⁶ 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 (year of publication, FEEDAP opinion unless otherwise indicated). They are all authorised for use in feed⁷ and food⁸ unless otherwise indicated

CG	Chemical group	Product (EU register name)	FLAVIS no	EFSA opinion,* Year
01	Straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes	Octan-1-ol	02.006	2013
		Heptan-1-ol	02.021	
		Octanal	05.009	
		Decanal	05.010	
		Dodecanal	05.011	
		Nonanal	05.025	
		Octyl acetate	09.007	
		Decyl acetate	09.009	

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

⁷ 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

⁸ 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.

CG	Chemical group	Product (EU register name)	FLAVIS no	EFSA opinion,* Year
03	a, β-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters	Geraniol	02.012	2016a
		(Z)-Nerol	02.058	
		Neral	05.170	
		trans-3,7-Dimethylocta-2,6-dienal (geranial)	05.188	
		Geranyl acetate	09.011	
		Neryl acetate	09.213	
04	Non-conjugated and accumulated unsaturated straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids, acetals and esters	Citronellol	02.011	2016b
		Citronellal	05.021	
		Citronellyl acetate	09.012	
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		2-(4-Methylphenyl)propan-2-ol	02.042	
		4-Terpinenol	02.072	
		1-Terpinenol	02.096	
		β-Terpineol ^(a)	02.097	JECFA
		(I)-α-Bisabolol ^(a)	02.129	
		d,l-Borneol	02.016	
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Fenchyl alcohol	02.038	2016c
		d,l-Isoborneol	02.059	
		d,l-Bornyl acetate	09.017	
		Trans-Carveol ^(a)	02.062	
		Carvone ^(a)	07.012	2014, SC
		1,8-Cineole	03.001	
16	Aliphatic and alicyclic ethers	1,4-Cineole ^(a)	03.007	2011b, CEF
		p-Cymene	01.002	
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	Terpinolene	01.005	2015
		α-Phellandrene	01.006	
		1-Isopropenyl-4-methylbenzene	01.010	
		α-Terpinene	01.019	
		γ-Terpinene	01.020	
		Bisabola-1,8,12-triene	01.027	
		d-Limonene	01.045	
		I-Limonene	01.046	
		Pin-2(10)-ene (β-pinene)	01.003	2016d
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		Valencene	01.017	
		trans-β-Ocimene	01.018	
		δ-3-Carene	01.029	

CG	Chemical group	Product (EU register name)	FLAVIS no	EFSA opinion,* Year
		δ-Cadinene ^{(a),(b)}	01.021	2011c, CEF
		β-Bisabolene ^(a)	01.028	
		β-Cubebene ^{(a),(b)}	01.030	
		δ-Elemene ^(a)	01.039	
		δ-Germacrene ^{(a),(b)}	01.042	
		3,7,10-Humulatriene ^{(a),(b)}	01.043	
		β-Phellandrene ^{(a),(b)}	01.055	
		Tricyclene ^{(a),(b)}	01.060	
		4(10)-Thujene (sabinene) ^(a)	01.059	2015b, CEF
		cis-β-Ocimene ^(a)	01.064	
		α-Farnesene ^(a)	01.040	
		β-Farnesene ^(a)	01.041	2015a, CEF
		1-Methyl-1,3-cyclohexadiene ^(a)	01.077	

(*): 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): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). No longer authorised for use as flavours in food.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁹ in support of the authorisation request for the use of expressed lemon oil and distilled lemon oil from *C. limon* (L.) Burm. f., and lime oil from *C. aurantiifolia* (Christm.) Swingle as feed additives.

The FEEDAP Panel 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 use the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 8.¹⁰

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

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the three preparations expressed lemon oil and distilled lemon oil from *C. limon* (L.) Burm. f., and lime oil from *C. aurantiifolia* (Christm.) Swingle is in line with the principles laid down in Regulation (EC) No 429/2008¹² 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, 2012c), Guidance on studies concerning the safety of use of the additive for users/ workers (EFSA FEEDAP Panel, 2012d), Guidance on the identity, characterisation and conditions of use of

⁹ FEED dossier reference: FAD-2010-0322.

¹⁰ Technical dossier FAD-2010-0335/Supplementary information February 2018/2018-01-30_SInReply_cardamom.

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

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

feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b), Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019c).

3. Assessment

The preparations under assessment are expressed lemon oil from fruit peels of *C. limon* (L.) Osbeck (isonym *C. limon* (L.) Burm. f.) and its fractions obtained after distillation, and lime oil from *C. aurantiifolia* (Christm.) Swingle. 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 of the additives

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 hybrids derived from three ancestral species now represented by the cultivars described as the mandarin, pomelo and citron. Thus, lemon is a natural hybrid of citron (*Citrus medica*) and the sour orange (*Citrus aurantium*), which is itself derived from a cross made with pomelo (*Citrus maxima*). The currently preferred botanical name for lemon is *Citrus limon* (L.) Osbeck but the isonym *C. limon* (L.) Burm. f. is still widely used. Limes form a more diverse series of hybrids than lemons with at least five distinct groups recognised. The present application concerns an extract made from limes belonging to 'small fruit acid limes' (*C. aurantiifolia* (Christm.) Swingle).

Citrus species are thought to have originated in northern India and then to have spread naturally throughout the tropical and subtropical areas of Australasia. After domestication, the many varieties of *Citrus* including limes and lemons were further distributed and can now be found growing in most parts of the world.

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 lemon 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. In addition to the direct use of cold-pressed lemon oil, the applicant also uses distillation of the expressed oil to obtain two further preparations – a volatile fraction collected after condensation and a residual fraction.¹³

Although lime oil can be expressed, the applicant uses steam distillation as the preferred method of extraction. Intact limes are crushed in a screw press and the juice containing the lime oil recovered. The juice is then steam distilled and the volatile fraction condensed and collected. It may then be filtered and polished as required.¹⁴

The FEEDAP Panel considers that two types of preparations fall into the definition 'lemon oil distilled': (i) lemon oil distilled (residual fraction) which corresponds to the residual fraction (i.e. the residues) from the distillation of lemon oil expressed and (ii) lemon oil distilled (volatile fraction), which corresponds to the volatile fraction (i.e. the distillate) from the distillation of lemon oil expressed.

Therefore, four additives are considered in the next sections, expressed lemon oil (Section 3.2), the residual fraction obtained from the distillation of expressed lemon oil (Section 3.3), the distilled fraction of expressed lemon oil (Section 3.4) and lime oil (Section 3.5).

3.2. Expressed lemon oil

This application concerns the essential oil derived by cold expression from fruit peel of *C. limon* from multiple origin (Argentina, Brazil, South Africa, Spain, France and Italy).

¹³ Technical dossier/Supplementary information June 2019.

¹⁴ Technical dossier/Supplementary information August 2019.

3.2.1. Characterisation of expressed lemon oil

Expressed lemon oil is a yellow clear mobile liquid with a characteristic aroma. In 10 batches of the additive (five originate from the Mediterranean area, five from other regions outside the EU, e.g. Argentina, Brazil and South Africa), the optical rotation at 20°C ranged between + 65.1° and + 66.6° (specification: + 57.0° to + 68.0°), refractive index between 1.4732 and 1.4747 (specification: 1.4710–1.4760) and the density (20°C) ranged between 0.847 and 0.851 kg/L (specification: 0.843–0.860).¹⁵ Expressed lemon oil is identified with the single Chemical Abstracts Service (CAS) number 84929-31-7 and Flavor Extract Manufacturers Association (FEMA) number 2625.

Volatile components

The product specifications are based on the International Standard developed by the International Organisation for Standardization (ISO) 855:2003 for essential oil of *C. limon*,¹⁶ which were adapted to reflect the concentrations expressed as % of gas chromatographic peak area (% GC area) of the main volatile components of the essential oil, namely d-limonene (60–73%, the phytochemical marker), pin-2(10)-ene (hereinafter referred to as β-pinene, 9–18%), γ-terpinene (6–12%), pin-2(3)-ene (hereinafter referred to as α-pinene, 1.3–3.0%), 4(10)-thujene (hereinafter referred to as sabinene, 0.5–3.0%), trans-3,7-dimethylocta-2,6-dienal (hereinafter referred to as geranial, 0.1–2%) and neral (cis-3,7-dimethylocta-2,6-dienal, 0.1–1.8%). Analysis of 10 batches of the additive¹³ showed compliance with these specifications (Table 2). These seven compounds account for about 94.2% on average (range 92.4–95.5%) of % GC area.

Table 2: Major volatile constituents of expressed essential oil from the fruit peels of *Citrus limon* (L.) Osbeck as defined based on ISO standard (855;2003) specifications and batch to batch variation based on the analysis of 10 batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent EU register name	CAS no	FLAVIS no	Specification (%)	% of GC area	
				Mean ^(a)	Range
d-Limonene	5989-27-5	01.045	60–73	64.8	61.2–66.7
β-Pinene (pin-2(10)-ene)	127-91-3	01.003	9–18	13.3	11.0–16.8
γ-Terpinene	99-85-4	01.020	6–12	9.04	7.66–11.5
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	1.3–3.0	2.74	2.23–3.63
Sabinene (4(10)-thujene)	3387-41-5	01.059	0.3–3.0	2.21	1.41–2.65
Geranial	141-27-5	05.188	0.1–2.0	1.30	0.65–2.08
Neral	106-26-3	05.170	0.1–1.8	0.87	0.39–1.43
Total				94.2	92.4–95.5

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

(a): Mean calculated on 10 batches.

The applicant provided the full characterisation of the volatile constituents in 10 batches obtained by gas chromatography coupled with mass spectrometry (GC-MS).¹⁷ In total, up to 88 constituents were detected, 71 of which were identified and accounted on average for 99.7% (99.5–99.9%) of the GC area. Besides the seven compounds indicated in the product specifications, 13 other compounds were detected at individual levels > 0.1% and are listed in Table 3. These 20 compounds together

¹⁵ Technical dossier/Supplementary information June 2019/Annex_II_SIn_Reply_lemon_oil_expressed_CoA.

¹⁶ Essential oil obtained by expression, without the aid of heat and with or without previous separation of the pulp and the peel, from the fresh fruit of *Citrus limon* (L.) Burm. f., of the Rutaceae family, growing mainly in Argentina, Brazil, Cyprus, Italy, Ivory Coast, Spain, South Africa and United States.

¹⁷ Technical dossier/Supplementary information June 2019/Annex_III_SIn_Reply_lemon_oil_expressed_chromatograms.

account on average for 99.0% (98.4–99.3%) of the GC area. The remaining volatile 51 compounds (ranging between 0.002% and 0.1% and accounting together for < 1%) are listed in the footnote.¹⁸

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

Constituent EU register name	CAS no	FLAVIS no	% of GC area	
			Mean^(a)	Range
Myrcene	123-35-3	01.008	1.53	1.25–2.14
β-Bisabolene	495-61-64	01.028	0.52	0.10–0.87
α-Thujene	2867-05-2	–	0.41	0.11–0.56
1-Isopropyl-4-methylbenzene	99-87-6	01.002	0.36	0.20–0.59
Neryl acetate	141-12-8	09.213	0.35	0.07–0.68
(E)-α-Bergamotene	13474-59-4	–	0.31	0.07–0.57
Terpinolene	586-62-9	01.005	0.28	0.07–0.56
Geranyl acetate	105-87-3	09.011	0.27	0.04–0.51
β-Caryophyllene	87-44-5	01.007	0.23	0.06–0.33
α-Terpinene	99-86-5	01.019	0.19	0.05–0.30
I-Fenchone	7787-20-4	–	0.12	0.06–0.17
Linalool	78-70-6	02.013	0.11	0.01–0.45
cis-3,7-Dimethyl-1,3,6-octatriene (cis-β-Ocimene)	3338-55-4	01.064	0.11	0.10–0.12
Total			4.80	2.96–6.23

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

(a): Mean calculated on 10 batches.

Among the volatile components, some substances of concern were detected. α-Thujone, a substance which shall not be added as such to food (see Section 3.2.2.2),¹⁹ was identified and quantified (0.006%) in one batch and 5,7-dimethoxycoumarin (hereinafter referred to as citropten, also known as limettin) was detected (0.007–0.057%) in five batches of Mediterranean origin. p-Mentha-1,8-dien-7-al (hereinafter referred as to perillaldehyde) a substance for which EFSA identified a concern for genotoxicity (EFSA CEF Panel, 2015c) was also detected in one batch (0.023%).

Non-volatile components

The non-volatile residue (residue on evaporation) of expressed lemon oil accounts for 1.8–3.6% 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 lemon oil.²⁰ The non-volatile fraction contains furocoumarins and methoxycoumarins, which together account for 0.5–0.8% of the oil (Comments on the PhEur, 2012). Besides furocoumarins and coumarins, the presence of hydrocarbons, sterols, waxes, carotenoids and flavonoids has been reported (Mehl et al., 2014; Russo et al., 2015). Marti et al. (2014) identified compounds belonging to the classes of methoxylated flavones (e.g. 4',5,7-trimethoxyflavone) and fatty acids (hydroxylated and unsaturated)

¹⁸ Additional volatile constituents: trans-3,7-dimethyl-1,3,6-octatriene, alpha-terpineol, alpha-phellandrene, camphene, octanal, para-mentha-3,8-diene, citronellol, octan-1-ol, valencene, decanal, nonanal, citronellal, delta-3-carene, citropten, 4-terpinenol, bisabola-1,8,12-triene, bicyclogermacrene, p-mentha-1,8-dien-7-al, cis-limonene oxide, nerol, decyl acetate, β-copaene, geraniol, dodecanal, (E)-α-bisabolene, citronellyl acetate, α-copaene, cyclohexene 4-methyl-1-(1-methylethyl)-, (R)-δ-cadinene, octyl acetate, trans-d-limonene oxide, (I)-α-bisabolol, 1,8-cineole, 1,4-cineole, carvone, β-elemene, germacra-1(10),4(14),5-triene, trans-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, trans-carveol, p-mentha-1,8(10)-dien-9-ol, 3,7-dimethyl-3,6-octadienal (isogeranial), β-cubebene, 1,1,7-trimethyltricyclo [2.2.1.0.(2.6)]heptane, bornyl acetate, α-thujone, cis-para-2,8-menthadien-1-ol, cis-carveol, heptan-1-ol, (Z)-γ-bisabolene, β-sinensal, α-sinensal.

¹⁹ Regulation (EC) NO 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC, OJL 345, 31.12.2008, pp. 34–50.

²⁰ Technical dossier/Supplementary information July 2020/Request of clarification_Annex_IV_non volatile search.

on a qualitative basis in non-volatile lemon oil residues. However, in the quantitative study by Russo et al. (2015), methoxylated flavones were not detected in expressed lemon oil.

The literature is mainly focused on furocoumarins and coumarins, which are considered substances of concern. According to the Comments on the PhEur (2012), the main furocoumarins identified and quantified in lemon oil are bergamottin (5-geranoxypsoralen) (0.12–0.4%), oxypeucedanin hydrate (0.1–0.22%), byakangelicol (0.06–0.16%) and byakangelicin (no % is given) and the main methoxycoumarins are 5-geranyloxy-7-methoxycoumarin (0.23–0.27%) and citropten (0.05–0.15%).

An additional literature search regarding substances of concern and chemical composition of the plant species *C. limon* and its preparations was performed by the applicant.²¹ The occurrence of phellopterin, 8-geranoxypsoralen, psoralen, 5-methoxypsoralen (5-MOP, also known as bergapten), 8-methoxypsoralen (8-MOP, also known as xanthotoxin), dimethoxypsoralen (also known as isopimpellin), imperatorin, oxypeucedanin and 5-geranoxypsoralen (also known as bergamottin) has been reported in the EFSA Compendium (EFSA, 2012).

Analysis of the 10 batches²² by high-performance liquid chromatography (HPLC) with UV detector showed detectable concentrations of 12 of the 14 linear derivatives, with high variability between batches of different origin (Mediterranean origin vs. other regions outside the EU) (see Table 4).

Table 4: Furocoumarins and methoxycoumarins (as mg/kg) in the essential oil from the fruit peels of *Citrus limon* (L.) Osbeck based on the analysis of 10 batches of different origins

Congener	Average (mg/kg)	Range (mg/kg)	
		Outside EU ^(a)	Mediterranean
Furocoumarins			
Psoralen	45.3	85–92	4.4–18.5
5-Methoxypsoralen (bergapten)	23.6	3.9–5.8	7.5–75.4
5-Geranoxypsoralen (bergamottin)	497.3	1.0–1.4	250–1,272
Oxypeucedanin	214.7	0.2–0.4	249–456
8-Methoxypsoralen (xanthotoxin)	187.3	0.2–0.5	152–430
8-Geranoxypsoralen	100.6	< 250	0.2–0.5
Imperatorin	31.5	0.7–11.4	27.1–63.9
5,8-Dimethoxypsoralen (isopimpellin)	6.04	0.1–0.3	7.4–13.9
Phellopterin	0.37	< 0.4	0.1–1.0
Oxypeucedanin hydrate	20.2	< 0.1	7.8–53.4
Byakangelicol	178.7	n.d. ^(c)	93.9–273
Byakangelicin	< 0.1	< 0.1	< 0.1
Methoxycoumarins			
Citropten ^(b)	163.0	0.1–1.0	70–570
5-Geranyloxy-7-methoxycoumarin	< 0.1	< 0.1	< 0.1

(a): Argentina, Brazil and South Africa.

