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SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of an essential oil obtained from the fruit of *Apium graveolens* L. (celery seed oil) for all animal species (FEFANA asbl)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of an essential oil obtained from the fruit of *Apium graveolens* L. (celery seed oil), when used as a sensory additive in feed and water for drinking for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel concluded that the use of celery seed oil is of no concern up to the following concentrations in complete feed: 1.6 mg/kg for chickens for fattening, 2.3 mg/kg for laying hens, 2.1 mg/kg for turkeys for fattening, 2.8 mg/kg for piglets, 3.3 mg/kg for pigs for fattening, 4.1 mg/kg for sows, 6.5 mg/kg for veal calves (milk replacer), 6.2 mg/kg for cattle for fattening, sheep, goats and horses, 4.0 mg/kg for dairy cows, 2.5 mg/kg for rabbits, 6.8 mg/kg for salmonids and 7.2 mg/kg for dogs. These conclusions were extrapolated to other physiologically related species. For cats, ornamental fish and other species, no conclusion can be drawn. The use of celery seed oil in animals feed is not expected to pose concern for the consumers and for the environment. The additive under assessment should be considered as an irritant to skin and eyes, and as a respiratory and skin sensitiser. When handling the essential oil, exposure of unprotected users to perillaldehyde and bergapten may occur. Therefore, to reduce the risk, the exposure of the users should be minimised. Since *A. graveolens* and its preparations were recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

KEYWORDS

Apium graveolens L., bergapten, celery seed oil, flavouring compounds, limonene, perillaldehyde, safety, sensory additives

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1 | INTRODUCTION

1.1 | Background and Terms of Reference

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

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 29 additives (namely dill herb oil, dill seed extract, dill tincture, dong quai tincture, celery seed oil, celery seed extract (oleoresin), celery tincture, hares ear tincture, caraway seed oil, caraway oleoresin/extract, coriander oil, cumin oil, taiga root extract (solvent-based, sb), taiga root tincture, fennel oil, fennel tincture, common ivy extract (sb), opoponax oil, ginseng tincture, parsley oil, parsley tincture, anise oil, anise tincture, ajowan oil, Ferula Assa-foetida oil, anise star oil, anise star tincture, anise star terpenes and omicha tincture) belonging to botanically defined group (BDG) 02 – Apiales/Austrobaileyales 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 additives.[3] These additives were deleted from the register of feed additives.[4] During the course of the assessment, this application was split and the present opinion covers only one out of the 20 remaining additives under application: celery seed oil from the fruit of *Apium graveolens* L. for all animal species.

The remaining 19 additives belonging to botanically defined group (BDG) 02 – Apiales/Austrobaileyales under application are assessed in separate opinions.

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 24 June 2019.

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 the product celery seed oil (*A. graveolens*), when used under the proposed conditions of use (see **Section 3.3.3**).

1.2 | Additional information

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

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[5] in support of the authorisation request for the use of celery seed oil from *A. graveolens* as a feed additive. The dossier was received on 02/February 2024 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2024-00060.[6]

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.

[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]Dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, opoponax oil (27 February 2019); parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil (2 April 2020); celery tincture (9 December 2020).

[4]Register of feed additives, Annex II, withdrawn by OJ L162, 10.5.2021, p. 5.

[5]FEED dossier reference: FAD-2010-0221.

[6]The original application EFSA-Q-2010-01286 was split on 02/02/2024 and a new EFSA-Q-2024-00060 was generated.

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings (CDGs). 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 additives belonging to BDG 2, including the one under assessment.[7]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance/agent in the additive. The evaluation report is related to the methods of analysis for each feed additive included in BDG 02 (Apiales and Austrobaileyales).[8] During the assessment, the EURL issued a partial report[9] and an addendum of the report.[10] In particular, for the characterisation of *celery seed oil*, the EURL report recommended a method based on gas chromatography coupled with flame ionisation detection (GC-FID) for the quantification of the phytochemical marker *limonene* in *celery seed oil*.

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of celery seed oil from *A. graveolens* is in line with the principles laid down in Regulation (EC) No 429/2008[11] 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 on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023a), 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), General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021a).[12]

3 | ASSESSMENT

The additive under assessment, celery seed oil, is an essential oil obtained from the fruit of *Apium graveolens* L., intended for use as a sensory additive (functional group: flavouring compounds) in feed and in water for drinking for all animal species.

3.1 | Origin and extraction

Apium graveolens L. is a biennial herbaceous marshland plant belonging to the Apiaceae family. Originally native to the Mediterranean region and the Middle East, the plant has a long history of use as a vegetable and now is extensively cultivated in most temperate parts of the world. Depending on the cultivar, either the long fibrous stem (celery), the leaves (leaf celery) or the bulbous hypocotyl (celeriac) may be consumed in a raw or cooked form. In common with most members of the Apiaceae, multiple small flowers are produced in dense compound umbels, each giving rise to a small fruit (1.5–2 mm in length). These, when ripe, are collected, ground and used directly as a spice or mixed with salt (celery salt). It should be noted that most references to celery seed are to the fruit.

The essential oil is obtained from the fruit of *A. graveolens* by steam distillation. The volatile constituents are condensed and then separated from the aqueous phase by decantation.

[7]Technical dossier/Supplementary information/Letter dated 29/4/2021.

[8]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0221_en

[9]Additives included in the partial report: dill herb oil, dill tincture, dong quai tincture, cumin oil, fennel tincture, parsley tincture, anise tincture, star anise tincture and ferula assa-foetida oil.

[10]Additives included in the addendum: celery seed oil, caraway seed oil, coriander oil, taiga root tincture, fennel oil, common ivy extract (sb), ginseng tincture, anise oil, anise star oil, anise star terpenes and omicha tincture.

[11]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.

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

3.2 | Uses other than feed flavouring

There is no specific EU authorisation for any *A. graveolens* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008,[13] flavouring preparations produced from food may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer.'