(b): Determined in the volatile fraction by GC-MS.

(c): LOD not given.

Impurities

Data on chemical and microbial impurities were provided in five batches of lemon oil expressed of different origin (three of Mediterranean origin).²³ The concentrations of heavy metals were below the corresponding limit of quantification (LOQ) in five batches. 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 chlorpyrifos ethyl (0.14–0.9 mg/kg), chlorpyrifos methyl (0.11 and 0.43 mg/kg, detected in two batches), 2-phenylphenol (0.74–4.6 mg/kg detected in three batches) and imazalil

²¹ Technical dossier/Supplementary information June 2019/Literature search_lemon_oil.

²² Technical dossier/Supplementary information June 2019/Annex VI_Sin reply_lemon_oil_expressed_SOC_COA and SIN July 2020/Supplement_1_lemon_oil.

²³ Technical dossier/Supplementary information June 2019/Annex VII_Sin reply_lemon_Oil_expressed_impurities. Limit of quantification (LOQ) in mg/kg for heavy metals and arsenic: 0.005 for mercury, 0.01 for cadmium, 0.05 for lead and 0.1 for arsenic; LOQ for individual pesticides: 0.1 mg/kg; LOQ for mycotoxins: < 0.1 µg/kg for aflatoxins B1, B2, G1 and G2.

(0.33 mg/kg, detected in one batch). In three batches of different origin (two of Mediterranean origin), 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 the World Health Organization (WHO) PCCD/F + PCB (Toxic Equivalent) TEQ ranged between 0.83 and 0.92 pg/g wet weight. None of the data on chemical impurities raised concerns.

Analysis of microbial contamination of three batches of lemon oil expressed indicated that *Salmonella* spp. was absent in 25 g, *Enterobacteriaceae*, total viable count, yeasts, moulds were < 10 colony forming unit (CFU)/g.

3.2.1.1. Shelf-life

The typical shelf-life of lemon oil expressed 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).²⁴ No separate or additional stability studies for flavouring additives were performed.

3.2.1.2. Conditions of use

Lemon oil expressed is intended to be added to feed for all animal species without withdrawal. The maximum proposed use level in complete feed is 40 mg/kg for chickens for fattening and turkeys for fattening, 55 mg/kg for laying hens, 75 mg/kg for piglets, 90 mg/kg for pigs for fattening, veal calves, cattle for fattening and dairy cows, 110 mg/kg for lactating sows, 155 mg/kg for horses, 30 mg/kg for sheep, goats and rabbits, 40 mg/kg for salmonids and 20 mg/kg for dogs, cats and ornamental fish.

The proposed use in water for drinking is 5–10 mg/kg.²⁵

3.2.2. Safety

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

Many of the major volatile components, accounting for about 99% of the % GC areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for food⁷ and feed⁸ uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Six compounds, δ-cadinene [01.021], β-cubebene [01.030], δ-germacrene [01.042], 3,7,10-humulatriene [01.043], β-phellandrene [01.055] and tricyclene [01.060], evaluated in FGE25.Rev2 by applying the Procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional toxicity data (EFSA CEF Panel, 2011c). In the absence of such toxicological data, the EFSA CEF Panel was unable to complete its assessment. As a result, these compounds are no longer authorised for use as flavours in food. In the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach (EFSA FEEDAP Panel, 2017b) or read-across from structurally related substances.

Among the volatile components accounting for > 0.1% of the % GC area, α-thujene, (E)-α-bergamotene and l-fenchone have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and 8 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastro-intestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2016c,d). The compounds were screened with the Organization for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox and no alert were identified for *in vitro* mutagenicity, for genotoxic and non-genotoxic carcinogenicity and for other endpoints.¹³

Only the compounds not previously assessed are considered in detail in the following sections, based on the evidence provided by the applicant in the form of several literature searches.²⁶ The focus will be on the volatile components α-thujone and perillaldehyde and on the non-volatile components of the essential oil, mainly furocoumarins and methoxycoumarins, which were analytically determined in the oil under assessment and for which a quantitative risk assessment is performed. The safety of 1,8-cineole will also be reviewed as the FEEDAP Panel identified new toxicological data (Xu et al., 2014; Caldas et al., 2016) published since the last assessment (EFSA FEEDAP Panel, 2012b).

²⁴ Technical dossier/Section II.

²⁵ Technical dossier/Supplementary information/July 2020.

²⁶ Technical dossier/Supplementary information/June 2020 and Supplementary information July 2020.

Other components like fatty acids, sterols, waxes and carotenoids, whose presence in lemon oils has been reported in the literature (see Section 3.2.1) but were not quantified in the additive under assessment, are ubiquitous in natural feeds and foods and not further addressed.

3.2.2.1. Absorption, distribution, metabolism and excretion

Volatile components: perillaldehyde and α -thujone

Perillaldehyde is quickly metabolised, largely by oxidation of the side chain to a carboxylic acid, which is excreted unchanged and as 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 (reviewed in EFSA FEEDAP Panel, 2015).

As summarised by the European Medicines Agency (EMA) in the public statement on the use of herbal medicinal products containing thujone (EMA, 2012), 'Metabolism of thujone has been investigated in mouse, rat and human liver preparations *in vitro* and in mice, rats and (partially) rabbits *in vivo*. Hydroxylation occurs at various positions, mainly at 7- and 4-positions, followed to a different extent by glucuronidation, and reductions as minor reactions are principal metabolic pathways, although *in vitro* and *in vivo* metabolic profiles do not necessarily agree with each other (Ishida et al., 1989; Höld et al., 2000, 2001). After *in vitro* liver microsomal incubations with α -thujone, 7-hydroxy- α -thujone seems to be a major metabolite in mice, rats and humans, whereas with β -thujone, formation of 4-hydroxy- β -thujone exceeded that of the 7-hydroxymetabolite in all species. 2-Hydroxy-thujone was observed only in mouse liver microsomes.' (...) 'In mice, treated orally with α -thujone *in vivo*, 2-hydroxy- α -thujone (mostly as a glucuronide) was the principal metabolite in urine, whereas 7-hydroxy- β -thujone was by far the most abundant urinary metabolite after β -thujone administration. In the rat, 4-hydroxythujones were principal urinary metabolites after administration of thujones (Höld et al., 2001).'

Furocoumarins and methoxycoumarins

The applicant submitted published reviews on the absorption, distribution, metabolism and excretion (ADME) of furocoumarins and methoxycoumarins (Melough et al., 2018; Kozioł and Skalicka-Woźniak, 2016). Generally, furocoumarins are well and rapidly absorbed, both in experimental animals and humans. Some of the furocoumarins present in the additive under assessment, i.e. psoralen, 5-MOP, 8-MOP, oxypeucedanin and imperatorin, were administered by the oral route to rats at 10 mg/kg body weight (bw), and their presence in plasma analysed by GC-MS. The T_{max} ranged between 0.5 h and 2 h and the highest concentrations were lower than 10 μ g/mL (Zhao et al., 2013, as referenced in Kozioł and Skalicka-Woźniak, 2016).

Kornhauser et al. (1981) observed a linear relationship between the serum and skin concentration of 8-MOP and a non-linear correlation with 5-MOP, which correlated with their phototoxicity.

After oral administration of 40 mg of ^{14}C labelled 8-MOP to humans, the plasma T_{max} was achieved about 2 h after dosing and the radioactivity recovery in urine was 74% within 48 h and 14% in faeces after 3 days (Busch et al., 1978, as referenced in Melough et al., 2018a). Data on oral absorption in humans indicate that bioavailability and kinetics vary greatly between individuals and cannot be predicted (Herfst and De Wolff, 1982, as referenced in SKLM, 2006).

Furocoumarins can be metabolised pre-systemically in the small intestine of humans and laboratory animals (reviewed by Melough and Chun, 2018). Furocoumarins (8-MOP) exhibited non-linear kinetics and a saturable first-pass effect in macaque monkeys, particularly relevant at the low doses (Rozman et al., 1989, as referenced in SKLM, 2006). Humans also exhibited a saturable first-pass effect (Schmid et al. 1980 and Brickl et al., 1984, as referenced in SKLM, 2006).

Furocoumarins are extensively biotransformed predominantly in the liver via cytochrome P450 (CYP)-dependent monooxygenases and excreted mainly in urine. In mice and humans, more than 90% of an orally administered dose of 8-MOP was found within 12 h as metabolites in the urine. The main biotransformation pathways are epoxidation, hydroxylation, glucuronide conjugation and hydrolytic opening of the lactone ring (SKLM, 2006). By using an LC/time of flight (TOF) mass spectrometry system, the extensive metabolism of imperatorin was confirmed and 51 metabolites were identified in urine of rats after a single oral administration of 80 mg/kg bw (Qiao et al., 2013, as referenced in Kozioł and Skalicka-Woźniak, 2016). Several metabolic pathways were proposed, mainly oxidation, cleavage of side chain and ring hydrolysis followed by conjugation reactions with sulfate and glucuronide.

Furocoumarins from grapefruit juice have been reported to inhibit CYPs, especially CYP3A4 at the intestine level, and to compete for substrate binding, increasing the bioavailability of drugs

metabolised by these enzymes (Meluogh and Chun, 2018). On the other hand, furocoumarins have been reported to induce hepatic glutathione S-transferases (GSTs) and CYP activities in mice. Particularly, the linear furocoumarins bergamottin and isopimpinellin have the potential to induce substantially increased the activity of hepatic murine CYP2B and CYP3A, suggesting a modulating effect of furocoumarins on hepatic drug-metabolising enzymes depending on their chemical structure (Kleiner et al., 2008).

Bergaptol was found as the principal metabolite in human urine by liquid chromatography tandem mass spectrometry (LC-MS-MS) analysis after ingestion of grapefruit juice containing bergamottin and 6',7'-dihydroxybergamottin (Messer et al., 2011; Lee et al., 2016, as referenced in Melough et al., 2018a).

Due to the similarity of the furocoumarins present in the additive, it is anticipated that they all follow similar biotransformation pathways, with formation of polar metabolites excreted in urine.

No specific data exist about the ADME of methoxycoumarins. Because of its lipophilic nature, it is expected that citropten is also rapidly and completely absorbed after oral uptake and distributed to tissues. It is assumed that the same metabolic pathways that are relevant for furocoumarins, such as demethylation followed by conjugation also apply to methoxycoumarins, such as citropten.

3.2.2.2. Toxicology

Genotoxicity and carcinogenicity

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

Volatile components: perillaldehyde and α -thujone

Expressed lemon oil contains **perillaldehyde** (0.023%, detected in one batch), a substance for which EFSA identified a concern for genotoxicity (EFSA CEF Panel, 2015c), which was confirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 2018). No information is available on the carcinogenicity of perillaldehyde.

The genotoxicity and carcinogenicity data of **thujone** (α - and β -thujone) have been summarised in the EMA public statement on the use of herbal medicinal products containing thujone (EMA, 2012). Thujone was tested in the framework of the National Toxicology Program (NTP) of the US Department of Health and Human Services (NTP, 2011).

As summarised by EMA (2012), 'In connection with the NTP study (NTP, 2011, TR No. 570), the genotoxic potential of racemic thujone²⁷ (used in the carcinogenicity study) and α -thujone were investigated according to the NTP protocols. The Ames test results of both compounds were negative in the presence or absence of the activating enzyme system. *In vivo*, daily exposure by gavage to racemic thujone (6.25, 12.5, 25, 50 or 75 mg (α,β -thujone/kg bw) for 3 months did not result in an increase in micronucleated erythrocytes in the peripheral blood of male B6C3F1 mice. However, female mice had a small but significant increase in micronucleated erythrocytes in the peripheral blood at the end of the 3-month study. Racemic thujone did not induce bone marrow toxicity.'

According to the NTP report (NTP, 2011) on 2-year gavage studies with rats (dose levels 12.5, 25 and 50 mg/kg) and mice (dose levels 3, 6, 12, 25 mg/kg), 'there was some evidence of carcinogenic activity of α,β -thujone in male F344/N rats based on increased incidences of preputial gland neoplasms at the dose level of 25 mg/kg (all rats at 50 mg/kg died before the end of the study);²⁸ increased incidences of benign pheochromocytoma of the adrenal medulla may have been related to administration of α,β -thujone in male F344/N rats administered 12.5 or 25 mg/kg.²⁹ There was no evidence of carcinogenic activity of α,β -thujone in female F344/N rats administered 12.5 or 25 mg/kg. There was no evidence of carcinogenic activity of α,β -thujone in male or female B6C3F1 mice administered 3, 6, or 12 mg/kg.'

Furocoumarins and methoxycoumarins +/- UVA

The genotoxicity and carcinogenicity data of psoralens (linear furocoumarins) have been summarised by International Agency for Research on Cancer (IARC) in monographs 40 (IARC, 1986) and 100A (IARC,

²⁷ According to the NTP report, the mixture tested in the 3-month and 2-year carcinogenicity study was not racemic thujone but an α,β -thujone mixture containing 70% β -thujone, 11% β -thujone, 16% fenchone, 2% camphor and 0.5% of unidentified impurities.

²⁸ Preputial gland: incidences of carcinoma (1/49, 0/49, 5/50); adenoma or carcinoma (3/49, 1/49, 9/50).

²⁹ Adrenal medulla: incidences of benign pheochromocytoma (6/50, 8/50, 12/49).

2012) and in the report of the Senate Commission of the Deutsche Forschungsgemeinschaft (SLKM) on the Toxicological Assessment of Furocoumarins in Foodstuff (SKLM, 2006).

Because of their structure including a linear condensed aromatic ring system, furocoumarins form noncovalent intercalation complexes with DNA, like anthraquinones and anthracyclines. Upon irradiation with ultraviolet A (UVA), the compounds are activated to form covalent monoadducts with DNA bases (mainly thymidine). Further activation with UVA can lead linear furocoumarins (psoralen, 5-MOP and 8-MOP) to form a second covalent linkage to cause DNA-crosslinks. Angular furocoumarins, such as angelicin, can also form mono-adducts with DNA but no DNA-crosslinks, which explains the lower genotoxic potency of these compounds compared to linear furocoumarins. Furocoumarins irradiated with UVA may produce reactive oxygen species (ROS), which induce photo-oxidative damage to various macromolecules (IARC, 2012). No comparative information is available for methoxycoumarins, however, because of their structural similarity, it can be expected that methoxycoumarins show a similar behaviour as furocoumarins. Psoralen, 8-MOP and angelicin were shown to be weak frameshift mutagens in *E. coli lac⁻* (lactose operon) in the dark, which reflects the DNA-intercalation properties of these compounds. Upon UVA irradiation 5-MOP and 8-MOP bind covalently to DNA of bacteria and yeasts and exhibit strong mutagenic effects. Mutagenic effects in bacteria were also observed for other psoralens, such as imperatorin.

Mutagenicity, chromosomal aberrations and sister chromatid exchange (SCE) have been observed with 8-MOP in Chinese hamster ovary (CHO) cells in the dark. Exposure of CHO cells treated with 5-MOP and 8-MOP to UVA leads to the formation of DNA adducts, DNA-interstrand crosslinks, SCE and mutations. Raquet and Schrenk (2014) compared the cytotoxicity and genotoxicity of a combined treatment of 5-MOP, 8-MOP, angelicin and citropten and UVA irradiation in V79 Chinese hamster lung fibroblasts and found concentration additivity. 5-MOP was the most potent, followed by 8-MOP. The relative potency of the angular furocoumarin angelicin and the methoxycoumarin citropten for gene-mutation and clastogenicity after UVA exposure was 50-fold and > 100-fold lower than that of the most potent furocoumarin, 5-MOP.

Mice of both sexes orally treated with 8-MOP (300 and 600 mg/kg bw) without UVA irradiation showed an induction of micronuclei in peripheral erythrocytes, which was highest between 2 and 5 days after exposure (Stivala, 1995 as referenced in SKLM, 2006).

The carcinogenicity of 8-MOP without UVA irradiation was studied in F344/N rats (males and females) given oral doses of 0, 37.5 or 75 mg/kg per day for 5 days a week for a total of 103 weeks (NTP, 1989). Treated male rats showed significantly increased numbers of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney and of Zymbal gland carcinomas but without dose dependency.³⁰ There was a positive trend in the occurrence of alveolar/bronchiolar adenomas as well as of subcutaneous tissue fibroadenomas. No increase in tumour incidences were observed in treated females. The carcinogenicity data in male rats are not suitable for benchmark dose modelling as only two doses were tested and both doses led to a similar increase of the tumour incidence. Therefore, there is little dose-response information in these data and no reference point can be calculated to be used in a quantitative risk assessment of oral furocoumarin exposure.

The topical application of 5-MOP or 8-MOP combined with UVA irradiation led to the formation of papillomas and carcinomas of the squamous epithelium of hairless mice (Cartwright and Walter, 1983; Young et al., 1983 as referenced in SKLM, 2006) or inbred mice (Zajdela and Bisagni, 1981 as referenced in SKLM, 2006).

Human psoriasis patients treated orally with 8-MOP followed by skin UVA irradiation developed dose-dependent increases in the formation of squamous cell carcinomas, basal cell carcinomas and melanomas (summarised by IARC, 2012). However, these data do not allow conclusions to be drawn for the general population, which may show differences in susceptibility compared to patients suffering from psoriasis.