3.3 | Characterisation

3.3.1 | Characterisation of celery seed oil

Celery seed oil is a yellow to amber clear slightly viscous liquid with a characteristic odour. In seven batches of the additive (of Chinese or Indian origin), the density (20°C) ranged between 926 and 929 kg/m^3 (four batches), the refractive index (20°C) between 1.483 and 1.492 (specification: 1.474–1.492, seven batches) and the specific optical rotation (at 20°C, four batches) between −46.36° and 49.88°.[14] Celery seed oil is identified with the single Chemical Abstracts Service (CAS) number 8015-90-5, the European Inventory of Existing Chemical Substances (EINECS) number 289-668-4, the Flavor Extract Manufacturers Association (FEMA) 2271 and the Council of Europe (CoE) number 52.

For celery seed oil, the specifications used by the applicant are based on the standard developed by the International Organisation for Standardization (ISO) 3760:2002 for essential oil of celery seeds (*A. graveolens*),[15] adapted to reflect the concentrations of selected volatile components. Four components contribute to the specifications as shown in Table 1, with limonene selected as phytochemical marker. Analysis of seven batches of the additive from two different suppliers showed compliance with the specifications when analysed by GC-FID and expressed as percentage of gas chromatographic peak area (% GC area).

TABLE 1 Major constituents of the essential oil from the fruit of *Apium graveolens* L. as defined by specifications and batch to batch variation based on the analysis of seven batches by gas chromatography with flame ionisation detector (GC-FID). The content of each constituent is expressed as the area percent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

Constituent			% GC area		
EU register name	**CAS no**	**FLAVIS no**	**Specification[a]**	**Mean**	**Range**
d-Limonene[b]	5989-27-5	01.045	35–79	61.1	56.7–68.2
β-Selinene	17066–67-0	–	5–20	12.4	9.2–17.8
Senkyunolide A	62006–39-7	–	1.5–22	9.77	3.8–15.2
β-Pinene (pin-2(10)-ene)	127–91-3	01.003	0.3–2	0.56	0.46–0.72
Total				83.8	81.7–87.0[c]

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

[a]Specifications defined based on GC-FID analysis.

[b]Stereochemistry not specified, however considering that the naturally occurring limonene is typically *d*-limonene, it is assumed that this form is also in celery seed oil.

[c]The values given for the total are the lowest and the highest values of the sum of the components in the seven batches analysed.

The applicant provided the full characterisation of the volatile constituents in seven batches obtained by gas chromatography–mass spectrometry (GC–MS).[16] In total, 72 peaks were identified and accounted on average for 97.9% (97.3%–99.1%) of the % GC area. The four compounds indicated in the product specifications account for about 73.6% on average (range 69.9%–84.3%) of % GC area. Besides the four compounds indicated in the product specifications, 28 other compounds were detected at individual levels > 0.1% and are listed in Table 2. The 32 compounds together account on average for 96.7% (95.9%–98.7%) of the % GC area. The remaining 40 compounds (ranging between 0.1% and 0.013%) and accounting for 1.24% are listed in the footnote.[17] Based on the available data on the characterisation, celery seed oil is considered a fully defined mixture (EFSA Scientific Committee, 2019a).

[13]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.

[14]Technical dossier/Supplementary information April 2022/Annex_II_SIn_Reply_celery_seed_oil_COA_chromatograms.

[15]Technical dossier/Supplementary information April 2022/Annex_III_SIn_reply_celery_seed_oil_ ISO_3760_2002.

[16]Technical dossier/Supplementary information May 2020/Annex_II_SIn_ Reply_celery_seed_oil_COA_chromatograms.

[17]Additional constituents: constituents ($n = 11$) between < 0.1% and ≥ 0.05%: *α*-curcumene, 3-butyl hexahydrophthalide, myrtenyl acetate, *trans*-limonene epoxide, *α*-pinene, (−)-*α*-elemol, linalool, *β*-elemene, humulene oxide II, pinocarvyl acetate, tetrahydrophtalide derivative (MW 266 $C_{16}H_{26}O_3$). constituents ($n = 29$) between < 0.05% and ≥ 0.01%: carvyl acetate, *α*-terpineol, hexadecanoic acid, 4-hydroxy-4-methylpentan-2-one, *trans-p*-mentha-1(7),8-dien-2-ol, 1-(4-methylcyclohex-3-en-1-yl) ethenone, limonene 8,9-oxide, 1,8-*p*-menthadien-4-ol, *(E,Z)*-2,4-dodecadiene, 1-isopropyl-4-methylbenzene, γ-selinene, oct-1-en-3-yl-acetate, *cis*-dehydrocarvone, senkyunolide (isomer 1), *p*-mentha-1,8-dien-7-ol, cis-limonene epoxide, sabinene, 2-methoxy-4-vinylphenol, α-cedrene, 4-terpinenol, senkyunolide (isomer 2), p-mentha-1,8-dien-7-al, γ-terpinene, oct-1-en-3-ol, 1-isopropenyl-4-methylbenzene, *cis*-linalool oxide (5-ring), 1,5,8-*p*-menthatriene, *trans*-linalool oxide (5-ring), 3-octyl acetate.

TABLE 2 Constituents of the essential oil from the fruit of *Apium graveolens* L. accounting for > 0.1% of the composition (based on the analysis of seven batches by gas chromatography–mass spectrometry). The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

Constituent			% GC area	
EU register name	**CAS no**	**FLAVIS no**	**Mean**	**Range**
d-Limonene[a]	5989-27-5	01.045	41.6	37.5–59.2
β-Selinene	17066-67-0	–	17.1	11.4–27.7
Senkyunolide A	62006-39-7	–	14.3	5.74–20.8
β-Pinene (pin-2(10)-ene)	127-91-3	01.003	0.58	0.50–0.73
3-Butylphthalide	6066-49-5	10.025	10.16	3.30–15.2
α-Selinene	473-13-2	–	3.33	2.01–5.96
Amyl benzene	538-68-1	–	2.47	1.37–4.15
1-Pentyl-1,3-cyclohexadiene	76346-02-6	–	1.84	1.59–2.08
Valerophenone	1009-14-9	–	1.34	1.07–1.75
Sedanolide	6415-59-4	–	1.26	1.22–1.32
β-Eudesmol	473-15-4	–	1.12	1.07–1.16
β-Caryophyllene	87-44-5	01.007	0.88	0.28–1.90
Myrcene	123-35-3	01.008	0.65	0.54–0.78
1,8-Cineole	470-82-6	03.001	0.57	0.57[b]
5-Pentylcyclohexa-1,3-diene	56318-84-4	–	0.51	0.29–0.85
β-Caryophyllene epoxide	1139-30-6	16.043	0.46	0.20–0.63
(Z)-3-(Isobutylidene)phthalide	56014-87-0	–	0.42	0.31–0.49
α-Eudesmol	473-16-5	–	0.32	0.20–0.40
trans-Carveol	1197-07-5	–	0.22	0.09–0.31
$C_{12}H_{16}O_3$ (dihydrophtalide derivative)	–	–	0.21	0.16–0.32
3,7,10-Humulatriene	6753-98-6	01.043	0.19	0.11–0.23
Carvone	99-49-0	07.012	0.15	0.08–0.23
(-)-*trans*-Isopiperitenol	74410-00-7	–	0.14	0.12–0.15
4-Pentylphenol	14938-35-3	–	0.13	0.13–0.14
Ligustilide	4431-01-0	–	0.13	0.09–0.16
β-Phellandrene	555-10-2	01.055	0.13	0.12–0.13
cis-Carveol	1197-06-4	–	0.11	0.09–0.12
cis-*p*-2,8-Menthadien-1-ol	3886-78-0	–	0.11	0.09–0.13
8-*p*-Menthene-1,2-diol	1946-00-5	–	0.11	0.06–0.16
trans-Dihydrocarvone	5948-04-9	–	0.10	0.03–0.18
trans-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol	7212-40-0	–	0.10	0.07–0.14
(E)-3-Butylidenephthalide	76681-73-7	–	0.10	0.09–0.11
Total			96.7	95.9–98.7[c]

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

[a]Stereochemistry not specified, however, considering that the naturally occurring limonene is typically *d*-limonene, it is assumed that this form is also in celery seed oil.

[b]Detected in one batch only.

[c]The values given for the total are the lowest and the highest values of the sum of the components in the seven batches analysed.

The applicant performed a literature search (see Section 3.4) for information on the chemical composition of *A. graveolens* and its preparations and the presence of compounds of known concern.[18] Furocoumarins (including bergapten, xanthoxin and isopimpinellin) and flavonoids (graveobioside A and B, apiin, isoquercetin) in fruit of *A. graveolens* are reported as present in the EFSA Compendium (EFSA, 2012)[19] based on one reference (PDR for Herbal Medicines, 2004), and in several publications by the same authors (e.g. Garg et al., 1978, 1979). Two publications also reported the presence of myristicin, a *p*-allylalkoxybenzene structurally related to safrole, in celery seed oil. In the first publication, myristicin accounted for 0.18% of the % GC area of an essential oil containing 85% limonene, 3.68% *β*-selinene and 0.9% *β*-pinene (senkyunolide not present) (Kubesckka & Formacek, 2002). Myristicin was not detected in the oil under assessment (limit of detection, LOD 0.001%).

[18]Technical dossier/Supplementary information April 2022/Literature search_celery_seed_oil.

[19]https://www.efsa.europa.eu/en/microstrategy/botanical-summary-report.

Furocoumarins (psoralen, xanthoxin, isopimpinellin, bergapten and isoimperatorin) were analysed in six batches of celery seed oil (from two different suppliers). Only bergapten was detected in all batches of celery seed oil at concentration of about 0.001% (range: 0.0008%–0.0011%, maximum expected concentration 0.0015%). The other furocoumarins were below the corresponding LODs.[20]

Although the occurrence of *p*-mentha-1,8-dien-7-al [05.117] (hereinafter referred to as perillaldehyde) is not reported in the EFSA compendium or in the literature retrieved by the applicant,[21] low concentrations of perillaldehyde were detected as part of the full characterisation of celery seed oil. Perillaldehyde was detected in three batches of one of the two essential oils used as examples of the oil under assessment in concentration ranging from 0.016% to 0.025% (maximum expected concentration 0.05%). In the other oil, perillaldehyde was not detected (LOD, 0.011%).[22]

3.3.1.1 | *Impurities*

The applicant referred to the 'periodic testing' of some representative flavourings premixtures for mercury, cadmium, lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorus pesticides, aflatoxins (B1, B2, G1, G2) and ochratoxin A. However, no data have been provided on the presence of these impurities.

3.3.2 | Shelf-life

The typical shelf-life of celery seed oil is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light). However, no data supporting this statement were provided.

3.3.3 | Conditions of use

Celery seed oil is intended to be added to feed and water for drinking for all animal species without a withdrawal period. Maximum use levels in complete feed were proposed for the animal species and categories listed in Table 3. No use level has been proposed by the applicant for the use in water for drinking.

TABLE 3 Conditions of use for the essential oil from the fruit of *Apium graveolens* L.: Maximum proposed use levels in complete feed for the intended animal species and categories.

Animal category	Maximum use level (mg/kg complete feed)
Chickens for fattening	10
Laying hens	10
Turkeys for fattening	10
Piglets	20
Pigs for fattening	20
Sows	20
Calves (milk replacers)	15
Cattle for fattening	15
Dairy cows	15
Sheep/goats	15
Horses	20
Rabbit	10
Fish (salmon)	10
Dogs	20
Cats	20
Ornamental fish	10

[20]Technical dossier/Supplementary information April 2022/Annex_VIII_SIn_reply_celery_seed_oil_SOC_COA. Limits of detection (LODs): psoralen, xanthoxin and isopimpinellin, 2.5 mg/kg; isoimperatorin 5 mg/kg; xanthoxin, bergapten and isompinellin, 10 mg/kg.

[21]Technical dossier/Supplementary information April 2022/Literature search_Celery_seed_oil/Literature_search_summary_ Celery_Seed_Oil.

[22]Technical dossier/Supplementary information February 2024/20240223_Sin_FAD-2010-0221_request of clarification.

3.4 | Safety

The assessment of safety of celery seed oil is based on the maximum use levels in complete feed proposed by the applicant for the species listed above (Table 3).