IARC (2012) concluded that there is sufficient evidence in experimental animals and in humans for the carcinogenicity of 8-MOP plus UVA radiation (Group 1). However, for 8-MOP without UVA radiation, there is only limited evidence for the carcinogenicity in experimental animals. No epidemiological data relevant to the carcinogenicity of 5-MOP were available, whereas on the basis of experiments designed to test the carcinogenicity to mouse skin of 5-MOP (mainly dermal application) in combination with UVA radiation or solar-simulated radiation, there is sufficient evidence of carcinogenicity in experimental animals. The evidence is insufficient in the absence of UVA radiation. The overall evaluation was that 5-MOP in the presence of UVA radiation is probably carcinogenic to humans (Group 2A; IARC 1986, 1987). However,

³⁰ Incidences of tubular cell adenomas (1/50, 11/50 and 8/49) and adenocarcinomas (0/50, 1/50 and 3/49) of the kidney; Incidences of Zymbal gland carcinomas (1/50, 7/50 and 4/49).

oral dietary furocoumarin exposures of the general population below the phototoxicity threshold dose have not been associated with an additional risk of skin cancer (SKLM, 2006).

For angelicin and some of its methyl derivatives, limited evidence for the carcinogenicity to experimental animals in combination with UVA radiation was obtained, but no evaluation could be made of the carcinogenicity to humans (IARC, 1986, 1987).

Other toxicity studies in laboratory animals

Volatile components: 1,8-cineole, perillaldehyde and α-thujone

The safety of **1,8-cineole** has been previously assessed (EFSA FEEDAP Panel, 2012b). Meanwhile new toxicological data have been published which are reviewed below. In a 50-day repeated-dose toxicity and reproductive study, 1,8-cineole was administered to Wistar rats by gavage at doses of 100, 500 or 1,000 mg 1,8-cineole/kg bw per day (Caldas et al., 2016). Examination included daily observation; weekly body weight, food and water intakes; and haematology,³¹ clinical chemistry,³² organ weights and gross and microscopic pathology at the end of the study. Results showed an effect on body weight at the highest two doses (mainly a result of an initial reduced growth and a failure to restore that) accompanied by some effects on food and water intake. Differences were seen in haematological parameters in male rats, i.e. higher mean corpuscular volume (at 1,000 mg/kg bw per day), higher platelet count (at 500 and 1,000 mg/kg bw per day), lower mean corpuscular haemoglobin concentration (at 500 and 1,000 mg/kg per day) and lower mean platelet volume at all doses as compared to controls. None of these differences showed significant dose-related trends. Similar differences were not seen in females. No consistent differences were seen in serum chemistry. Treatment-related changes in organ weights were a decrease in the absolute weight of the spleen and lungs in males at doses of 500 and 1,000 mg/kg bw, and an increase in the absolute and relative weight of the liver in females at 1,000 mg/kg bw. Histopathological examination showed eosinophilic and/or lymphocytic infiltrates in the lungs and liver of males and females and in the uterus of female rats at all doses; however, these infiltrations were minimal and were not considered treatment-related. Overall, based on the effects on body weight observed at 500 and 1,000 mg/kg bw per day, the FEEDAP Panel identifies the lowest dose tested at 100 mg/kg bw per day as the no observed adverse effect level (NOAEL).

In a reproductive toxicity study reported by the same authors (Caldas et al., 2016), pregnant rats were randomly distributed into eight groups ($n = 7\text{--}10$ per group), four of which were assigned to treatment during the preimplantation period (day 1–6 of pregnancy) and four to treatment during organogenesis (day 7–14 of pregnancy). In both periods, the control group received a 1% Tween-80 aqueous solution (0.1 mL/10 g), while the treated groups received 1,8 cineole by gavage at doses of 250, 500 or 1,000 mg/kg bw per day for 7 days. High-dose females gained significantly less weight during the treatment period and this was associated with a higher level of dead fetuses and lower total fetus mass in the preimplantation group. The study suggests that possibly 1,8-cineole presents maternal and fetal toxicity at doses ≥ 250 mg/kg bw.

In another study (Xu et al., 2014), groups of 10 mice of undefined sex were given 1,8 cineole in solution daily by gavage for 30 days to provide doses of 0, 21.38, 64.15 or 192.45 mg/kg per day. Examination included daily observation, body weight, food and water intakes; organ weights and gross and microscopic pathology. No changes in body weight gain and the absolute and relative weight of organs were observed in treated mice compared to controls. Histopathological examination showed that the highest dose (192.5 mg/kg per day) of 1,8-cineole caused serious damages in liver and kidneys, with main lesions of granular and vacuolar degeneration and vascular congestion, whereas the two lower doses (21.4 and 64.2 mg/kg per day) caused no or only mild such damages in liver and kidneys. Due to the limitations in the study design and the limited background knowledge of the mice used, this kind of studies does not allow to derive an NOAEL, but the FEEDAP Panel considers that the results confirm the liver and the kidneys as target organs, similar as in the rat study.

No information is available on the subchronic toxicity of **perillaldehyde**.

As summarised by EMA (2012), **thujone** was tested in the framework of the NTP of the US Department of Health and Human Services (TR 570, NTP, 2011).

³¹ Including: haematocrit, haemoglobin, erythrocyte count, reticulocyte count, total leucocyte count (including differential), platelet count, mean platelet volume, mean corpuscular haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin concentration.

³² Including: sodium, potassium, glucose, cholesterol, urea nitrogen, creatinine, uric acid, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, direct and indirect bilirubin.

' α -Thujone and isomeric mixture³³ were administered by gavage to B6C3F1 mice and to Fischer 344 rats at doses of 0, 1, 3, 10, 30 or 100 mg/kg for 14 days. In both species, the increased mortality observed in the top dose group was associated with indications of neurotoxicity (hyperactivity, tremors, tonic seizures). The 3-month NTP study was essentially similar to the 2-week study except the duration. α -Thujone and isomeric mixture were administered by gavage to B6C3F1 mice and to Fischer 344 rats at doses of 0, 6.25, 12.5, 25, 50 or 100 mg/kg for 13 weeks. In both species the increased mortality observed in the higher dose groups (from 25 or 50 and greater) was associated with seizures.'

In the chronic study, 'an isomeric mixture of thujone²³ was administered by gavage to B6C3F1 mice at doses of 0, 3, 6, 12 and 25 mg/kg body weight/day and to Fischer 344 rats at doses of 0, 12.5, 25 and 50 mg/kg body weight/day for 2 years. In both species, increased mortality was observed in the top dose group, and in the rat also in the middle dose group. Clonic and tonic seizures were observed in the middle and top dose groups in rats and in the top dose group in mice. A small increase in clonic seizures was observed also in the low dose group in rats. The administration of α,β -thujone resulted in increased incidences of non-neoplastic lesions in the brain³⁴ and spleen³⁵ of male and female F344/N rats, the kidneys³⁶ of male F344/N rats and the pituitary gland³⁷ of female F344/N rats usually at the two highest dose levels. In the rat, the no observed effect level (NOEL) value was 12.5 mg/kg bw for mortality and tonic seizures (no NOEL for clonic seizures). In the mouse, the NOEL was 12 mg/kg bw for seizures and mortality.'

Lachenmeier and Uebelacker (2010, unpublished report, as referenced in EMA 2012) 'have performed a detailed re-evaluation of the available evidence using the benchmark dose (BMD) approach and found that the application of the appropriate dose-response modelling on the long-term chronic toxicity study of the NTP, using clonic seizures as a response, yielded a BMD lower confidence limit for a benchmark response of 10% (BMDL₁₀) as 11 mg/kg body weight per day.' Considering that the mixture tested contained 70% of α -thujone, a BMDL₁₀ of 8 mg/kg bw per day is calculated for α -thujone.

Furocoumarins and methoxycoumarins

As summarised in the toxicological assessment performed by SKLM (SKLM, 2006), limited data are available on the subchronic toxicity of furocoumarins. When 8-MOP was tested in a 90-day study in rats at daily doses of 0, 25, 50, 100, 200 or 400 mg/kg bw per day, in the absence of UV light, a dose-dependent increase in the relative liver weight was observed at all doses tested. At the two highest doses tested, several adverse effects were observed, including increased mortality, reduced body weight, lipid deposits in the liver and the adrenal glands, atrophy of the prostate, seminal vesicles and the seminiferous tubules (Dunnik et al., 1984, as reported in SKLM, 2006; NTP, 1989). Other studies with 5-MOP in dogs and rats were considered insufficient to identify an NOAEL. From the available data, the SKLM concluded that a NOAEL for the repeated intake of furocoumarins could not be identified.

No specific data are available for citropten. However, considering the mechanistic data showing that citropten is much less potent compared with linear furocoumarins with respect to its mutagenicity, it is assumed that this is also true for toxicity not related to genotoxic effects.

Several single generation reproduction studies have been reported in rats and rabbits for 5-MOP and 8-MOP in the presence of UVA light, using doses ranging from 20 to 560 mg/kg bw per day. In all of the studies, there was an adverse effect on the parent generation, sometimes resulting in reduced litter size and weight. Effects on offspring were limited to reduction in body weight; one study claiming malformations in offspring was rejected by IARC as being inadequate.

Human phototoxicity

Furocoumarins and methoxycoumarins

Most of the information available on the toxicity of furocumarins in humans is from studies with patients suffering from skin diseases, such as vitiligo or psoriasis. SKLM (2006) reports on therapeutic

³³ α,β -thujone mixture containing 70% β -thujone, 11% β -thujone, 16% fenchone, 2% camphor and 0.5% of unidentified impurities.

³⁴ Male rats: necrosis (0/50, 0/50, 1/50, 3/50), pigmentation (0/50, 1/50, 0/50, 3/50); female rats: pigmentation (1/50, 3/50, 5/50, 19/50).

³⁵ Male rats: pigmentation (19/50, 24/50, 30/49, 46/48); female rats: pigmentation (39/48, 40/49, 39/48, 45/50).

³⁶ Male rats: mineralisation (17/48, 33/48, 41/44, 38/49).

³⁷ Female rats: pars distalis, atrophy (0/50, 0/49, 2/49, 12/48); Rathke's cleft, dilatation (7/50, 1/49, 13/49, 26/48).

oral doses in the treatment of psoriasis in the range of 0.5–0.6 mg 8-MOP/kg bw or 1.2 mg 5-MOP/kg bw administered in combination with UVA (0.5–7 J/cm², wavelength range 315–400 nm, maximum at 355 nm).

Acute phototoxic effects (e.g. erythema, oedemas and blisters) have been observed after oral exposure to furocoumarins in combination with sunlight or UVA light in doses, such as 50 mg 8-MOP (0.83 mg/kg bw for 60 kg bw) or 45 mg psoralen (0.75 mg/kg bw for 60 kg bw) (Fitzpatrick and Pathak, 1984; Ljunggren, 1990, as referenced in SKLM, 2006).

From exposure studies in adult volunteers, it is concluded that the oral threshold dose for furocoumarin mixtures for the induction of erythemas in combination with sunlight is in the order of 10 mg 8-MOP plus 10 mg 5-MOP or 15 mg 8-MOP equivalents (0.25 mg/kg bw for 60 kg bw, corresponding to blood levels of 8-MOP and 5-MOP at 30 min after intake of approximately 10–15 ng/ml each) (Schlatter et al., 1991, as referenced in SKLM, 2006). This phototoxic threshold dose was not reached by normal conventional dietary intake of vegetables, such as celery roots (experimental intake of 2–8 mg furocoumarin mixture per person resulting in blood levels of 8-MOP and 5-MOP which were below the limit of detection (LOD) of 2 ng/ml) (Schlatter et al., 1991 as referenced in SKLM, 2006).

A similar oral phototoxicity threshold dose of 14 mg 8-MOP (~ 0.23 mg/kg bw for 60 kg bw) has been reported for the occurrence of erythema in humans after exposure in combination with sunlight (Brickl et al., 1984, as referenced in SKLM, 2006).

No data were identified for methoxycoumarins.

Conclusions on toxicology

Perillaldehyde is genotoxic. No data are available on the carcinogenicity and the subchronic toxicity of perillaldehyde.

The FEEDAP Panel identified an NOAEL of 100 mg/kg bw per day for 1,8-cineole and a BMDL₁₀ of 8 mg/kg bw per day for α -thujone.

Furocoumarins are genotoxic *in vitro* and *in vivo*, especially in combination with UVA radiation. In the absence of UVA radiation, the potency is low. Linear furocoumarins in combination with UVA radiation are carcinogenic in rats and mice. Dietary furocoumarin exposures of the general population below the phototoxicity threshold dose have not been associated with an additional risk of skin cancer. The available carcinogenicity data (both in humans and experimental animals) are not suitable for benchmark dose modelling and no reference point can be calculated to be used in a quantitative risk assessment of oral furocoumarin exposure.

The available data set for the subchronic toxicity of furocoumarins is limited and does not allow the identification of an NOAEL. High oral doses of linear furocoumarins in animals have negative effects on reproduction but only limited data are available. No specific data are available for citropten.

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 (except perillaldehyde) of expressed lemon oil. For furocoumarins and citropten, the only methoxycoumarin congener detected in the additive and therefore considered as representative for the class, their toxicological properties and the available data set do not allow to identify a reference point for the risk assessment or to derive a safe level. On the other hand, feeding animals citrus by-products is a common practice with no report of adverse effects (Bampidis and Robinson, 2006; Feedipedia³⁸). Therefore, the assessment of the safety for target species is based on the comparison between the combined furocoumarin and citropten intake via the consumption of citrus by-products as feed material and that via the use of lemon essential oil as a feed additive. The same approach is applied for perillaldehyde, occurring in citrus peel.

³⁸ <https://www.feedipedia.org/node/680>

Volatile components

Based on considerations related to structural and metabolic similarities, the components were allocated to 11 assessment groups, five of which correspond to the chemical groups (CGs) 31 (and related subgroups), 8 and 1, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 (aliphatic and aromatic hydrocarbons), the application of subassessment 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 5.

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 5.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in the assessment group, toxicological data were available to derive NOAEL values. Structural and metabolic similarity among the components in the assessment groups was 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 for subchronic studies, from which NOAEL values could be derived, were available for myrcene [01.008], limonene [01.045], 1-isopropyl-4-benzene [01.002] and β -caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016d), octyl acetate [09.007] in CG 1 (EFSA FEEDAP Panel, 2013), citral [05.020] in CG 3 (EFSA FEEDAP Panel, 2016a), citronellol [02.011] and related citronellyl derivatives in CG 4 (EFSA FEEDAP Panel, 2016b), terpineol [02.230] and linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a) and 1,8-cineole [03.001] in CG 16 (EFSA FEEDAP Panel, 2012b). For 1,8-cineole, the FEEDAP Panel notes that more toxicological evidence became available after its evaluation of CG 16 (EFSA FEEDAP Panel, 2012b). An NOAEL of 100 mg/kg bw per day was identified/selected (see Section 3.2.2.2) and applied to 1,8-cineole.

Considering the structural and metabolic similarities, the NOAELs for the representative compounds of CG 31, myrcene [01.008], limonene [01.045] and β -caryophyllene [01.007] were applied, respectively, using read-across to the compounds within subassessment group II (*cis*- β -ocimene [01.064] and *trans*- β -ocimene [01.018]), group III (γ -terpinene [01.020], β -bisabolene [01.028], terpinolene [01.005], α -terpinene [01.019], α -phellandrene [01.006], p-mentha-3,8-diene) and group V (β -pinene [01.003], α -pinene [01.004], sabinene [01.059], α -thujene, camphene [01.009], valencene [01.017] and δ -3-carene [01.029]) (EFSA CEF Panel, 2015a,b).

For α -thujone the FEEDAP Panel retains a BMDL₁₀ of 8 mg/kg bw per day, recalculated from the BMDL₁₀ of 11 mg/kg bw per day derived from the long-term chronic toxicity study in mice and rats using clonic seizures as a response (see Section 3.2.2.2) as described in EMA (2012).

Considering the structural and metabolic similarities in CG 6, read-across was applied using the NOAEL of 250 mg/kg bw per day for terpineol [02.230] to extrapolate to α -terpineol [02.014] and terpinen-4-ol [02.072].

Read-across was also applied using the NOAEL of 120 mg/kg bw per day for octyl acetate [09.007] to octanal [05.009], octan-1-ol [02.006], nonanal [05.025], decanal [05.010], decyl acetate [09.009], dodecanal [05.011] and heptan-1-ol [02.021], 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 gerarial [05.188], geranyl acetate [09.011], neral [05.170], neryl acetate [09.213] and Z-nerol [02.058] and selected it as the reference point for CG 3.

For the remaining compounds, namely bisabola-1,8,12-triene, (E)- α -bergamotene, bicyclogermacrene, tricyclene [01.060], δ -germacrene [01.042], (I)- α -bisabolol, l-fenchone, bornyl acetate [09.017], trans-carveol, cis-carveol [02.062] and cis-para-2,8-menthadien-1-ol, toxicity studies and NOAEL values performed with the compounds under assessment were not available and read-across was not possible. Therefore, the TTC approach was applied (EFSA FEEDAP Panel, 2017b).

As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from *in vivo* toxicity study or read across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e.

3, 0.91 and 0.15 mg/kg bw per day, respectively, for Cramer Class I, II and III compounds). Reference points selected for each compound are shown in Table 5.

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

For risk characterisation, the margin of exposure (MOE) was calculated for each component as the ratio between the reference point and the exposure. For each assessment group, the combined (total) margin of exposure (MOET) was calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances (EFSA Scientific Committee, 2019a). An MOET > 100 allowed for interspecies- and intra-individual variability (as in the default 10 × 10 uncertainty factor). The compounds resulting individually in an MOE > 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible.³⁹

The approach to the safety assessment of lemon oil expressed for the target species is summarised in Table 5. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 40 mg/kg.