No studies to support the safety for target animals, consumers and users were performed with the additive under assessment. The applicant carried out an extensive database search (no time limits) to identify data related to the chemical composition and the safety of preparations obtained from *A. graveolens*.[23] Four cumulative databases (LIVIVO, NCBI, OVID and ToxInfo), 13 single databases including PubMed and Web of Science and 12 publishers' search facilities including Elsevier, Ingenta, Springer and Wiley were used. The keywords used covered different aspects of safety and the inclusion and exclusion criteria were provided by the applicant.

Many of the individual components of the essential oil have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC); the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); and/or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The flavouring compounds currently authorised for feed[24] and/or food[25] use, together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[26] and the corresponding EFSA opinion are listed in Table 4.

TABLE 4 Flavouring compounds already assessed by EFSA and/or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 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).

CG	Chemical group	Product – EU register name (common name)	FLAVIS No	EFSA* or JECFA opinion, year
01	Straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes	Hexadecanoic acid	08.014	2013
05	Saturated and unsaturated aliphatic secondary alcohols, ketones and esters with esters containing secondary alcohols	Oct-1-en-3-ol	02.023	2020
		Oct-1-en-3-yl acetate	09.281	
		3-Octyl acetate[a]	09.254	WHO 2000 (JECFA)
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		4-Terpinenol	02.072	
		(–)-α-Elemol[a]	02.149	2011a, CEF 2015a, CEF
07	Primary alicyclic saturated and unsaturated alcohols/aldehydes/acids/acetals/esters with esters containing alicyclic alcohols	*p*-Mentha-1,8-dien-7-ol[a] (perillyl alcohol)	02.060	2017, CEF
		Myrtenyl acetate[a]	09.302	
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Carvone[a]	07.012	2014, SC
		d-Carvone	07.146	2016a
		Carvyl acetate[b]	09.215	
		Dihydrocarvone[a,c]	07.128	WHO 2000 (JECFA)
10	Secondary aliphatic saturated or unsaturated alcohols, ketones, ketals and esters with a second secondary or tertiary oxygenated functional group	4-Hydroxy-4-methylpentan-2-one[a]	07.165	2011b, CEF
11	Alicyclic and aromatic lactones	3-Butylidenephthalide	10.024	2009, CEF
		3-Butylphthalide	10.025	
13	Furanones and tetrahydrofurfuryl derivatives	Linalool oxide[d]	13.140	2012b
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012c, 2021b

[23]Technical dossier/Supplementary information April 2022/Literature_search_celery_seed_oil.

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

[25]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.

[26]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 1 80, 19.7.2000, p. 8.

TABLE 4 (Continued)

CG	Chemical group	Product – EU register name (common name)	FLAVIS No	EFSA* or JECFA opinion, year
25	Phenol derivatives containing ring-alkyl, ring-alkoxy and side chains with an oxygenated functional group	2-Methoxy-4-vinylphenol	04.009	2012d
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	1-Isopropyl-4-methylbenzene (*p*-cymene)	01.002	2015
		1-Isopropenyl-4-methylbenzene	01.010	
		γ-Terpinene	01.020	
		d-Limonene	01.045	
		Pin-2(10)-ene (*β*-pinene)	01.003	2016b
		Pin-2(3)-ene (*α*-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		α-Cedrene[a,e]	01.022	2011c, CEF
		3,7,10-Humulatriene[a,e]	01.043	
		β-Phellandrene[a,e]	01.055	
		4(10)-Thujene (Sabinene)[a]	01.059	2015b, CEF
32	Epoxides	*β*-Caryophyllene epoxide	16.043	2014, CEF

*FEEDAP opinion unless otherwise indicated.

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

[b]EFSA evaluated carvyl acetate [09.215] as a mixture of isomers (related to *(1R,5R)*-carvyl acetate or *cis*-l-carvyl acetate).

[c]JECFA evaluated dihydrocarvone [07.128] as a mixture of *cis*- and *trans*-dihydrocarvone (WHO, 2000).

[d]Linalool oxide [13.140]: A mixture of *cis*- and *trans*-linalool oxide (5-ring) was evaluated [13.140].

[e]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.

As shown in Table 4, a number of components of celery seed oil, accounting for about 56% of the % GC area, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food[27] without limitations and for use in feed[28] at individual use levels higher than those resulting from the intended use of the essential oil in feed. Linalool oxide has been assessed in CG 13 as a mixture of *cis*- and *trans*- isomers (EFSA FEEDAP Panel, 2012b).

Three compounds listed in Table 4, *α*-cedrene [01.022], 3,7,10-humulatriene [01.043] and *β*-phellandrene [01.055], have been evaluated in Flavouring Group Evaluation 25 Revision 2 (FGE25.Rev2) by applying the procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional subchronic toxicity data (EFSA CEF Panel, 2011c). In the absence of such toxicological data, the EFSA CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015b). As a result, these compounds are not authorised for use as flavours in food. In the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach or read-across from structurally related substances, following the approach recommended in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a).

Forty additional components have not been previously assessed for use as flavourings. The FEEDAP Panel notes that 16 of them[29] accounting for 27% of the GC area are aliphatic monoterpenes or sesquiterpenes structurally related to flavourings already assessed in CG 6, 8 and 31 and a similar metabolic and toxicological profile is expected. These lipophilic compounds are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2012a, 2015, 2016a, 2016b). Three additional compounds, *cis*- and *trans*-limonene epoxide, and humulene oxide are epoxides structurally related to *β*-caryophyllene epoxides in CG 32.

Two main constituents, senkyunolide A and 3-butylphthalide [10.025], and additional nine phthalide derivatives (sedanolide, *(Z)*-3-(isobutylidene)phthalide, ligustilide, *(Z)*-3-butylidenephthalide, 3-butyl hexahydrophthalide, a dihydrophthalide derivate, a tetrahydro derivative and two senkyunolide isomers) have been identified in celery seed oil and account

[27]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.