Table 5: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual volatile components of lemon oil expressed classified according to assessment groups. Intake calculations for the individual components are based on the use level of 40 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 sum of the reciprocals of the MOE of the individual substances

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max Feed conc.	Daily Intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw/day	–	mg/kg bw/day	–	–
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	2.14	0.856	0.0768	I	44	573	
cis-β-Ocimene	01.064	0.12	0.050	0.0045	I	44	9,882	
trans-β-Ocimene	01.018	0.11	0.043	0.0039	I	44	11,346	
MOET CG 31, II				0.0852				517
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.045	66.7	26.68	2.3951	I	250	104	
γ-Terpinene	01.020	11.5	4.584	0.4115	I	250	608	
β-Bisabolene	01.028	0.87	0.346	0.0311	I	250	8039	
Terpinolene	01.005	0.55	0.222	0.0199	I	250	11,139	
α-Terpinene	01.019	0.30	0.121	0.0108	I	250	23,053	
α-Phellandrene	01.006	0.26	0.102	0.0092	I	250	27,302	
MOET CG 31, III				2.8777				87
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	0.59	0.236	0.0212	I	154	7,257	
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
α-Pinene	01.004	16.8	6.720	0.6033	I	222	368	
β-Pinene	01.003	3.63	1.452	0.1304	I	222	1,703	

³⁹ Compounds included in the assessment groups but not reported in the table: nonanal, decyl acetate, dodecanal, octyl acetate and heptan-1-ol (CG 1); isogeraniol, geraniol, nerol, -sinensal and -sinensal (CG 3); (I)-α-bisabolol, 4-terpineol and trans-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol (CG 6); carvone (CG 8); 1,8-cineole and 1,4-cineole (CG 16); p-mentha-3,8-diene, bisabola-1,8,12-triene, (E)-α-bisabolene, cyclohexene, 4-methyl-1-(1-methylethyl)-, (R)-, β-elemene and (Z)-γ-bisabolene (CG 31, III); camphene, valencene, δ-3-carene, β-copaene, α-copaene, δ-cadinene, β-cubebene and tricyclene (CG 31, V).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max Feed conc.	Daily Intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
Sabinene	01.059	2.65	1.060	0.0952	I	222	2,333	
α -Thujene	n.a.	0.56	0.223	0.0200	I	222	11,099	
β -Caryophyllene	01.007	0.33	0.130	0.0117	I	222	18,964	
(E)- α -bergamotene	n.a.	0.57	0.228	0.0204	I	3	147	
Bicyclogermacrene	n.a.	0.03	0.012	0.0011	I	3	2,785	
MOET CG 31, V				0.8820				91
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
δ -Germacrene	01.042	0.01	0.004	0.0003	I	3	9,283	
CG 1								
Decanal	05.010	0.19	0.0768	0.0069	I	120	17,405	
Octanal	05.009	0.14	0.0568	0.0051	I	120	23,534	
Octan-1-ol	02.006	0.11	0.0456	0.0041	I	120	29,314	
MOET CG 1				0.0161				7,459
CG 3								
Geranial	05.188	2.08	0.832	0.0747	I	345	4,619	
Neral	05.170	1.43	0.572	0.0514	I	345	6,719	
Neryl acetate	09.213	0.68	0.272	0.0244	I	345	14,150	
Geranyl acetate	09.011	0.51	0.203	0.0182	I	345	18,913	
MOET CG 3				0.1687				2,045
CG 4								
Citronellol	02.011	0.13	0.051	0.0046	I	50	10,878	
Citronellal	05.021	0.07	0.028	0.0025	I	50	20,180	
Citronellyl acetate	09.012	0.07	0.026	0.0023	I	50	21,422	
MOET CG 4				0.0094				5,315
CG 6								
Linalool	02.013	0.45	0.179	0.0161	I	117	7,273	
α -Terpineol	02.014	0.16	0.066	0.0059	I	250	42,451	
MOET CG 6				0.0220				6,209
CG 8								
I-Fenchone	n.a.	0.17	0.068	0.0061	II	0.9	148	
Bornyl acetate	09.017	0.01	0.005	0.0008	I	3	6,962	
Trans-Carveol	02.062	0.01	0.004	0.0004	I	3	7,595	
cis-p-2,8-Menthadien-1-ol	n.a.	0.01	0.002	0.0004	III	0.15	696	
Cis-Carveol	02.062	0.01	0.002	0.0002	I	3	13,924	
MOET CG 8				0.0073				117
CG 32 (epoxides)								
cis-Limonene oxide	n.a.	0.07	0.026	0.0023	I	3	1,285	
trans-Limonene oxide	n.a.	0.03	0.013	0.0012	I	3	2,532	
MOET CG 32								852
α -Thujone	n.a.	0.006	0.002	0.0002	III	8	37,131	

(a): Intake calculations for the individual components are based on the use level of 5 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

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

As shown in Table 5, for all the assessment groups, the MOET calculated for chickens for fattening at the proposed use level of 40 mg/kg complete feed was ≥ 87 . From the lowest MOET of 87 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 6.

Table 6: Combined margin of exposure (MOET) calculated for the other target species at the corresponding proposed use level in feed of expressed lemon oil

	Body weight (kg)	Daily feed intake (g DM/kg bw)	Feed intake (g DM/day)	Use level (mg/kg feed)	Lowest MOET
Chickens for fattening	2	79	158	40	87
Laying hens	2	53	106	55	94
Turkeys for fattening	3	59	176	40	116
Piglets	20	44	880	75	83
Pigs for fattening	60	37	2,200	90	83
Sow lactating	175	30	5,280	110	83
Veal calves (milk replacer)	100	19	1,890	90	172
Cattle for fattening	400	20	8,000	90	153
Dairy cows	650	31	20,000	90	100
Sheep/goat	60	20	1,200	30	458
Horse	400	20	8,000	155	89
Rabbit	2	50	100	30	183
Salmon	0.12	18	2.1	40	382
Dogs	15	17	250	20	809
Cats	3	20	60	20	687
Ornamental fish	0.012	5	0.054	20	2,749

Table 6 shows an MOET below the value of 100 for chickens for fattening, laying hens, pigs and horses. In order to ensure an MOET ≥ 100 , the concentration in feed should be reduced to 35 mg/kg for chickens for fattening, 52 mg/kg for laying hens, 62 mg/kg for piglets, 74 mg/kg for pigs for fattening, 92 mg/kg for sows and 137 mg/kg for horses. For the other species, the MOET is close to or exceeds the value of 100 at the maximum proposed concentrations. This excludes a concern for the target species arising from the volatile components of expressed lemon oil (except perillaldehyde). Because glucuronidation is an important metabolic reaction to facilitate the excretion of these compounds (see Section 3.2.2.1), their use as additives in cat feed needs a wider margin of exposure. Considering that cats have an unusually low capacity for glucuronidation (Court and Greenblatt, 1997), an MOET of 500 is considered adequate.

When used in water for drinking, the intake of the additive via water would be two to 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 10 mg/kg for the use in water for drinking, which would ensure a comparable or lower exposure. Therefore, the FEEDAP Panel concludes that with respect to the exposure to the volatiles present in the additive the use of expressed lemon oil at the maximum proposed use levels in feed and in water for drinking is safe for all animal species.

Volatile components: Perillaldehyde

Low concentrations of perillaldehyde were detected in one batch of the additive under assessment (0.023%). The use of expressed lemon oil at the proposed use levels in feed would result in a perillaldehyde intake up to 0.8 µg/kg bw for poultry, 0.9 µg/kg bw for pigs, 0.7 µg/kg bw for ruminants, 0.8 µg/kg bw for horses, 0.4 µg/kg bw for rabbit and 0.2 µg/kg bw for fish.⁴⁰

On the other hand, perillaldehyde occurs in citrus by-products, which are used in diets at different concentrations depending on the target species (from 5% up to 30% in ruminants).⁴¹ Taking into account an inclusion level of 10% for poultry and 20% for the other species and considering the

⁴⁰ Intake values calculated considering the concentration of perillaldehyde in the additive (0.023%), the default values for feed intake (Table 6), the proposed use levels in feed for the different species (Table 6) and that complete feed contains 88% DM except milk replacer for veal calves (94.5%).

⁴¹ Technical dossier/Supplementary information July 2020/SIn FAD-2010-322-request of clarification.

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 rabbit and 3.6 g DM/kg bw for fish.

Based on the literature data provided by the applicant⁴² on the occurrence of perillaldehyde in citrus peel (e.g. 0.00038% in mandarin and lemon, and 0.001% in orange 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-product⁴³ (Bampidis and Robinson, 2006), the occurrence of perillaldehyde in lemon by-products was estimated to be 0.0002%. Based on citrus by-product intake (see above), the intake of perillaldehyde via feed was calculated to be 16 µg/kg bw for poultry, 18 µg/kg bw for pigs, 12 µg/kg bw for ruminants, 8 µg/kg bw for horses, 20 µg/kg bw for rabbit and 7 µg/kg bw for fish.

These intake values are at least 10-fold higher than those resulting from the high use level of expressed lemon oil in feed as proposed by the applicant (20–155 mg expressed lemon oil/kg feed).

Non-volatile components: Furocoumarins and methoxycoumarins

Despite the different relative potency for cytotoxicity, mutagenicity and clastogenicity after UVA irradiation (Raquet and Schrenk, 2014), all furocoumarins and citropten (as representative for methoxycoumarins) are currently grouped together and considered equally potent as the most potent furocoumarin (i.e. 5-MOP) in a worst-case scenario.

The average and maximum intake of individual furocoumarins and citropten and their sum, calculated for chickens for fattening fed diets containing the additive at the maximum proposed use level of 40 mg/kg is reported in Table 7. The average and maximum intake of total furocoumarins and citropten for the different target species are reported in Table 8.⁴⁴

Table 7: Concentration of furocoumarins and citropten in feed for chickens for fattening (as µg/kg complete feed) and their intake (as µg/kg bw per day) calculated at the maximum proposed use level of the additive in feed (40 mg/kg), considering the average and the maximum analysed value for each congener in the additive

Congener	Concentration in the additive		Concentration in complete feed		Intake (chickens for fattening)	
	Average	Max	Average	Max	Average	Max
	mg/kg additive		µg/kg feed		µg/kg bw/day	
Furocoumarins						
Psoralen	45.3	92.4	1.8	3.7	0.16	0.33
5-Methoxysoralen (bergapten)	23.63	75.4	0.9	3.0	0.08	0.27
Bergamottin (5-geranoxypsalen)	497	1,272	19.9	50.7	1.79	4.57
Oxypeucedanin	215	455	8.6	18.2	0.77	1.63
8-Methoxysoralen (xanthotoxin)	187	430	7.5	17.2	0.67	1.55
8-Geranoxypsalen	100.59	< 250	4.0	10.0	0.36	0.90
Imperatorin	31.5	63.9	1.3	2.6	0.11	0.23
5,8-Dimethoxysoralen (isopimpinellin)	6.04	13.9	0.2	0.6	0.02	0.05
Phellopterin	0.37	1.0	0.01	0.04	0.001	0.004
Oxypeucedanin hydrate	20.2	53.5	0.8	2.1	0.07	0.19
Byakangelicol	179	273	7.1	10.9	0.64	0.98
Methoxycoumarins						
Citropten	163	570	0.007	0.023	0.59	2.05
Total			59	142	5.27	12.75

⁴² Technical dossier/Supplementary information/November 2020.

⁴³ 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.

⁴⁴ Intake values calculated considering the average and maximum concentration of furocoumarins and citropten in the additive (Table 7), the default values for feed intake (Table 6), the proposed use levels in feed for the different species (Table 6) and that complete feed contains 88% DM except milk replacer for veal calves (94.5%).

Table 8: Target animal intake of furocoumarins and methoxycoumarins (as µg/kg bw per day) at the maximum proposed use level of the additive in feed for each species (ranging from 20 to 155 mg/kg complete feed). Total values of furocoumarins and methoxycoumarins in feed are calculated considering the average and the maximum analysed value for each furocoumarin congener in the additive

Target species	Daily feed intake	Body weight	Use level in feed	Intake furocoumarins	
	kg DM/day			Average	Max
Chickens for fattening	0.158	2	40	5.3	12.8
Laying hens	0.106	2	55	4.9	11.8
Turkey for fattening	0.176	3	40	3.9	9.5
Piglet	0.88	20	75	5.5	13.3
Pig for fattening	2.2	60	90	5.5	13.3
Sow lactating	5.28	175	110	5.5	13.4
Veal calf (milk replacer)	1.89	100	90	2.6	6.4
Cattle for fattening	8	400	90	3.0	7.3
Dairy cows	20	650	90	4.6	11.2
Sheep/goat	1.2	60	30	1.0	2.4
Horse	8	400	155	5.2	12.5
Rabbit	0.1	2	30	2.5	6.1
Salmon	0.0021	0.12	40	1.2	2.8
Dog	0.25	15	20	0.6	1.3
Cat	0.06	3	20	0.7	1.6
Ornamental fish	0.000054	0.012	20	0.2	0.4

The use of expressed lemon oil at the proposed use level in feed would result in an average intake of total furocoumarins and methoxycoumarins ranging from 0.2 µg/kg bw per day in ornamental fish and 5.5 µg/kg bw per day in pigs (maximum intake 13.4 µg/kg bw per day in sows).

Furocoumarins and methoxycoumarins occur in citrus by-products. Based on the literature data provided by the applicant²³ on the occurrence of furocoumarins and methoxycoumarins in citrus peel (e.g. 0.003% in lemon, 0.02% in grapefruit, 0.057% in mandarin and 0.009% in orange according to Russo et al., 2014; Mercolini et al., 2013; Ramirez-Pelayo et al., 2019), in citrus pulp (e.g. 0.0003% in grapefruit and 0.012% in mandarin as reported by Chebrolu et al., 2016; Scordino et al., 2011), and in citrus seeds (0.001% in lemon, Miyake et al., 1999), the occurrence of furocoumarins and methoxycoumarins in lemon by-products was estimated to be 0.0018%.⁴⁵ Based on citrus by-product intake (7.9 g 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 rabbit and 3.6 g DM/kg bw for fish), the intake of furocoumarins and methoxycoumarins via feed was calculated to be 140 µg/kg bw per day for poultry, 160 µg/kg bw per day for pigs, 100 µg/kg bw per day for ruminants and 70 µg/kg bw per day for horses, 180 µg/kg bw per day for rabbits and 65 µg/kg bw per day for fish.

These intakes are about 10-fold higher than those resulting from the high use level of expressed lemon oil in feed as proposed by the applicant (20–155 mg/kg, see Table 8). The FEEDAP Panel also notes that these intakes are below the phototoxicity threshold dose (0.25 mg/kg bw for 60 kg bw) established from human studies (see Section 3.2.2.2 *Human phototoxicity*).

Conclusions on safety for the target species

The FEEDAP Panel concludes that expressed lemon oil is safe up to the maximum proposed use level in complete feed of 40 mg/kg for turkeys for fattening, 90 mg/kg for veal calves, cattle for fattening and dairy cows, 30 mg/kg for sheep, goats and rabbits and 40 mg/kg for salmonids. For the other species, the calculated safe concentrations are 35 mg/kg for chickens for fattening, 52 mg/kg for laying hens, 62 mg/kg for piglets, 74 mg/kg for pigs for fattening, 92 mg/kg for sows and 137 mg/kg

⁴⁵ Composition of fresh citrus by-products: 62.5% citrus peel, 32.5% pulp and 5% seeds. Assuming similar proportions in dried citrus by-products, the occurrence of furocoumarins in lemon by-products is calculated to be 0.0018% ($0.003\% \times 0.625 + 0 \times 32.5 + 0.0001 \times 0.05$).

for horses. These target species are fed citrus by-products as part of daily feed. For these species, the use of the expressed lemon oil in feed is not expected to increase the exposure to furocoumarins and methoxycoumarins and perillaldehyde to a relevant extent (< 10%). For companion animals and ornamental fish not normally exposed to citrus by-products, no conclusion can be drawn.

The FEEDAP Panel also concludes that the use of expressed lemon oil at the maximum proposed use levels in water for drinking of 10 mg/kg is safe for all species, except companion animals.

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

3.2.2.4. Safety for the consumer

Lemon oil obtained by cold expression of the peel is added to a wide range of food categories for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 3.66 mg/kg bw per day for lemon oil expressed.

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

Concerning furocoumarins intake from food, in Great Britain, the furocoumarin intake has been estimated by the Committee on Toxicity (COT) to be up to 1.2 mg per person per day corresponding to approximately 0.02 mg/kg bw per day for 60 kg bw (COT, 1996 as cited by SKLM, 2010). In Germany, the estimated average total daily intake of furocoumarins from non-flavoured and flavoured foods is approximately 0.56 mg/person per day (approximately 0.01 mg/kg bw per day for 60 kg bw), flavoured foods contributing to about 10% to the total intake (SKLM, 2006, 2010). Reported exposure levels for furocoumarin intake from food for the general population are below the phototoxicity threshold dose (0.25 mg/kg bw for 60 kg bw) and have not been associated with an additional risk of skin cancer.

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the FEEDAP Panel recognises that the constituents of lemon oil expressed 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 the individual constituents by humans consuming products of animal origin is not expected.

3.2.2.5. Safety for user

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

The applicant produced a safety data sheet⁴⁶ for lemon oil expressed where hazards for users have been identified. Since expressed lemon oil contains furocoumarin derivatives, the additive may cause phototoxicity after skin contact (NTP, 2000).

3.2.2.6. Safety for the environment

C. limon 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. Residual fraction from the distillation of expressed lemon oil (referred to as the residual fraction)

This application concerns the residual fraction (i.e. the residues) from the distillation of lemon oil expressed which is obtained from fruit peel of *C. limon* (L.) Osbeck from multiple geographic origins.

3.3.1. Characterisation of the residual fraction

The residual fraction is a greenish yellow clear mobile liquid with a characteristic odour. In five batches of the additive, the refractive index ranged between 1.4774 and 1.4788 (specification: 1.4770–1.4815).⁴⁷

⁴⁶ Technical dossier/Supplementary Information June 2019/Annex_IX_SIn_reply_lemon_oil_expressed_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1).

⁴⁷ Technical dossier/Supplementary information June 2019/Annex_II_SIn_Reply_lemon_oil_distilled_CoA.

Volatile components

The product specification for the residual fraction is based on the concentrations (expressed as % of GC area) of the main volatile components, namely d-limonene (51–63%, the phytochemical marker), γ -terpinene (8–17%), geranal (6–12%), neral (5–9%), pin-2(10)-ene (0.3–5.5%) and β -bisabolene (0.3–4.0%).⁴⁸ Analysis of five batches of the additive showed compliance with these specifications with the exception of one batch, which showed a lower d-limonene content and a higher γ -terpinene content (Table 9). These six compounds account for about 90.7% on average (range 85.0–92.9%) of the product, expressed as area % of the gas chromatographic (GC) profile.

Table 9: Major volatile constituents of the residual fraction of expressed lemon oil obtained from the fruit peels of *Citrus limon* (L.) Osbeck 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	Specification (%)	% of GC area	
				Mean^(a)	Range
d-Limonene	5989-27-5	01.045	51–63	53.3	49.3–56.8
γ -Terpinene	99-85-4	01.020	8–17	16.9	12.8–23.3
Geranal	141-27-5	05.188	6–12	10.4	9.5–11.2
Neral	106-26-3	0.8724	5–9	7.8	6.2–8.9
β -Pinene (pin-2(10)-ene)	127-91-3	01.003	0.3–5.5	1.24	0.32–3.38
β -Bisabolene	495-61-4	01.028	0.3–4	1.11	0.61–2.71
Total				90.7	85.0–92.9

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

(a): Mean calculated on five batches.

The applicant provided the full characterisation of the volatile constituents in five batches of the residual fraction obtained by GC-MS.⁴⁸ In total, up to 126 constituents were detected, 74 of which were identified and accounted on average for 99.5% (99.0–99.3) of the product (as the GC area). Besides the six compounds indicated in the product specifications, 20 other compounds were detected at levels > 0.1% and are listed in Table 10. The remaining 48 compounds ranging between 0.002% and 0.1% are listed in the footnote.⁴⁹ Among these substances, α -thujone was detected in four batches (0.009–0.016%), and perillaldehyde in one batch (0.092%).

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

Constituent EU register name	CAS no	FLAVIS no	% of GC area	
			Mean^(a)	Range
Geranyl acetate	105-87-3	09.011	1.02	0.81–1.57
Neryl acetate	141-12-8	09.213	0.79	0.37–2.32
(E)- α -bergamotene	13474-59-4	–	0.62	0.30–2.05
β -Caryophyllene	87-44-5	01.007	0.58	0.32–1.28
Terpinolene	586-62-9	01.005	0.51	0.11–0.86

⁴⁸ Technical dossier/Supplementary information June 2019/Annex III_SIn _Reply_lemon_oil_distilled_chromatograms.

⁴⁹ Additional volatile constituents: l-fenchone, decyl acetate, 4-terpineol, p-mentha-1,8-dien-7-al, citronellal, nonanal, carvone, cis-3,7-dimethyl-1,3,6-octatriene, bisabola-1,8,12-triene, valencene, bicyclogermacrene, trans-d-limonene oxide, pin-2(3)-ene, (l)- α -bisabolol, octan-1-ol, octyl acetate, trans-carveol, spathulenol, (-)-trans- γ -bergamotene, β -elemene, α -copaene, dodecanal, (E)- α -bisabolene, cis-carveol, β -cubebene, α -phellandrene, octanal, pinocarveol, δ -selinene, trans-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, α -thujene, δ -3-carene, p-camphorene, (E)- γ -bisabolene, cis-para-2,8-menthadien-1-ol, δ -cadinene, α -thujone, 1,4-cineole, 1,8-cineole, neryl propionate, p-mentha-1,8(10)-dien-9-ol, geranyl propionate, camphene, α -selinene, 7-epi- γ -selinene, β -copaene, germacra-1(10),4(14),5-triene, (E,Z)-2,6-dimethylocta-2,4,6-triene.

Constituent EU register name	CAS no	FLAVIS no	% of GC area	
			Mean ^(a)	Range
1-Isopropyl-4-methylbenzene	99-87-6	01.002	0.45	0.32–0.58
Citronellol	106-22-9	02.011	0.40	0.16–0.55
Myrcene	123-35-3	01.008	0.39	0.25–0.58
Linalool	78-70-6	02.013	0.36	0.34–0.46
Geraniol	106-24-1	02.012	0.30	0.20–0.36
α -Terpineol	98-55-5	02.014	0.29	0.20–0.48
Citronellyl acetate	150-84-5	09.012	0.25	0.10–0.34
trans- β -Ocimene	3779-61-1	01.064	0.16	0.11–0.22
para-Mentha-3,8-diene	586-67-4	—	0.14	0.04–0.21
Sabinene	3387-41-5	01.059	0.13	0.02–0.45
Decanal	112-31-2	05.010	0.12	0.10–0.15
3,7-dimethyl-3,6-octadienal	55722-59-3	—	0.12	0.08–0.16
Citropten	487-06-9	—	0.11	0.07–0.22
α -Terpinene	99-86-5	01.019	0.10	0.08–0.13
cis-Limonene oxide	13837-75-7	01.002	0.10	0.07–0.13
Total			6.95	4.82–11.40

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

(a): Mean calculated on five batches.

Non-volatile components

The literature search performed by the applicant identified 12 furocoumarins and two methoxycoumarins that have been reported to occur in expressed lemon oil (see Section 3.2.1). HPLC analysis of the 10 batches⁵⁰ of the residual fraction of expressed lemon oil also showed detectable concentrations of all the substances except byakangelicin and 5-geranyloxy-7-methoxycoumarin. Phellopterin was detected in one batch only. The concentrations of furocoumarins and methoxycoumarins in the residual fraction of lemon oil expressed are summarised in Table 11.

Table 11: Furocoumarins and methoxycoumarins in the residual fraction of expressed lemon oil obtained from the fruit peels of *Citrus limon* (L.) Osbeck (based on the analysis of five batches)

Congener	Average	Range
	mg/kg	mg/kg
Furocoumarins		
Psoralen	23.9	22.1–26.2
5-Methoxysoralen (bergapten)	71.1	31.9–144
Bergamottin (5-geranoxypsoralen)	3.0	3.0
Oxypeucedanin	2,825	2,061–4,183
8-Methoxysoralen (xanthotoxin)	30.2	17–56.1
8-Geranoxypsoralen	649	425–1,361
Imperatorin	1,213	928–1,489
5,8-Dimethoxysoralen (isopimpinellin)	113	108–119
Phellopterin	3.0	3.0
Oxypeucedanin hydrate	80	51.7–109
Byakangelicol	88	0.5–134
Byakangelicin	< 0.1	< 0.1

⁵⁰ Technical dossier/Supplementary information June 2019/Annex VI_Sin reply_lemon_oil_distilled_SOC_COA and SIn July 2020/Supplement_1_lemon_oil.

Congener	Average	Range
	mg/kg	mg/kg
Methoxycoumarins		
Citropten (5,7-dimethoxycoumarin) ^(a)	690	220–970
5-Geranyloxy-7-methoxycoumarin	< 0.1	< 0.1

(a): By GC-MS.

Impurities

Data on chemical and microbial impurities were provided in three batches of the residual fraction.⁵¹ The concentrations of heavy metals were below the corresponding LOQ, with the exception of cadmium in one batch (0.08 mg/kg). 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 endosulfan (0.029–0.032 mg/kg), endosulfan sulfate (0.033 mg/kg), chlorpyrifos ethyl (0.63–0.70 mg/kg) and 2-phenylphenol (3.2–3.9 mg/kg). In the same batches, PCDD, PCDF and PCBs were below the corresponding LOQ and the calculated upper bond for the sum of the WHO PCCD/F + PCB TEQ ranged between 1.67 and 1.87 pg/g fat. None of the data on chemical impurities raised concerns.

Analysis of microbial contamination of three batches of the residual fraction indicated that *Salmonella* spp. was absent in 25 g, *Enterobacteriaceae*, total viable count, yeasts, moulds were < 10¹ colony forming unit (CFU)/g.

3.3.1.1. Shelf-life

The typical shelf-life of the residual fraction 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).²² No separate or additional stability studies for flavouring additives were performed.

3.3.1.2. Conditions of use

The residual fraction of expressed lemon oil is intended to be added to feed for all animal species without withdrawal. The maximum proposed use level in complete feed is 12 mg/kg for poultry, rabbits, fish, dogs and cats, 30 mg/kg for pigs, 20 mg/kg for ruminants, 35 mg/kg for horses. No use level has been proposed by the applicant for the use in water for drinking.

3.3.2. Safety

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

Many of the major volatile components, accounting for about 99% 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 lemon 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 lemon oil have been already described in Section 3.2.2.2.

3.3.2.1. Safety for the target species

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

As the additive under assessment is sufficiently characterised (> 99%), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the residual fraction of expressed lemon oil, considering the volatile components separately from the non-volatiles (furocoumarins and citropten) as described in Section 3.2.2.3.

⁵¹ Technical dossier/Supplementary information June 2019/Annex VII_Sin reply_lemon-Oil_distilled_impurities. Limit of quantification (LOQ) in mg/kg for heavy metals and arsenic: 0.005 for mercury, 0.01 for cadmium, 0.05 for lead and 0.1 for arsenic LOQ for individual pesticides: 0.1 mg/kg; LOQ for mycotoxins: < 0.1 µg/kg for aflatoxins B1, B2, G1 and G2.

Volatile components

The approach followed, i.e. the allocation of the components to the (sub-)assessment groups, the estimate of exposure for the target species, the identification of a reference point for each constituent (hazard characterisation) and the calculation of the MOET for each assessment group (risk characterisation), is described in Section 3.2.2.3. 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.⁵²

The approach to the safety assessment of the residual fraction for the target species is summarised in Table 12. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight at the use level of 12 mg/kg.

Table 12: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual volatile components of the residual fraction of expressed lemon oil classified according to assessment groups. Intake calculations for the individual components are based on the use level of 12 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 sum of the reciprocals of the MOE of the individual substances

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max feed conc.	Daily intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 31 II (Acyclic alkanes)								
Myrcene	01.008	0.56	0.069	0.0062	I	44	7,091	
trans-Ocimene	01.018	0.22	0.027	0.0024	I	44	18,234	
cis-Ocimene	01.064	0.11	0.013	0.0012	I	44	36,796	
MOET CG 31, II				0.0098				4,483
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.045	56.8	6.816	0.6119	I	250	409	
γ-Terpinene	01.020	23.3	2.796	0.2510	I	250	996	
β-Bisabolene	01.028	2.71	0.325	0.0292	I	250	8,563	
Terpinolene	01.005	0.86	0.104	0.0093	I	250	26,891	
MOET CG 31, III				0.9014				277
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	0.58	0.070	0.0063	I	154	24,605	
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
β-Pinene	01.003	3.38	0.406	0.0364	I	222	6,097	
(E)-α-bergamotene	n.a.	2.05	0.246	0.0221	I	3	136	
β-Caryophyllene	01.007	1.28	0.154	0.0138	I	222	16,100	
Bicyclogermacrene	n.a.	0.91	0.023	0.0021	I	3	1,458	
Sabinene	01.059	0.45	0.053	0.0048	I	222	46,309	
α-trans β-bergamotene	n.a.	0.10	0.012	0.0011	I	3	2,678	
MOET CG 31, V				0.0803				115

⁵² Compounds included in the assessment groups but not reported in the table: decanal, decyl acetate, nonanal, octan-1-ol, octyl acetate, dodecanal and octanal (CG 1); geraniol, isogeraniol, neryl propionate and geranyl propionate (CG 3); 4-terpineol, (E)-β-bisabolol and trans-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol(CG 6); carvone (CG 8); 1,8-cineole and 1,4-cineole (CG 16); (E,Z)-2,6-dimethylocta-2,4,6-triene (CG 31, II); bisabola-1,8,12-triene, p-mentha-3,8-diene, α-terpinene, β-elemene, (E)-α-bisabolene, α-phellandrene, p-camphorene and (E)-γ-bisabolene (CG 31, III); valencene, α-pinene, α-copaene, δ-selinene, δ-3-carene, β-cubebene, α-thujene, δ-cadinene, camphene, γ-selinene, 7-epi-α-selinene and β-copaene (CG 31, V).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max feed conc.	Daily intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
Germacra-1(10),4(14),5-triene	n.a.	0.006	0.001	0.0001	I	3	46,414	
CG 3								
Geranial	05.188	11.2	1.344	0.1207	I	345	2,859	
Neral	05.170	8.94	1.073	0.0963	I	345	3,582	
Neryl acetate	09.213	2.32	0.278	0.0250	I	345	13,804	
Geranyl acetate	09.011	1.57	0.188	0.0169	I	345	20,398	
MOET CG 3				0.2589				1,333
CG 4								
Citronellol	02.011	0.55	0.066	0.0059	I	50	8,424	
Citronellyl acetate	09.012	0.34	0.041	0.0037	I	50	13,571	
Citronellal	05.021	0.12	0.015	0.0013	I	50	37,735	
MOET CG 4				0.0109				4,568
CG 6								
α -Terpineol	02.014	0.48	0.058	0.0052	I	250	48,247	
Linalool	02.013	0.46	0.055	0.0049	I	117	23,817	
MOET CG 6				0.0115				14,601
CG 8								
I-Fenchone	n.a.	0.17	0.020	0.0018	II	<i>0.91</i>	518	
Spathulenol	n.a.	0.09	0.011	0.0010	III	<i>0.15</i>	148	
Trans-Carveol	02.062	0.06	0.007	0.0006	I	<i>3</i>	5,063	
Cis-Carveol	02.062	0.03	0.004	0.0004	I	<i>3</i>	8,191	
Pinocarveol		0.03	0.004	0.0004	I	<i>3</i>	7,957	
cis-p-2,8-Menthadien-1-ol	n.a.	0.03	0.003	0.0003	III	<i>0.15</i>	557	
MOET CG 8				0.0044				92
CG 32 (epoxides)								
cis-Limonene oxide	n.a.	0.13	0.015	0.0014	I	<i>3</i>	2,210	
trans-Limonene oxide	n.a.	0.09	0.010	0.0009	I	<i>3</i>	3,201	
MOET CG 32								1,307
α -Thujone	n.a.	0.016	0.002	0.0002	III	<i>8</i>	46,414	

(a): Intake calculations for the individual components are based on the use level of 12 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

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

As shown in Table 12, for all the assessment groups, the MOET calculated for chickens for fattening at the proposed use level of 12 mg/kg complete feed was ≥ 92 . From the lowest MOET of 92 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 13.

Table 13: Combined margin of exposure (MOET) calculated for the other target species at the proposed use level of the residual fraction of expressed lemon oil

	Body weight (kg)	Daily feed intake (g DM/kg bw)	Feed intake (g DM/day)	Use level (mg/kg feed)	Lowest MOET
Chickens for fattening	2	79	158	12	92
Laying hens	2	53	106	12	137
Turkeys for fattening	3	59	176	12	123
Piglets	20	44	880	30	66
Pigs for fattening	60	37	2,200	30	79
Sow lactating	175	30	5,280	30	97
Veal calves (milk replacer)	100	19	1,890	20	246
Cattle for fattening	400	20	8,000	20	218
Dairy cows	650	31	20,000	20	141
Sheep/goat	60	20	1,200	20	218
Horse	400	20	8,000	35	125
Rabbit	2	50	100	12	145
Salmon	0.12	18	2.1	12	404
Dogs	15	17	250	12	428
Cats	3	20	60	12	363
Ornamental fish	0.012	5	0.54	12	1,454

Table 13 shows an MOET below the value of 100 for chickens for fattening, piglets, pigs for fattening and sows. In order to ensure an MOET ≥ 100 , the concentration in feed should be reduced to 11 mg/kg for chickens for fattening, 20 mg/kg for piglets, 24 mg/kg for pigs for fattening and 29 mg/kg for sows. For the other species, the FEEDAP Panel concludes with respect to the exposure to the volatiles (except perillaldehyde) present in the additive that the use of the residual fraction of expressed lemon oil at the maximum proposed use levels in feed is safe for all animal species, except for cats. Owing to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997), an MOET of 500 is considered adequate. No specific proposals have been made by the applicant for the use level in water for drinking. Therefore, the FEEDAP Panel considered the same use level in water for drinking as proposed for feed. When used in water for drinking, the intake of the additive via water would be two to three times higher than the intake via feed for poultry, pigs and rabbits (EFSA FEEDAP Panel, 2010). The Panel considers that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

Volatile components: Perillaldehyde

Low concentrations of perillaldehyde were detected in one batch of the additive under assessment (0.092%). The use of the residual fraction of expressed lemon oil at the proposed use level in feed (12–35 mg/kg) would result in an intake of perillaldehyde up to 1.0 µg/kg bw for poultry, 1.4 µg/kg bw for pigs, 0.6 µg/kg bw for ruminants, 0.7 µg/kg bw for horses, 0.6 µg/kg bw for rabbit and 0.2 µg/kg bw for fish.⁵³

These concentrations are at least 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 (calculated to be 16 µg/kg bw for poultry, 18 µg/kg bw for pigs, 12 µg/kg bw for ruminants, 8 µg/kg bw for horses, 20 µg/kg bw for rabbit and 7 µg/kg bw for fish, see Section 3.2.2.3, *Volatile components: perillaldehyde*).