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

[29]β-Eudesmol and α-eudesmol (CG 6); *trans*-carveol, *cis*-carveol, *trans*-dihydrocarvone and *cis*-dihydrocarvone (CG 8); *(E,Z)*-2,4-dodecadiene, 1-pentyl-1,3-cyclohexadiene, 5-pentylcyclohexa-1,3-diene, *β*-elemene, 1,5,8-*p*-menthatriene, amyl benzene, *α*-curcumene, *β*-selinene, *α*-selinene and *γ*-selinene (CG 31).

together on average for 26.7% of the GC area. They are structurally related or identical to the authorised flavourings 3-butylphthalide [10.025], 3-propylidenephthalide [10.005] and 3-butylidenephthalide [10.024] (EFSA CEF Panel, 2009; EFSA FEEDAP Panel, 2012e, 2022). The information on the absorption, distribution, metabolism and excretion (ADME) is summarised in Section 3.4.1.

Celery seed oil contains perillaldehyde (average: 0.021%, range: 0.016%–0.025%), a substance for which EFSA identified a concern for genotoxicity (EFSA CEF Panel, 2015d, 2017), which was confirmed by JECFA (WHO, 2018). The applicant submitted a publication on the in vivo and in vitro mutagenicity of perillaldehyde (Honma et al., 2021), which was not available at the time of the previous assessments and is described below.

Bergapten (5-methoxypsoralen) occurs in celery seed oil only in trace amounts (see Section 3.3.1). For furocoumarins, reference is made to the safety evaluation of furocoumarins documented in the EFSA opinion on expressed lemon oil and its fractions and on lime oil (EFSA FEEDAP Panel, 2021b).

The following sections focus on the ADME and on the toxicology of perillaldehyde, bergapten, phthalide derivatives and the other 11 compounds[30] not assessed for use in food, based on the information provided by the applicant in the form of literature searches and quantitative structure–activity relationship (QSAR) analysis.

3.4.1 | Absorption, distribution, metabolism and excretion

3.4.1.1 | *Perillaldehyde*

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

3.4.1.2 | *Bergapten*

The ADME of furocoumarins including bergapten has been reviewed by the FEEDAP Panel in the opinion on lemon oil and its fractions and lime oil (EFSA FEEDAP Panel, 2021b).

Furocoumarins are extensively biotransformed predominantly in the liver via cytochrome P450 (CYP)-dependent monooxygenases and excreted mainly in urine. The main biotransformation pathways are epoxidation, hydroxylation, glucuronide conjugation and hydrolytic opening of the lactone ring.

3.4.1.3 | *Phthalides*

ADME data available for 3-propylidenephthalide indicate that the γ-lactone is hydrolysed in vivo in mammals to 2-(2-hydroxyalkyl)benzoic acid which may be excreted directly, or the side chain oxygenated functional group (alcohol or enolic alcohol) may be oxidised (alcohol) or reduced (enol). The reduced form is subsequently conjugated and excreted. The benzoic acid moiety may conjugate with glycine or other amino acids (e.g. ornithine in birds) and be excreted mainly as the hippurate, while the ketone function may be reduced to the corresponding alcohol and excreted as the glucuronic acid conjugate (EFSA FEEDAP Panel, 2012e). A similar pathway is expected for *(Z)*-3-(isobutylidene)phthalide and *(E)*-3-butylidenephthalide.

Pharmacokinetic studies are available for senkyunolide A (Yan et al., 2007) and for *(Z)*-ligustilide (Yan et al., 2008). After oral administration to rats, senkyunolide A was rapidly absorbed, but showed a low bioavailability (8%) (Yan et al., 2007). After 100 mg/kg senkyunolide A oral administration, plasma c_{max} was 1.66 μg/mL at a t_{max} of 0.21 h, being $t_{1/2}$ of 0.52 h. In vitro assays performed in simulated gastric and intestinal fluids indicated that senkyunolide A was unstable, resulting in more than 60% loss of the compound, this being partly responsible for its low bioavailability. In S9 and microsomes from rat liver, senkyunolide A was partly biotransformed, pointing to a role of first-pass metabolism of the compound, also contributing to its low bioavailability. In the in vivo study, five minor metabolites were detected in plasma, although only for 3-butylphthalide the structure was unequivocally established by HPLC coupled with mass spectrometry (HPLC-MS). Two metabolites were tentatively identified as 11-hydroxysenkyunolide A and 11-hydroxy-3-butylphthalide, and two as glutathione and cysteine conjugates of 7-hydroxysenkyunolide A. The three non-conjugated metabolites were also identified in vitro.

(Z)-Ligustilide was rapidly absorbed by rats after oral administration although the bioavailability was very low (2.6%) (Yan et al., 2008). After oral administration of 500 mg/kg body weight, plasma C_{max} was 0.66 μg/mL at a t_{max} of 0.36 h, with $t_{1/2}$ of 3.43 h. In vitro, extensive biotransformation of *(Z)*-ligustilide was observed in S9 and microsomes of rat liver (more than 90%), pointing to an extensive first pass metabolism. No degradation was observed in simulated gastric and intestinal fluids. Eight metabolites of *(Z)*-ligustilide were detected in vitro, three of which were unequivocally characterised

[30] cis-*p*-2,8-menthadien-1-ol, *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, 1,8-*p*-menthadien-4-ol, 8-*p*-menthene-1,2-diol, *trans*-*p*-mentha-1(7),8-dien-2-ol, 1-(4-methylcyclohex-3-en-1-yl)ethenone, (–)-*trans*-isopiperitenol, pinocarvyl acetate, valerophenone, 4-pentylphenol and limonene 8,9-epoxide.

by HPLC-MS as 3-butylidenephthalide, senkyunolide I and senkyunolide H. 11-Hydroxyligustilide and two isomers of hydroxyligustilide glutathione conjugate were tentatively identified. In vivo, among several putative metabolites detected in plasma, eight were common to those formed in vitro.

Overall, the available evidence indicates that both senkyunolide A and *(Z)*-ligustilide are poorly absorbed, extensively metabolised, mainly by oxidative pathway, forming hydroxyl compounds that are presumed to be conjugated and rapidly excreted. Some metabolites are themselves constituents of the oil (3-butylphthalide, 3-butylidenephthalide, senkyunolide I and senkyunolide H). These compounds, being hydroxylated, are expected to be conjugated and excreted.

3.4.2 | Genotoxicity

For fully defined mixtures, the EFSA Scientific Committee (EFSA SC) recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential using all available information, including read-across and QSAR considerations about their genotoxic potential (EFSA Scientific Committee, 2019b). Therefore, the potential genotoxicity of identified constituents is first considered.

3.4.2.1 | *Perillaldehyde*

The EFSA CEF Panel concluded that perillaldehyde raises a concern for genotoxicity based on the positive results observed in vivo with a Comet assay performed in liver of male rats. In the present opinion, the FEEDAP Panel evaluated a publication provided by the applicant (Honma et al., 2021), which was not available at the time the CEF Panel evaluated perillaldehyde (EFSA CEF Panel, 2015d, 2017).

In the publication by Honma et al. (2021), it is reported an Ames test performed with perillaldehyde using Salmonella Typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and *Escherichia* coli strain WP2 uvrA in the presence and absence of metabolic activation. According to the applicant, the good laboratory practice (GLP) study followed the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 471 (1997). Eight perillaldehyde concentrations were tested (i.e. 9.77, 19.5, 39.1, 78.1, 156, 313, 625 and 1250 µg/plate) applying the preincubation method. Cytotoxicity was observed at 313 µg/plate and above in the presence and absence of metabolic activation. No increase in the number of revertant colonies was observed at any concentration tested in any strain and experimental condition (Honma et al., 2021). The FEEDAP Panel concluded that perillaldehyde did not induce gene mutations in bacterial cells under the experimental conditions applied in this study.

An in vivo mutagenicity study was also performed applying the transgenic rodent somatic cell gene mutation assay in compliance with OECD TG 488 (2020). The study was performed in 9-week-old male Muta™ Mouse (CD2-LacZ80/HazfBR) administered perillaldehyde by oral gavage at 250, 500, 1000 mg/kg per day for 28 consecutive days. Mutation frequency (MF) was estimated via the lacZ positive selection method in liver and glandular stomach. The positive control ethyl nitrosourea (ENU) significantly increased MF in liver and glandular stomach confirming the sensitivity of the test system. MFs were comparable between treated and vehicle control groups. The FEEDAP Panel concluded that perillaldehyde did not induce gene mutations in vivo in liver and glandular stomach of transgenic rodents under the experimental conditions applied in the study.

The FEEDAP Panel noted the discrepancy between the positive results obtained in vivo with the Comet assay in liver, reported in the CEF opinions, and the negative outcomes of the TGR assay (Honma et al., 2021) and emphasised that the TGR assay detects gene mutations, while the Comet assay reveals primary DNA lesions such as single and double strand breaks that may give rise to gene mutations as well as to chromosome aberrations. Therefore, a negative TGR assay does not necessarily overrule the results of a positive in vivo Comet assay. On this basis, the FEEDAP Panel concluded that the publication by Honma et al. (2021) does not give reason to modify the conclusion drawn on the genotoxicity of perillaldehyde (*p*-mentha-1,8-dien-7-al) by the CEF Panel (EFSA CEF Panel, 2015d, 2017).

3.4.2.2 | *Bergapten*

Bergapten (5-methoxypsoralen) is the most potent furocoumarin congener. The genotoxicity and carcinogenicity of bergapten and furocoumarins in general have been reviewed by the FEEDAP Panel in the opinion on lemon oil and its fractions and on lime oil (EFSA FEEDAP Panel, 2021b).

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 such as bergapten 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.

3.4.2.3 | *Other compounds*

The genotoxic potential for 11 compounds (cis-*p*-2,8-menthadien-1-ol, *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, 1,8-*p*-menthadien-4-ol, 8-*p*-menthene-1,2-diol, *trans*-*p*-mentha-1(7),8-dien-2-ol, 1-(4-methylcyclohex-3-en-1-yl) ethenone, (-)-*trans*-isopiperitenol, pinocarvyl acetate, valerophenone, 4-pentylphenol and limonene 8,9-epoxide) was

predicted by the applicant using the QSAR Toolbox. No alerts were identified for in vitro mutagenicity (Ames test), for genotoxic and non-genotoxic carcinogenicity and for other toxicity endpoints for 1-(4-methylcyclohex-3-en-1-yl)ethenone and valerophenone. Structural alerts for mutagenicity for *cis*-*p*-2,8-menthadien-1-ol, *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, 1,8-*p*-menthadien-4-ol, *trans*-*p*-mentha-1(7),8-dien-2-ol and (–)-*trans*-isopiperitenol were due to the presence of the vinyl/allyl alcohol group, while for pinocarvyl acetate alerts were due to the presence of a vinyl/allyl ester group. For the other three compounds, structural alerts were due to the presence of 'H acceptor-path 3-H acceptor' (8-*p*-menthene-1,2-diol), phenol group 4-pentyl phenol or of an epoxide (limonene 8,9-epoxide). For these substances, predictions of Ames mutagenicity (with and without S9) were made by 'read-across' analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Read-across-based predictions were found to be consistently negative for all categories of analogues. On this basis, the alerts raised were discounted.[31]

The genotoxicity of (+)-limonene epoxide, investigated in the Ames test and the SOS Chromotest, gave negative results (Basler et al., 1989 as referenced in EFSA CEF Panel, 2014). When V79 Chinese hamster cells were incubated with (+)-limonene epoxide, no increase in sister chromatid exchange was observed (von der Hude et al., 1991, as referenced in EFSA CEF Panel, 2014). No genotoxic concern is therefore expected for *cis*-limonene epoxide and *trans*-limonene epoxide.

3.4.3 | Safety for the target species

Tolerance studies with the target species and/or toxicological studies in laboratory animals 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 and that 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 (EFSA Scientific Committee, 2019a).

As the additive under assessment is a fully defined mixture (the identified components represent about 98% of the % GC area, see Section 3.3.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the components of the essential oil, except for perillaldehyde and bergapten, which is assessed separately. For perillaldehyde and bergapten, the available data set do not allow to identify a reference point for the risk assessment or to derive a safe level. Therefore, the assessment of the safety for target species is based on the comparison between the intake of perillaldehyde and bergapten via the consumption of citrus by-products, commonly used as feed material, and that via the use of celery seed oil as a feed additive. For citrus by-products, data on the occurrence of perillaldehyde and furocoumarins are available and have been evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2021b).