Non-volatile components: Furocoumarins and methoxycoumarins

The approach followed for furocoumarins and methoxycoumarins is based on the comparison between the combined furocoumarin and methoxycoumarin intake via the consumption of citrus by-products as feed material and that via the use of the residual fraction of expressed lemon oil as a feed

⁵³ Calculated considering the concentration of perillaldehyde in the additive (0.092%), the default values for feed intake (Table 13) 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%).

additive, as described in Section 3.2.2.3 (*Non-volatile components: Furocoumarins and methoxycoumarins*).

The average and maximum intake of individual furocoumarins and citropten and their sum, calculated for chickens for fattening fed diets containing the additive at the maximum proposed use level of 12 mg/kg is reported in Table 14. The average and maximum intake of total furocoumarins and methoxycoumarins for the different target species is reported in Table 15.⁵⁴

Table 14: Concentration of furocoumarins and methoxycoumarins in feed for chickens for fattening (as µg/kg complete feed) and intake of chickens for fattening (as µg/kg bw per day) calculated at the maximum proposed use level of the additive in feed (12 mg/kg) and considering the average and the maximum analysed value for each congener in the additive

Congener	Concentration in the additive		Concentration in complete feed		Intake (chickens for fattening)	
	Average	Max	Average	Max	Average	Max
	mg/kg additive		µg/kg feed		µg/kg bw/day	
Furocoumarins						
Psoralen	23.9	26.2	0.29	0.31	0.03	0.03
5-Methoxysoralen (bergapten)	71.1	144.4	0.85	1.73	0.08	0.16
Bergamottin (5-geranoxysoralen)	3.0	3.0	0.036	0.036	0.003	0.003
Oxypeucedanin	2825	4183	33.90	50.19	3.04	4.51
8-Methoxysoralen (xanthotoxin)	30.2	56.1	0.36	0.67	0.03	0.06
8-Geranoxysoralen	649	1361	7.79	16.33	0.70	1.47
Imperatorin	1213	1489	14.6	17.86	1.31	1.60
5,8-Dimethoxysoralen (isopimpinellin)	113	119	1.35	1.42	0.12	0.13
Phellopterin	3.0	3.0	0.036	0.036	0.003	0.003
Oxypeucedanin hydrate	80	109	0.96	1.30	0.09	0.12
Byakangelicol	88	134	1.06	1.61	0.09	0.14
Methoxycoumarins						
Citropten	690	970	8.28	11.64	0.74	1.04
Total			69	103	6.24	9.26

Table 15: Target animal intake of total furocoumarins and methoxycoumarins at the maximum proposed use level of the additive in feed for each target species (ranging from 12 to 35 mg/kg complete feed). Total values of furocoumarins and methoxycoumarins in feed are calculated considering the average and the maximum analysed value for each congener in the additive lemon oil expressed

Target species	Daily feed intake	Body weight	Use level in feed	Intake furocoumarins	
	Average			Average	Max
	kg DM/day	kg	mg/kg	µg/kg bw/day	
Chickens for fattening	0.158	2	12	6.2	9.3
Laying hens	0.106	2	12	4.2	6.2
Turkey for fattening	0.176	3	12	4.6	6.9
Piglet	0.88	20	30	8.7	12.9
Pig for fattening	2.2	60	30	7.2	10.7
Sow lactating	5.28	175	30	6.0	8.8
Veal calf (milk replacer)	1.89	100	20	2.3	3.4

⁵⁴ Intake values calculated considering the average and maximum concentration of furocoumarins and citropten in the additive (Table 14), the default values for feed intake (Table 13), 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%).

Target species	Daily feed intake	Body weight	Use level in feed	Intake furocoumarins	
	kg DM/day	kg	mg/kg	Average	Max
Cattle for fattening	8	400	20	2.6	3.9
Dairy cows	20	650	20	4.0	6.0
Sheep/goat	1.2	60	20	2.6	3.9
Horse	8	400	35	4.6	6.8
Rabbit	0.1	2	12	3.9	5.9
Salmon	0.0021	0.12	12	1.4	2.1
Dog	0.25	15	12	1.3	2.0
Cat	0.06	3	12	1.6	2.3
Ornamental fish	0.000054	0.012	12	0.4	0.5

The use of the residual fraction of expressed lemon oil at the proposed use level in feed (12–35 mg/kg) would result in an average intake of total furocoumarins and methoxycoumarins ranging from 0.4 µg/kg bw per day in ornamental fish and 8.7 µg/kg bw per day in piglets (maximum intake 12.9 µg/kg bw per day).

These concentrations are about 10-fold lower than those resulting from the intake of furocoumarins and methoxycoumarins from citrus by-products, when used in diets at concentrations of 10% for poultry and 20% for the other species (calculated to be 140 µg/kg bw per day for poultry, 160 µg/kg bw per day for pigs, 100 µg/kg bw per day for ruminants and 70 µg/kg bw per day for horses, 180 µg/kg bw per day for rabbits and 65 µg/kg bw per day for fish, see Section 3.2.2.3).

Conclusions on safety for the target species

The FEEDAP Panel concludes that the residual fraction of expressed lemon oil is safe up to the maximum proposed use level in complete feed of 12 mg/kg for laying hens, turkeys for fattening, rabbits and salmonids, 20 mg/kg for ruminants and 35 mg/kg for horses. For the other species, the calculated safe concentrations are 11 mg/kg for chickens for fattening, 20 mg/kg for piglets, 24 mg/kg for pigs for fattening and 30 mg/kg for sows. These target species are fed citrus by-products as part of daily feed. For these species, the use of the residual fraction of expressed lemon oil in feed is not expected to increase the exposure to furocoumarins and methoxycoumarins and perillaldehyde to a relevant extent (< 10%). For companion animals and ornamental fish 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 provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed, except companion animals.

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

3.3.2.2. Safety for the consumer

Considering the similarity in the composition of expressed lemon oil and its residual fraction, 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 residual fraction of expressed lemon 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 sheet⁵⁵ for lemon oil 'distilled' (combined residual and distilled fractions of expressed lemon oil) where hazards for users have been identified. Since lemon oil expressed contains furocoumarin derivatives, the additive may cause phototoxicity (NTP, 2000).

⁵⁵ Technical dossier/Supplementary Information June 2019/AAnnex_IX_SIn reply_lemon_oil_distilled_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1), serious eye damage/eye irritation (H319, category 2).

3.3.2.4. Safety for the environment

C. limon (L.) Burm. f. 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. Distilled fraction of expressed lemon oil (referred to as the distilled fraction)

This application concerns the volatile fraction (i.e. the distillate) from the distillation of lemon oil expressed, which is obtained from fruit peel of *C. limon* (L.) Osbeck from multiple geographic origins.

3.4.1. Characterisation of the distilled fraction

The distilled fraction is a colourless clear mobile liquid with a characteristic odour. In five batches of the additive, the refractive index varied between 1.4730 and 1.4735 (specification: 1.4715–1.4850).¹⁷

The product specification for the distilled fraction is based on the concentrations (expressed as % of GC area) of the main components, namely d-limonene (66–78%, the phytochemical marker), pin-2(10)-ene (5–20%), α -terpinene (1.5–9.5%), pin-2(3)-ene (0.5–3.0%) and/or sabinene (0.5–3.0%). Analysis of five batches of the additive¹¹ showed compliance with these specifications (Table 16). These five compounds account for about 90.7% on average (range 85.0–92.9%) of the product, expressed as area % of GC area.

Table 16: Major constituents of the distilled fraction of expressed lemon oil obtained from the fruit peels of *Citrus limon* (L.) Osbeck 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	Specification (%)	% of GC area	
				Mean ^(a)	Range
d-Limonene	5989-27-5	01.045	66–78	66.6	65.8–68.5
β -Pinene (pin-2(10)-ene)	127-91-3	01.003	5–20	16.6	14.6–17.6
γ -Terpinene	99-85-4	01.020	1.5–9.5	8.02	7.27–9.23
α -Pinene (pin-2(3)-ene)	80-56-8	01.004	0.5–3.0	2.80	2.71–3.08
Sabinene (4(10)-thujene)	3387-41-5	01.059	0.3–3.0	1.89	1.13–2.90
Total				95.9	95.6–96.1

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

(a): Mean calculated on five batches.

The applicant provided the full characterisation of the five batches of the distilled fraction obtained by GC-MS.⁴⁸ In total, up to 53 constituents were detected, 36 of which were identified and accounted on average for 99.95% (99.88–99.98%) of the product (as the GC area). Besides the five compounds indicated in the product specifications, 15 other compounds were detected at levels > 0.5% and are listed in Table 17. These 20 compounds > 0.5% together account on average for 99.9% (99.6–100%) of the product. The remaining 16 compounds (ranging between 0.002% and 0.5%) are listed in the footnote.⁵⁶

⁵⁶ Additional constituents: linalool, para-mentha-3,8-diene, cyclohexene, 4-methyl-1-(1-methylethyl)-, (R)-, cis-limonene oxide, octan-1-ol, 1,4-cineole, trans-d-limonene oxide, alpha-terpineol, 1,8-cineole, nonanal, beta-caryophyllene, trans-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, heptan-1-ol, carvone, citronellal, trans-carveol.

Table 17: Constituents of the distilled fraction of expressed lemon oil obtained from the fruit peels of *Citrus limon* (L.) Osbeck accounting for > 0.5% of the composition (based on the analysis of 10 batches) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent EU register name	CAS no	FLAVIS no	% of GC area	
			Mean ^(a)	Range
Myrcene	123-35-3	01.008	1.95	1.42–2.26
1-Isopropyl-4-methylbenzene	99-87-6	01.002	0.57	0.38–0.81
α-Thujene	3387-41-5	01.059	0.29	0.18–0.61
α-Terpinene	99-86-5	01.019	0.21	0.18–0.23
Terpinolene	586-62-9	01.005	0.19	0.12–0.26
cis-β-Ocimene	3338-55-4	01.064	0.11	0.10–0.13
Camphene	79-92-5	01.009	0.09	0.09–0.11
trans- β-Ocimene	141-27-5	05.188	0.09	0.07–0.10
Octanal	124-13-0	05.009	0.09	0.02–0.15
Neral	106-26-3	05.170	0.09	0.09
Trimethyltricyclo [2.2.1.0.(2.6)] heptane	508-32-7	01.060	0.08	0.003–0.32
trans-3,7-Dimethylocta-2,6-dienal	141-27-5	05.188	0.08	0.003–0.197
α-Phellandrene	99-83-2	01.006	0.07	0.004–0.318
α-3-Carene	13466-78-9	01.029	0.06	0.006–0.095
4-Terpineol	562-74-3	02.072	0.05	0.049
Total			4.02	3.74–4.12

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

(a): Mean calculated on five batches.

The HPLC analysis of five batches of the distilled fraction of expressed lemon oil showed that furocoumarins and methoxycoumarins are below the corresponding LOQ.⁵⁷

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, organo-chloride pesticides, organo-phosphorous pesticides, aflatoxin B1, B2, G1, G2 and ochratoxin A. However, no data has been provided. Since the distilled fraction of lemon oil is produced by steam distillation, the likelihood of any measurable carry-over of heavy metals is low except for mercury (Tascone et al., 2014).

3.4.1.1. Shelf-life

The typical shelf-life of the distilled fraction 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).²² No separate or additional stability studies for flavouring additives were performed.

3.4.1.2. Conditions of use

The distilled fraction of expressed lemon oil is intended to be added to feed for all animal species without withdrawal. The maximum proposed use level in complete feed is 40 mg/kg for chickens for fattening, 55 mg/kg for turkeys for fattening, 60 mg/kg for laying hens, 75 mg/kg for piglets, 90 mg/kg for pigs for fattening, 105 mg/kg for lactating sows, 95 mg/kg for ruminants, 165 mg/kg for horses, 60 mg/kg for rabbits, salmons, dogs, cats and ornamental fish. No use level has been proposed by the applicant for the use in water for drinking.

3.4.2. Safety

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

⁵⁷ Technical dossier/Supplementary information June 2019/Annex VI_Sin reply_lemon_oil_distilled_SOC_COA and SIn July 2020/Supplement_1_lemon_oil. LOQ: 0.1 mg/kg.

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

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

3.4.2.1. Safety for the target species

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

As the distilled fraction of expressed lemon oil is sufficiently characterised (> 99.9%), the FEEDAP Panel applied a component-based approach to assess the safety for target species. The approach followed, i.e. the allocation of the components to the (sub-)assessment groups, the estimate of exposure for the target species, the identification of a reference point for each constituent (hazard characterisation) and the calculation of the MOET for each assessment group (risk characterisation), is described in Section 3.2.2.3. 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.⁵⁸

The approach to the safety assessment of the distilled fraction for the target species is summarised in Table 18. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight at the use level of 40 mg/kg.

Table 18: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of the distilled fraction of expressed lemon oil classified according to assessment groups. Intake calculations for the individual components are based on the use level of 40 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 sum of the reciprocals of the MOE of the individual substances

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max Feed conc.	Daily intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw per day	–	mg/kg bw per day	–	–
CG 31 II (Acyclic alkanes)								
Myrcene	01.008	2.26	0.904	0.0812	I	44	542	
cis-β-Ocimene	01.064	0.13	0.051	0.0046	I	44	9,648	
trans-β-Ocimene	01.018	0.10	0.042	0.0037	I	44	11,782	
MOET CG 31, II				0.0894				492
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.045	68.5	27.40	2.4598	I	250	102	
γ-Terpinene	01.020	9.23	3.692	0.3314	I	250	754	
Terpinolene	01.005	0.26	0.104	0.0093	I	250	26,880	
α-Terpinene	01.019	0.23	0.092	0.0083	I	250	30,139	
MOET CG 31, III				2.8088				89
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	0.814	0.326	0.0292	I	154	5,269	
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
β-Pinene	01.003	16.58	7.040	0.6320	I	222	351	

⁵⁸ Compounds included in the assessment groups but not reported in the table: octan-1-ol, nonanal and heptan-1-ol (CG 1); citronellal (CG 4); 4-terpineol, α-terpineol and trans-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol (CG 6); carvone (CG 8); 1,8-cineole and 1,4-cineole (CG 16); α-phellandrene, p-mentha-3,8-diene and cyclohexene, 4-methyl-1-(1-methylethyl)-, (R)- (CG 31, III); camphene, δ-3-carene and β-caryophyllene (CG 31, V).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max Feed conc.	Daily intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
α -Pinene	01.004	2.80	1.232	0.1106	I	222	2,007	
Sabinene	01.059	1.89	1.160	0.1041	I	222	2,132	
α -Thujene	n.a.	0.29	0.246	0.0220	I	222	10,069	
Tricyclene	01.060	0.20	0.079	0.0071	I	222	31,382	
MOET CG 31, V			7.040	0.8759				260
CG 1								
Octanal	05.009	0.15	0.061	0.0055	I	120	21,842	
CG 3								
Neral	05.170	0.32	0.128	0.0115	I	345	30,024	
Geranial	05.188	0.32	0.127	0.0114	I	345	30,213	
MOET CG 3				0.0229				15,059
CG 6								
Linalool	02.013	0.08	0.032	0.0028	I	117	41,243	
CG 8								
Trans-Carveol	02.062	0.002	0.001	0.0001	I	3	41,772	
CG 32 (epoxides)								
cis-Limonene oxide	n.a.	0.04	0.015	0.0012	I	3	2,566	
trans-limonene oxide	n.a.	0.09	0.014	0.0011	I	3	2,712	
MOET CG 32								1,319

(a): Intake calculations for the individual components are based on the use level of 40 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

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

As shown in Table 18, for all the assessment groups, the MOET calculated for chickens for fattening at the proposed use level of 40 mg/kg complete feed was ≥ 89 . From the lowest MOET of 89 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 19.

Table 19: Combined margin of exposure (MOET) calculated for the other target species at the proposed use level of the distilled fraction of expressed lemon oil in feed

	Body weight (kg)	Daily feed intake (g DM/kg bw)	Feed intake (g DM/day)	Use level (mg/kg feed)	Lowest MOET
Chickens for fattening	2	79	158	40	89
Laying hens	2	53	106	60	88
Turkeys for fattening	3	59	176	55	87
Piglets	20	44	880	75	85
Pigs for fattening	60	37	2,200	90	84
Sow lactating	175	30	5,280	105	89
Veal calves (milk replacer)	100	19	1,890	95	167
Cattle for fattening	400	20	8,000	95	148
Dairy cows	650	31	20,000	95	95
Sheep/goat	60	20	1,200	95	148
Horse	400	20	8,000	165	85
Rabbit	2	50	100	60	94

	Body weight (kg)	Daily feed intake (g DM/kg bw)	Feed intake (g DM/day)	Use level (mg/kg feed)	Lowest MOET
Salmon	0.12	18	2.1	60	260
Dogs	15	17	250	60	276
Cats	3	20	60	60	234
Ornamental fish	0.012	5	0.54	60	937

Table 19 shows an MOET below the value of 100 for poultry, pigs, dairy cows, rabbits and horses. In order to ensure an MOET ≥ 100 , the concentration in feed should be reduced to 36 mg/kg for chickens for fattening, 53 mg/kg for laying hens, 48 mg/kg for turkeys for fattening, 64 mg/kg for piglets, 76 mg/kg for pigs for fattening, 94 mg/kg for sows, 91 mg/kg for dairy cows, 56 mg/kg for rabbits and 141 mg/kg for horses. For the other species, the FEEDAP Panel concludes that the use of the distilled fraction at the maximum proposed use levels in feed is safe for all animal species, except for cats. Owing to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997), safe concentration in feed should be reduced to 30 mg/kg to ensure an MOET of about 500. No specific proposals have been made by the applicant for the use level in water for drinking. Therefore, the FEEDAP Panel considered the same use level in water for drinking as proposed for feed. When used in water for drinking, the intake of the additive via water would be two to three times higher than the intake via feed for poultry, pigs and rabbits (EFSA FEEDAP Panel, 2010). The Panel considers that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

Conclusions on safety for the target species

The FEEDAP Panel concludes that the use of the distilled fraction of expressed lemon oil is safe up to the maximum proposed use levels of 95 mg/kg for veal calves, cattle for fattening, sheep and goats, and 60 mg/kg for dogs, salmons and ornamental fish. For the other species, the calculated safe concentrations are 36 mg/kg for chickens for fattening, 53 mg/kg for laying hens, 48 mg/kg for turkeys for fattening, 64 mg/kg for piglets, 76 mg/kg for pigs for fattening, 94 mg/kg for sows, 91 mg/kg for dairy cows, 56 mg/kg for rabbits, 141 mg/kg for horses and 30 mg/kg for cats.

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

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

3.4.2.2. Safety for the consumer

Considering the similarity in the composition of the volatile fraction of expressed lemon 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 fraction of expressed lemon oil up to the highest safe use level in feed.

3.4.2.3. 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 sheet⁵⁹ for lemon oil 'distilled' (combined residual and distilled fractions of expressed lemon oil) where hazards for users have been identified.

3.4.2.4. Safety for the environment

C. limon (L.) Osbeck 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.

⁵⁹ Technical dossier/Supplementary Information June 2019/Annex_IX_SIn_reply_lemon_oil_distilled_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1), serious eye damage/eye irritation (H319, category 2).

3.5. Lime oil

This application concerns the essential oil obtained by steam distillation from unpeeled fruits of the plant species *C. aurantiifolia* (Christm.) Swingle from a single origin (Mexico). Briefly, lime fruits are pressed, and the peels are removed. Steam is passed through the remaining lime juice and oil. The steam carries up the volatile constituents which are condensed. The essential oil is then separated from water by decantation.

3.5.1. Characterisation of lime oil

The lime oil distilled is a pale yellow clear mobile liquid, with a characteristic odour of fresh lime. In five batches of the additive, the refractive index was 1.48 and the relative density ranged between 0.859 and 0.860 g/mL.¹²

Volatile components

The product specification for lime essential oil is based on the concentrations (expressed as % of GC area) of the main components, namely d-limonene (45–52%, the phytochemical marker), α -terpinene (10–14%), terpinolene (5.5–10.5%), α -terpineol (6–8%) and β -caryophyllene (0.2–0.8%). Analysis of five batches of the additive showed compliance with these specifications when analysed by GC-FID.⁶⁰ When analysed by GC-MS, these five compounds account for about 74.8% on average (range 73.7–76.3%) of the product, expressed as area % of the GC profile (Table 20). The FEEDAP Panel notes that the concentration of d-limonene determined by GC-MS was underestimated, resulting in higher concentration of the other constituents.⁶¹

Table 20: Major constituents of lime essential oil obtained from the fruits of *Citrus aurantiifolia* (Christm.) Swingle as defined by ISO standard 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	Specification (%)	% of GC area	
				Mean ^(a)	Range
d-Limonene	5989-27-5	01.045	45–52	37.0	35.7–38.1 ^(b)
γ -Terpinene	99-85-4	01.020	10–14	14.5	13.5–16.9
Terpinolene	589-62-9	01.005	5.5–10.5	9.89	9.14–11.2
α -Terpineol	98-55-5	02.014	6–8	9.49	8.16–10.5
β -Caryophyllene	87-44-5	01.007	0.2–0.8	0.44	0.40–0.48
Total				74.8	73.7–76.3

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

(a): Mean calculated on five batches. (b) GC-FID 46.8–47.4%.

The applicant provided the full characterisation of the five batches obtained by GC-MS.⁶² In total, up to 87 constituents were detected, 60 of which were identified and accounted on average for 99.04% (98.74–99.12%) of the product (as the GC area). Besides the five compounds indicated in the product specifications, 29 other compounds were detected at levels > 0.1% and are listed in Table 21. These 34 compounds > 0.1% together account on average for 97.5% (97.2–97.6%) of the product. The remaining 25 compounds (ranging between 0.002% and 0.1%) are listed in the footnote.⁶³

⁶⁰ Technical dossier/Supplementary information August 2019 and July 2020.

⁶¹ Technical dossier/Supplementary information June 2020: According to the applicant, discrepancies between the specifications, defined by ISO based on GC-FID analysis, and the analyses by GC-MS reported in Table 20 are due to the use of different analytical methods.

⁶² Technical dossier/Supplementary information August 2019/Annex_III_SIn _Reply_lime_oil _chromatograms.

⁶³ Additional constituents: β -farnesene, 1-methyl-1,4-cyclohexadiene, 2-(4-methylphenyl)propan-2-ol, (1)- α -bisabolol, 3,7,10-humulatriene, decanal, (Z)- α -bisabolene, β -santalene, isoborneol, caryophyllene alcohol, δ -elemene, cis- α -bergamotene, β -selinene, nonanal, γ -eudesmol, germacrene B, β -elemene, 3-methylene-1,5,5-trimethylcyclohexene, α -cadinene, α -amorphene, sabinene, 1,1,7-trimethyltricyclo[2.2.1.0.(2.6)]heptane, 1-methyl-1,3-cyclohexadiene, (3R-trans)-3-methyl-6-(1-methylvinyl)cyclohexene, α -cadinol.

Table 21: Other constituents of lime essential oil obtained from the fruit peels of *Citrus aurantiifolia* (Christm.) Swingle accounting for > 0.1% of the composition (based on the analysis of 10 batches) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent EU register name	CAS no	FLAVIS no	% of GC area	
			Mean ^(a)	Range
1-Isopropyl-4-methylbenzene	99-87-6	01.002	2.59	2.35–3.07
Pin-2(10)-ene (β -pinene)	127-91-3	01.003	2.37	1.75–2.94
β -Bisabolene	495-61-4	01.028	1.99	1.64–2.44
1,4-Cineole	470-67-7	03.007	1.88	1.51–2.36
1,8-Cineole	470-82-6	03.001	1.67	1.40–1.96
Pin-2(3)-ene (α -pinene)	80-56-8	01.004	1.32	1.12–1.63
α -Terpineol	586-81-2	–	1.24	0.61–1.69
Myrcene	123-35-3	01.008	1.18	0.93–1.34
cis- β -Terpineol ^(b)	7299-41-4	–	0.71	0.52–0.96
α -Farnesene	502-61-4	01.040	0.80	0.62–0.89
1-Terpinenol	586-82-3	02.096	0.77	0.71–0.89
Borneol	507-70-0	02.016	0.56	0.30–0.69
(E)- α -Bergamotene	13474-59-4	–	0.66	0.62–0.79
β -Phellandrene	555-10-2	01.055	0.52	0.43–0.62
Fenchyl alcohol	1632-73-1	02.038	0.59	0.45–0.71
Camphene	79-92-5	01.009	0.50	0.38–0.62
α -Phellandrene	99-83-2	01.006	0.51	0.38–0.67
4-Terpinenol	532-74-3	02.072	0.56	0.50–0.67
trans- β -Terpineol ^(b)	7299-40-3	–	0.29	0.22–0.38
1-Isopropenyl-4-methylbenzene	1195-32-0	01.010	0.35	0.34–0.37
α -Selinene	28624-23-9	–	0.28	0.27–0.29
Geranal	141-27-5	05.188	0.24	0.21–0.26
cis- β -Ocimene	3338-55-4	01.064	0.21	0.19–0.23
α -Fenchene	471-84-1	–	0.19	0.15–0.23
α -Selinene	515-17-3	–	0.13	0.09–0.17
Linalool	78-70-6	02.013	0.16	0.14–0.18
Neral	106-26-3	05.170	0.17	0.11–0.32
Geranyl acetate	105-87-3	09.011	0.13	0.09–0.18
Selina-3,7(11)-diene	6813-21-4	–	0.13	0.10–0.15
Total			22.7	21.2–23.9

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

(a): Mean calculated on five batches.

(b): Isomers of β -Terpineol, FLAVIS number [02.097].

Non-volatile components

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *C. aurantiifolia* and its preparations.⁶⁴ The occurrence of 5-geranyloxyxpsoralen, 5-geranyloxy-7-methoxycoumarin, citropten, 5-MOP, 5,8-dimethoxysoralen and 3-methyl-1,2-cyclopentanediol (maple lactone) has been reported in several publications on preparations obtained from *C. aurantiifolia* (Dugo et al., 1997; NTP, 2000; Gorgus et al., 2010; Shalaby et al., 2011; Masson et al., 2016; Melough et al., 2017).

Analysis of the five batches⁶⁵ of the essential oil showed detectable concentrations of citropten (60.9–96.3 mg/kg), 5-geranyloxy-7-methoxycoumarin (< 6–185.7 mg/kg) and 5-MOP (4.12–8.32 mg/kg) in all

⁶⁴ Technical dossier/Supplementary information August 2019/Literature search_lime_oil.

⁶⁵ Technical dossier/Supplementary information August 2019/Annex VI_Sin reply_lime油_SOC_COA.

batches, whereas maple lactone was below the LOD (< 5 mg/kg), as were isopimpellin (< 1 mg/kg) and 5-geranyloxyxpsoralen (< 0.5 mg/kg).

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, organo-chloride pesticides, organo-phosphorous pesticides, aflatoxin B1, B2, G1, G2 and ochratoxin A. However, no data has been provided. Since lime oil is produced by steam distillation, the likelihood of any measurable carry-over of heavy metals is low except for mercury (Tascone et al., 2014).

3.5.1.1. Shelf-life

The typical shelf-life of the lime 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).²² No separate or additional stability studies for flavouring additives were performed.

3.5.1.2. Conditions of use

Lime oil is intended to be added to feed for all animal species without withdrawal. The maximum proposed use level in complete feed is 8.5 mg/kg for chickens for fattening, 12.5 mg/kg for laying hens, 11 mg/kg for turkeys for fattening, 15 mg/kg for piglets, 18 mg/kg for pigs for fattening, 22 mg/kg for lactating sows, 21.5 mg/kg for dairy cows, 33.5 mg/kg for cattle for fattening, sheep, goats and horses, 35.5 mg/kg for veal calves (milk replacers), 13.5 mg/kg for rabbits, 30 mg/kg for salmons, dogs, cats and ornamental fish.

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

3.5.2. Safety

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

The Panel notes that almost all the major volatile components of the additive, have been assessed and considered safe for use as flavourings, and are currently authorised for food⁸ and feed⁷ uses. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

Among the volatile components, 24 substances have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 31 and 8 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastro-intestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2016a, d). For 10 out of the 24 compounds (γ -eudesmol, α -cadinol, 3-methylene-1,5,5-trimethylcyclohexene, (3R-trans)-3-methyl-6-(1-methylvinyl)cyclohexene, cis- α -bergamotene, (E)- α -bergamotene, 1,1,7-trimethyltricyclo[2.2.1.0.(2.6)]heptane, α -fenchene, 3,7,10-humulatriene and germacrene B), the genotoxic potential of the was predicted using the OECD QSAR Toolbox.⁶⁶ No alerts were identified for *in vitro* mutagenicity, for genotoxic and non-genotoxic carcinogenicity and for other endpoints.

Lime oil contains trace amounts of 5-MOP and methoxycoumarins, mainly citroten and 5-geranyloxy-7-methoxycoumarin. The ADME of 5-MOP has been addressed in Section 3.2.2.1. In the absence of specific data on the ADME of methoxycoumarins, it is assumed that the same metabolic pathways described for furocoumarins also apply to citroten and 5-geranyloxy-7-methoxycoumarin (see Section 3.2.2.1).

The studies relevant to the assessment of the non-volatile individual components of lime oil (furocoumarins and citroten as representative of methoxycoumarins) have been already described in Section 3.2.2.2.

3.5.2.1. Safety for the target species

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

As the essential oil under assessment is sufficiently characterised (> 98.7%), the FEEDAP Panel applied a component-based approach to assess the safety for target species, considering the volatile components separately from the non-volatiles (methoxycoumarins and 5-methoxypsonalen) as described in Section 3.2.2.3.

⁶⁶ Technical dossier/Supplementary information August 2019/Annex_IX_Sin_reply_lime_oil_QSAR.

Volatile components

The approach followed, i.e. the allocation of the components to the (sub-)assessment groups, the estimate of exposure for the target species, the identification of a reference point for each constituent (hazard characterisation) and the calculation of the MOET for each assessment group (risk characterisation), is described in Section 3.2.2.3. 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.⁶⁷

The approach to the safety assessment of lime essential oil for the target species is summarised in Table 22. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/body weight and represent the worst-case scenario at the use level of 8.5 mg/kg.

Table 22: Compositional data, intake values, reference points and margin of exposure (MOE) for the individual components of lime essential oil classified according to assessment groups. Intake calculations for the individual components are based on the use level of 8.5 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 sum of the reciprocals of the MOE of the individual substances

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max feed conc.	Daily intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw/day	–	mg/kg bw/day	–	–
CG 31 II (Acyclic alkanes)								
Myrcene	01.008	1.34	0.114	0.0102	I	44	4,303	
α-Farnesene	01.040	0.89	0.076	0.0068	I	44	6,472	
cis-β-Ocimene	01.064	0.23	0.020	0.0018	I	44	24,748	
β-Farnesene	01.041	0.14	0.012	0.0011	I	44	40,043	
MOET CG 31, II				0.0199				2,221
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.045	38.1	3.239	0.2907	I	250	860	
γ-Terpinene	01.020	16.9	1.437	0.1290	I	250	1,939	
Terpinolene	01.005	11.2	0.952	0.0855	I	250	2,925	
α-Terpinene	01.019	3.79	0.322	0.0289	I	250	8,644	
β-Bisabolene	01.028	2.44	0.207	0.0186	I	250	13,427	
α-Phellandrene	01.006	0.67	0.057	0.0051	I	250	48,899	
1-Methyl-1,4-cyclohexadiene	n.a.	0.10	0.009	0.0008	I	3	3,854	
β-Elemene	n.a.	0.05	0.004	0.0004	I	3	7,561	
3-Methylene-1,5,5-trimethylcyclohexene	n.a.	0.05	0.004	0.0004	I	3	8,547	
1-Methyl-1,4-cyclohexadiene	01.077	0.02	0.002	0.0002	I	3	17,093	
(3R-trans)-3-methyl-6-(1-methylvinyl)cyclohexene	n.a.	0.02	0.002	0.0002	I	3	17,093	
MOET CG 31, III				0.5597				350

⁶⁷ Compounds included in the assessment groups but not reported in the table: decanal and nonanal (CG 1); neral, geranial and geranyl acetate (CG 3); trans-b-terpineol, linalool, 2-(4-methylphenyl)propan-2-ol, (l)-a-bisabolol, g-eudesmol and a-acidol (CG 6); caryophyllene alcohol (CG 8); b-phellandrene, d-elemene, and (Z)-a-bisabolene (CG 31, III); 4-isopropenyl-4-methylbenzene (CG 31, IV); d-selinene, a-fenchene, g-selinene, d-cadinene, sabinene, b-selinene, selina-3,7(11)-diene, d-amorphene and tricyclene (CG 31, V).