3.4.3.1 | *Components other than perillaldehyde and bergapten*

Based on considerations related to structural and metabolic similarities, the components were allocated to 11 assessment groups, corresponding to the chemical groups (CGs) 1, 5, 6, 7, 8, 10, 11, 13, 16, 21, 25, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000.[32] For chemical group 31 ('aliphatic and aromatic hydrocarbons'), subassessment groups as defined in FGE.25 and FGE.78 were established (EFSA CEF Panel, 2015a, 2015b). The allocation of the components to the (sub-)assessment groups is shown in Table 5 and in the corresponding footnote.

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 (bw) are used to express exposure in terms of mg/kg bw. 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 (Cramer et al., 1978). For some components in the assessment group, toxicological data were available to derive no observed adverse effect levels (NOAELs). Structural and metabolic similarity among the components in the assessment groups was evaluated to explore the application of read-across. If justified, extrapolation from a known NOAEL of components of an assessment group to the other components of the group with no available NOAEL was made. If sufficient evidence is available for the members of a (sub)assessment group, a (sub)assessment group NOAEL was derived.

[31]Technical dossier/Supplementary information April 2022/Annex VI_SIn_reply_celery_seed_oil_QSAR.

[32]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.

Toxicological data of subchronic studies, from which NOAEL values could be derived, were available for several compounds in CG 1 (EFSA FEEDAP Panel, 2013), for octen-3-one [07.081], the representative compound in CG 5 (EFSA FEEDAP Panel, 2020), linalool [02.013] and terpineol [02.230][33] in CG 6 (EFSA FEEDAP Panel, 2012a), *d*-carvone [07.146] in CG 08 (EFSA Scientific Committee, 2014), 1,8-cineole in CG 16 (EFSA FEEDAP Panel, 2021b), *d*-limonene [01.045], *p*-cymene [01.002], myrcene [01.008] and *β*-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016b), and *β*-caryophyllene oxide in CG 32 (EFSA CEF Panel, 2014).

For CG 1 compounds, a group NOAEL of 120 mg/kg bw was derived from the toxicological data available and extrapolated to hexadecanoic acid [08.014]. Similarly, the NOAEL of 6.7 mg/kg bw per day for oct-1-en-3-one [07.081] was extrapolated to oct-1-en-3-ol [02.023] and oct-1-en-3-yl acetate [09.281].

For the subgroup of terpinyl derivatives in CG 6, i.e. *α*-terpineol [02.072], 4-terpinenol [02.072], *β*-eudesmol and *α*-eudesmol, the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [02.230]. An uncertainty factor (UF) of 2 was applied to the NOAEL of 250 mg/kg bw per day to take into account the short duration (35 days) of the study with terpineol (EFSA FEEDAP Panel, 2012a).

For the subgroup of carvyl derivatives in CG 8, i.e. *trans*- and *cis*-dihydrocarvone, *trans*- and *cis*-carveol and carvyl acetate [09.215], the reference point was selected based on the benchmark dose (BMD) lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 60 mg/kg bw per day available for *d*-carvone [07.146].

For CG 11, the FEEDAP Panel notes that a NOAEL of 5.42 mg/kg bw per day was identified by JECFA for 3-propylidenephthalide [10.003] and was used by the FEEDAP Panel in the assessment of compounds in CG 11 (EFSA FEEDAP Panel, 2012e). The FEEDAP Panel already applied this NOAEL as group NOAEL for this assessment group (EFSA FEEDAP Panel, 2022).

The NOAELs of 250, 154 and 222 mg/kg bw per day for the representative compounds of CG 31, *d*-limonene [01.045], *p*-cymene [01.002] and *β*-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within subassessment groups III (*β*-elemene, *β*-phellandrene and γ-terpinene), IVe (*α*-curcumene) and V (*β*-selinene, *α*-selinene, *α*-pinene, *β*-pinene, sabinene γ-selinene and *α*-cedrene) (EFSA CEF Panel, 2015a, 2015b).

Read-across was also applied from *β*-caryophyllene [01.007] to 3,7,10-humulatriene [01.043] in CG 31, VI. For this compound, an UF of 2 was applied to the NOAEL of 222 mg/kg bw per day for *β*-caryophyllene [01.007] to take into account the uncertainty in read-across (EFSA FEEDAP Panel, 2023b).

The NOAEL of 109 mg/kg bw per day for *β*-caryophyllene epoxide [16.043] was extrapolated to humulene oxide II in CG 32.

For the remaining compounds,[34] toxicity studies performed with the compounds under assessment and NOAEL values derived from toxicity studies were not available and read-across was not possible. Therefore, the threshold of toxicological concern (TTC) approach was applied (EFSA FEEDAP Panel, 2017b; EFSA Scientific Committee, 2019c).

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, Munro et al., 1996). 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, 2019b, 2019c). An MOE(T) > 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. They are listed in the footnote.[35]

The approach to the safety assessment of celery seed oil 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 10 mg/kg complete feed.

[33]Terpineol is a mixture of four isomers: *α*-terpineol [02.014], a mixture of *(R)*-(+)-*α*-terpineol and *(S)*-(−)-*α*-terpineol, *β*-terpineol, *γ*-terpineol and 4-terpinenol [02.072] (or *δ*-terpineol). The specification for terpineol [02.230] covers *α*-, *β*-, *γ* and *δ*-terpineol. Composition of mixture: 55%–75% *α*-terpineol, 16%–23% *γ*-terpineol, 1%–10% cis-*β*-terpineol, 1%–13% trans-*β*-terpineol and 0%–1% *δ*-terpineol (EFSA CEF Panel, 2015c) FGE.18Rev 3.

[34]3-Octyl acetate, (−)-α-elemol, 8-*p*-menthene-1,2-diol, *trans*-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol, *cis*-*p*-2,8-menthadien-1-ol, 1,8-*p*-menthadien-4-ol, myrtenyl acetate, *p*-mentha-1,8-dien-7-ol, (−)-*trans*-isopiperitenol, pinocarvyl acetate, *trans*-*p*-mentha-1(7),8-dien-2-ol, 1-(4-methylcyclohex-3-en-1-yl)ethenone, 4-hydroxy-4-methylpentan-2-one, valerophenone, 1-pentyl-1,3-cyclohexadiene, 5-pentylcyclohexa-1,3-diene, 1,5,8-*p*-menthatriene, amyl benzene, *trans*-limonene epoxide, limonene 8,9-oxide and *cis*-limonene epoxide.