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS no	Max conc. in the oil	Max feed conc.	Daily intake ^(a)	Cramer class	NOAEL ^(b)	MOE	MOET
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	3.07	0.261	0.0234	I	154	5,862	
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
β-Pinene	01.003	2.94	0.250	0.0224	I	222	9,896	
α-Pinene	01.004	1.63	0.139	0.0124	I	222	17,849	
Camphene	01.009	0.62	0.052	0.0047	I	222	47,152	
β-Caryophyllene	01.007	0.48	0.041	0.0037	I	222	60,110	
(E)- α-bergamotene	n.a.	0.79	0.067	0.0060	I	3	499	
β-Santalene	n.a.	0.12	0.010	0.0009	I	3	3,360	
cis- α-Bergamotene	n.a.	0.11	0.010	0.0009	I	3	3,510	
MOET CG 31, V				0.0510				359
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
3,7,10-Humulatriene	01.043	0.14	0.011	0.0010	I	3	2,912	
Germacrene B	n.a.	0.06	0.005	0.0005	I	3	6,552	
MOET CG 31, VI				0.0086				2,016
CG 6								
α-Terpineol	02.014	10.50	0.893	0.0801	I	250	3,120	
α-Terpineol	n.a.	1.69	0.144	0.0129	I	250	19,386	
1-Terpinenol	02.096	0.89	0.075	0.0068	I	250	37,020	
cis-β-Terpineol	n.a.	0.96	0.081	0.0073	I	250	34,235	
4-Terpineol	02.072	0.67	0.057	0.0051	I	250	48,826	
MOET CG 6				0.1131				2,228
CG 8								
Fenchyl alcohol	02.038	0.71	0.060	0.0054	I	3	555	
Borneol	02.016	0.69	0.059	0.0053	I	3	566	
Isoborneol	02.059	0.07	0.006	0.0005	I	15	27,302	
MOET CG 8				0.0112				555
CG 16								
1,4-Cineole	03.007	2.36	0.201	0.0180	II	100	5,553	
1,8-Cineole	03.001	1.96	0.167	0.0150	II	100	6,686	
MOET CG 16				0.0330				3,034

(a): Intake calculations for the individual components are based on the use level of 8.5 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

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

As shown in Table 22, for all the assessment groups, the MOET was ≥ 100 . Therefore, no safety concern was identified for lime essential oil when used as a feed additive for chickens for fattening at the proposed use levels (8.5 mg/kg). From the lowest MOET of 350 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 23.

Table 23: Combined margin of exposure (MOET) calculated for the other target species at the proposed use level

	Body weight (kg)	Daily feed intake (g DM/kg bw)	Feed intake (g DM/day)	Use level (mg/kg feed)	Lowest MOET
Chickens for fattening	2	79	158	8.5	350
Laying hens	2	53	106	12.5	355
Turkeys for fattening	3	59	176	11	362
Piglets	20	44	880	15	356
Pigs for fattening	60	37	2,200	18	353
Sow lactating	175	30	5,280	22	356
Veal calves (milk replacer)	100	19	1,890	35.5	374
Cattle for fattening	400	20	8,000	33.5	351
Dairy cows	650	31	20,000	21.5	353
Sheep/goat	60	20	1,200	33.5	351
Horse	400	20	8,000	33.5	351
Rabbit	2	50	100	13.5	348
Salmon	0.12	18	2.1	30	435
Dogs	15	17	250	30	461
Cats	3	20	60	30	392
Ornamental fish	0.012	5	0.54	30	1,567

At the proposed use levels for the different species, the lowest MOET is ≥ 151 . Therefore, with respect to the exposure to the volatiles present in the additive, no safety concern was identified for lime oil when used as a feed additive at the proposed use levels except for cats. Owing to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997), safe concentration in feed should be reduced to 10 mg/kg to ensure an MOET of about 500. No specific proposals have been made by the applicant for the use level in water for drinking. Therefore, the FEEDAP Panel considered the same use level in water for drinking as proposed for feed. When used in water for drinking, the intake of the additive via water would be two to three times higher than the intake via feed for poultry, pigs and rabbits (EFSA FEEDAP Panel, 2010). The Panel considers that the use level in water for drinking is safe.

Methoxycoumarins and 5-methoxysoralen

The approach followed for methoxycoumarins and 5-MOP is based on the comparison between the combined furocoumarin and methoxycoumarin intake via the consumption of citrus by-products as feed material (see Section 3.2.2.3) and that via the use of lime oil as a feed additive.

The average and maximum intake of individual methoxycoumarins and 5-MOP and their sum, calculated for chickens for fattening fed diets containing lime oil at the maximum proposed use level of 8.5 mg/kg is reported in Table 24. The average and maximum intake of total furocoumarins and methoxycoumarins for the different target species is reported in Table 25.

Table 24: Concentration of methoxycoumarins and 5-methoxysoralen in feed for chickens for fattening (as µg/kg complete feed) and their intake (as µg/kg bw per day) calculated at the maximum proposed use level of the additive in feed (8.5 mg/kg), considering the average and the maximum analysed value for each congener in the additive

Congener	Concentration in the additive		Concentration in complete feed		Intake chickens for fattening	
	Average	Max	Average	Max	Average	Max
	mg/kg additive		µg/kg feed		µg/kg bw/day	
Methoxycoumarins						
Citropten	75.0	96.3	0.64	0.82	0.057	0.073
5-Geranoxy-7-methoxycoumarin	67.5	186	0.57	1.58	0.051	0.142

Congener	Concentration in the additive		Concentration in complete feed		Intake chickens for fattening	
	Average	Max	Average	Max	Average	Max
	mg/kg additive		µg/kg feed		µg/kg bw/day	
Furocoumarins						
5-Methoxysoralen (bergapten)	6.0	8.3	0.05	0.07	0.005	0.006
Total			1.26	2.47	0.113	0.222

Table 25: Target animal intake of total methoxycoumarins and 5-methoxysoralen (as µg/kg bw per day) at the maximum proposed use level of lime oil in feed for each species (ranging from 8.5 to 33.5 mg/kg complete feed). Total values of methoxycoumarins and 5-methoxysoralen in feed are calculated considering the average and the maximum analysed value for each congener in the additive

Target species	Daily feed intake	Body weight	Use level in feed	Intake furocoumarins	
	kg DM/day			Average	Max
Chickens for fattening	0.158	2	8.5	0.11	0.22
Laying hens	0.106	2	12.5	0.11	0.22
Turkey for fattening	0.176	3	11	0.11	0.21
Piglet	0.88	20	15	0.11	0.22
Pig for fattening	2.2	60	18	0.11	0.22
Sow lactating	5.28	175	22	0.11	0.22
Veal calf (milk replacer)	1.89	100	35.5	0.11	0.21
Cattle for fattening	8	400	33.5	0.11	0.22
Dairy cows	20	650	33.5	0.17	0.34
Sheep/goat	1.2	60	33.5	0.11	0.22
Horse	8	400	33.5	0.11	0.22
Rabbit	0.1	2	13.5	0.11	0.22
Salmon	0.0021	0.12	30	0.09	0.17
Dog	0.25	15	30	0.08	0.17
Cat	0.06	3	30	0.10	0.20
Ornamental fish	0.000054	0.012	30	0.02	0.05

The use of lime oil at the proposed use level in feed (8.5–35.5 mg/kg) would result in an average intake of total methoxycoumarins and 5-MOP ranging from 0.02 µg/kg bw per day in ornamental fish and 0.17 µg/kg bw per day in dairy cows (maximum intake 0.34 µg/kg bw per day in dairy cows).

These concentrations are about 100-fold lower than those resulting from the intake of furocoumarins and methoxycoumarins from citrus by-products, when used in diets at concentrations of 10% for poultry and 20% for the other species (calculated to be 140 µg/kg bw per day for poultry, 160 µg/kg bw per day for pigs, 110 µg/kg bw per day for ruminants, 70 µg/kg bw per day for horses, 180 µg/kg bw per day for rabbits and 65 µg/kg bw per day for fish, see Section 3.2.2.3).

Conclusions on safety for the target species

The FEEDAP Panel concludes that the lime oil is safe up to the maximum proposed use level in complete feed of 8.5 mg/kg for chickens for fattening, 12.5 mg/kg for laying hens, 11 mg/kg for turkeys for fattening, 15 mg/kg for piglets, 18 mg/kg for pigs for fattening, 22 mg/kg for lactating sows, 21.5 mg/kg for dairy cows, 33.5 mg/kg for cattle for fattening, sheep, goats and horses, 35.5 mg/kg for veal calves (milk replacers), 13.5 mg/kg for rabbits and 30 mg/kg for salmonids. These target species are fed citrus by-products as part of daily feed. For these species, the use of the lime oil in feed is not expected to increase the exposure to furocoumarins and methoxycoumarins to a relevant

extent (< 1%). For companion animals and ornamental fish 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, except for companion animals.

3.5.2.2. Safety for the consumer

Lime oil obtained by steam distillation of the peel is added to a wide range of food categories as spice or for flavouring purposes. Although individual consumption figures for the EU are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 1.62 mg/kg bw per day for lime 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 (see Table 1).

Concerning furocoumarins intake from food, in Great Britain, the furocoumarin intake has been estimated to be up to 1.2 mg per person per day corresponding to approximately 0.02 mg/kg bw per day for 60 kg bw (COT, 1996 as cited by SKLM, 2010). In Germany, the estimated average total daily intake of furocoumarins from non-flavoured and flavoured foods is approximately 0.56 mg/person per day (~ 0.01 mg/kg bw per day for 60 kg bw), flavoured foods contributing to about 10% to the total intake (SKLM, 2006, 2010). Reported exposure levels for furocoumarin intake from food for the general population are below the phototoxicity threshold dose (0.25 mg/kg bw for 60 kg bw) and have not been associated with an additional risk of skin cancer.

No data on residues in products of animal origin were made available for any of the constituents of the essential oil. However, the Panel recognises that the constituents of lime 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 the individual constituents by humans consuming products of animal origin is not expected.

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

3.5.2.3. Safety for user

No specific data were provided by the applicant regarding the safety of the additive for users. In the absence of specific data, the FEEDAP Panel cannot conclude on the safety for the users when handling the additive. Distilled lime oil was not irritating or sensitising when tested in volunteers at 15% or 100%. Lime oil is not phototoxic (Dosoky and Setzer, 2018).

The applicant produced a safety data sheet⁶⁸ for lime oil where hazards for users have been identified.

3.5.2.4. Safety for the environment

C. aurantiifolia is grown for lime production in Europe, particularly in Spain and Italy (FAO, 2019). Use of the essential oil under the proposed conditions of use in animal production is not expected to pose a risk for the environment.

3.6. Efficacy of the additives

C. limon and its extracts are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2625 (lemon oil obtained by cold expression of the peel, with or without previous separation of the pulp and the peel) and 2626 (lemon oil terpeneless).

C. aurantiifolia and its extracts are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2631 (lime oil distilled, prepared from the sour juice obtained by expression of the whole fruit) and 2632 (lime oil terpeneless).

Since lemon and lime and their extracts 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.

⁶⁸ Technical dossier/Supplementary Information August 2019/Annex_X_SIn reply_lime油_MSDS. Inhalation hazard (H304), Hazards for skin irritation (H315), eye irritation (H319), skin sensitisation (H317B).

4. Conclusions

Since lemon and lime oils may be produced by various processes resulting in preparations with different toxicological profiles, the following conclusions apply only to expressed lemon oil from fruit peels of *Citrus limon* (L.) Osbeck (isonym *Citrus limon* (L.) Burm. f.) and its fractions obtained after distillation, and to lime oil obtained by steam distillation from the fruits of *Citrus aurantiifolia* (Christm.) Swingle.

The use of the expressed lemon oil and its residual fraction and the use of lime oil in feed is not expected to increase the exposure to furocoumarins and methoxycoumarins and perillaldehyde of those target species that are already fed citrus by-products to a relevant extent (< 10%). For companion animals and ornamental fish, not normally exposed to citrus by-products, no conclusion can be drawn.

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

- Expressed lemon oil is safe at the maximum proposed use level in complete feed of 40 mg/kg for turkeys for fattening, 90 mg/kg for veal calves, cattle for fattening and dairy cows, 30 mg/kg for sheep, goats and rabbits and 40 mg/kg for salmonids. For the other species, the calculated safe concentrations are 35 mg/kg for chickens for fattening, 52 mg/kg for laying hens, 62 mg/kg for piglets, 74 mg/kg for pigs for fattening, 92 mg/kg for sows and 137 mg/kg for horses. The use level in water for drinking of 10 mg/kg is safe for all animal species (except companion animals).
- The residual fraction of expressed lemon oil is safe up to the maximum proposed use level in complete feed of 12 mg/kg for laying hens, turkeys for fattening, rabbits and salmonids, 20 mg/kg for ruminants and 35 mg/kg for horses. For the other species, the calculated safe concentrations are 11 mg/kg for chickens for fattening, 20 mg/kg for piglets, 24 mg/kg for pigs for fattening and 30 mg/kg for sows. The FEEDAP Panel considers that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed (except companion animals).
- The distilled fraction of expressed lemon oil is safe up to the maximum proposed use levels in complete feed of 95 mg/kg for veal calves, cattle for fattening, sheep and goats and 60 mg/kg for dogs, salmons and ornamental fish. For the other species, the calculated safe concentrations are 36 mg/kg for chickens for fattening, 53 mg/kg for laying hens, 48 mg/kg for turkeys for fattening, 64 mg/kg for piglets, 76 mg/kg for pigs for fattening, 94 mg/kg for sows, 91 mg/kg for dairy cows, 56 mg/kg for rabbits, 141 mg/kg for horses and 30 mg/kg for cats. The FEEDAP Panel considers that the use level in water for drinking is safe provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.
- Lime essential oil is safe up to the maximum proposed use levels in complete feed of 8.5 mg/kg for chickens for fattening, 12.5 mg/kg for laying hens, 11 mg/kg for turkeys for fattening, 15 mg/kg for piglets, 18 mg/kg for pigs for fattening, 22 mg/kg for lactating sows, 21.5 mg/kg for dairy cows, 33.5 mg/kg for cattle for fattening, sheep, goats and horses, 35.5 mg/kg for veal calves (milk replacers), 13.5 mg/kg for rabbits, 30 mg/kg for salmons and ornamental fish. The FEEDAP Panel considers that the use level in water for drinking is safe (except companion animals).

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

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

The additives under assessment should be considered as irritants to skin and eyes and the respiratory tract and as skin sensitisers. Since expressed lemon oil and its residual fraction contain furocoumarins, they may cause phototoxicity.

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

Since *C. limon* and *C. aurantiifolia* and their preparations are recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. Documentation as provided to EFSA/Chronology

Date	Event
05/11/2010	Dossier received by EFSA. Chemically defined flavourings from Botanical Group 08 – Sapindales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
14/12/2010	Reception mandate from the European Commission
26/02/2013	EFSA informed the applicant (EFSA ref. 7150727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission
24/06/2015	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products": data requirement for the risk assessment of botanicals
17/06/2016	Technical hearing during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products". Discussion on the ongoing work regarding the pilot dossiers BDG08 and BDG 09
27/04/2017	Trilateral meeting organised by the European Commission with EFSA and the applicant FEFANA on the assessment of botanical flavourings: characterisation, substances of toxicological concern present in the botanical extracts, feedback on the pilot dossiers
19/03/2018	Application validated by EFSA – Start of the scientific assessment
20/06/2018	Comments received from Member States
03/05/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterization, safety for the target species, safety for the consumer, safety for the user, safety for the environment</i>
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: Amyris oil, Cashew oil, Olibanum tincture, Neroli bigarade oil, Petitgrain bigarade absolute, Mandarin terpenes, Grapefruit oil expressed, Grapefruit extract (sb), Grapefruit extract.
03/06/2019	Reception of supplementary information from the applicant (partial submission)
01/08/2019	Reception of supplementary information from the applicant (partial submission)
01/07/2020	Reception of supplementary information from the applicant (partial submission)
28/07/2020	Reception of supplementary information from the applicant (partial submission)
24/11/2020	Reception of supplementary information from the applicant (partial submission)
12/03/2021	The application was split and a new EFSA-Q-2021-00126 was assigned to the preparations included in the present assessment.
17/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives- Scientific assessment re-started for the preparations included in the present assessment
18/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparations included in the present assessment

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Abbreviations

ADME	Absorption, distribution, metabolism and excretion
AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
BDG	botanically defined group
BELFRIT	BELgium, FRance, ITaly
BMD	benchmark dose
BMDL ₁₀	BMD lower confidence limit for a benchmark response of 10%
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CDG	chemically defined group
CHO	Chinese hamster ovary
COT	Committee on Toxicology
CYP	cytochrome P450
DFG	Deutsche Forschungsgemeinschaft
DM	dry matter
EC	European Commission
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EMEA	European Medicines Agencies
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food Additives and Flavourings

FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavor Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
FL-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography–mass spectrometry
GST	glutathione S-transferases
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
ISO	International Standard Organisation
lac ⁻	lactose operon
LC-MS-MS	liquid chromatography tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
MOET	combined margin of exposure (total)
MOP	methoxysoralen
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
PCBs	polychlorobiphenyls
PCDD	polychlorinated dibenzo- <i>p</i> -dioxin
PCDF	polychlorinated dibenzofuran
PhEur	European Pharmacopoeia
PPR	EFSA Panel on Plant Protection Products and their Residues
QSAR	Quantitative Structure-Activity Relationship
ROS	reactive oxygen species
SC	EFSA Scientific Committee
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food
SKLM	Senate Commission on Food Safety
TEQ	Toxic equivalent
TOF	Time of flight
TTC	threshold of toxicological concern
UF	uncertainty factor
UVA	ultraviolet A
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, 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 four 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, 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 08 (BDG 08)*¹, under the category/functional group 2(b) 'sensory additives'/'flavouring compounds', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species. For each preparation the Applicant indicated the corresponding phytochemical marker(s) and the corresponding range of content. The *feed additives* are intended to be incorporated into *feedingstuffs* or drinking water directly or through flavouring *premixtures* with no proposed minimum or maximum levels. However, the Applicant suggested the typical maximum inclusion level of the *feed additive* of 25 mg/kg *feedingstuffs*.

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

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

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

For the quantification of the phytochemical markers *linalyl acetate* and *linalool* in *petitgrain 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 *petitgrain bigarade oil*.

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

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

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

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

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

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

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

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

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