[35]Compounds included in the assessment groups but not reported in the table: hexadecenoic acid (CG 1); α-eudesmol, linalool, α-terpineol and 4-terpinenol (CG 6); *cis*-carveol and *cis*-dihydrocarvone (CG 8); 2-methoxy-4-vinylphenol (CG 25); *(E,Z)*-dodecadiene (CG 31, II); *β*-elemene, *β*-phellandrene and γ-terpinene (CG 31, III); α-curcumene, *p*-cymene and 1-isopropenyl-4-methylbenzene (CG 31, IVe); α-pinene, sabinene, γ-selinene and α-cedrene (CG 31, V); 3,7,10-humulatriene (CG31, VI); humulene oxide II (CG 32).

TABLE 5 Compositional data, intake values (calculated for chickens for fattening at 10 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of celery seed oil classified according to assessment groups, and combined margin of exposure (MOET) for each assessment group.

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	FLAVIS-No	Highest conc. in the oil	Highest feed conc.	Intake[a]	Cramer Class[b]	NOAEL/ $BMDL_{10}$[c]	MOE	MOET
Constituent	–	%	mg/kg	mg/kg bw/day	–	mg/kg bw/day	–	–
CG 3								
Oct-1-en-3-yl-acetate	09.281	0.04	0.004	0.0003	(I)	6.7	21,324	
Oct-1-en-3-ol	02.023	0.02	0.002	0.0002	(I)	6.7	35,539	
3-Octyl acetate	09.254	0.02	0.002	0.0001	I	*3*	22,278	
MOET CG 3								8339
CG 6								
β-Eudesmol	–	1.16	0.116	0.0104	(I)	125[d]	12,014	
α-Eudesmol	–	0.40	0.040	0.0036	(I)	125[d]	34,551	
(–)-*α*-Elemol	02.149	0.21	0.021	0.0019	I	*3*	1607	
8-*p*-Menthene-1,2-diol	–	0.16	0.016	0.0014	II	*0.91*	642	
trans-1-Methyl-4-(1-methyl vinyl) cyclohex-2-en-1-ol	–	0.14	0.014	0.0013	I	*3*	2353	
cis-*p*-2,8-Menthadien-1-ol	–	0.13	0.013	0.0011	I	*3*	2652	
1,8-*p*-Menthadien-4-ol	–	0.04	0.004	0.0004	I	*0.15*	380	
MOET CG 6								175
CG 7								
Myrtenyl acetate	09.302	0.11	0.011	0.0010	I	*3*	3094	
p-Mentha-1,8-dien-7-ol	02.060	0.03	0.003	0.0003	I	*3*	11,139	
MOET CG 7								2422
CG 8								
trans-Carveol	–	0.31	0.031	0.0027	(I)	60	21,913	
Carvone	07.012	0.23	0.023	0.0020	(II)	**60**	29,705	
(–)-*trans*-Isopiperitenol	–	0.15	0.015	0.0013	I	*3*	2289	
trans-Dihydrocarvone	–	0.18	0.018	0.0016	(II)	60	36,926	
Pinocarvyl acetate	–	0.07	0.007	0.0006	I	*3*	4988	
Carvyl acetate	09.215	0.13	0.013	0.0012	(I)	60	50,633	
trans-*p*-Mentha-1(7),8-dien-2-ol	–	0.06	0.006	0.0005	I	*3*	5863	
1-(4-Methylcyclohex-3-en-1-yl)ethanone	–	0.05	0.005	0.0004	I	*3*	6962	
MOET CG 8								907
CG 10								
4-Hydroxy-4-methylpentan-2-one	07.165	0.05	0.005	0.0004	I	*3*	6820	
CG 11								
Senkyunolide A	–	20.77	2.077	0.1864	(III)	5.42	29	
3-Butylphthalide	10.025	15.18	1.518	0.1363	(III)	5.42	40	
Sedanolide	–	1.32	0.132	0.0118	(III)	5.42	459	
(Z)-3-(Isobutylidene) phthalide	–	0.49	0.049	0.0044	(III)	5.42	1230	
Dihydrophthalide derivative ($C_{12}H_{16}O_3$)	–	0.32	0.032	0.0029	(III)	5.42	1893	
Ligustilide	–	0.16	0.016	0.0014	(III)	5.42	3773	
(E)-3-Butylidenephthalide	–	0.11	0.011	0.0009	(III)	5.42	5750	
3-Butyl hexahydrophthalide	–	0.10	0.010	0.0009	(III)	5.42	6289	

TABLE 5 (Continued)

Essential oil composition			Exposure		Hazard characterisation		Risk characterisation	
Assessment group	**FLAVIS-No**	**Highest conc. in the oil**	**Highest feed conc.**	**Intake[a]**	**Cramer Class[b]**	**NOAEL/ $BMDL_{10}$[c]**	**MOE**	**MOET**
Tetrahydrophthalide derivative ($C_{16}H_{26}O_3$)	–	0.06	0.006	0.0005	(III)	5.42	10,592	
Senkyunolide isomer 1	–	0.05	0.005	0.0005	(III)	5.42	11,611	
Senkyunolide isomer 2	–	0.04	0.004	0.0003	(III)	5.42	16,317	
MOET CG 11								16
CG 13								
cis-Linalool oxide	–	0.02	0.002	0.0002	II	*0.91*	5963	
trans-Linalool oxide	–	0.02	0.002	0.0001	II	*0.91*	6335	
								3072
CG 16								
1,8-Cineole	03.001	0.57	0.057	0.0051	(II)	**100**	19,715	
CG 21								
Valerophenone	–	1.75	0.175	0.0157	I	3	191	
CG 25								
4-Pentylphenol	–	0.14	0.014	0.0013	I	3	2321	
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	0.78	0.078	0.0070	(I)	**44**	6308	
CG 31, III (Cyclohexene hydrocarbons)								
d-Limonene	01.001	59.21	5.921	0.5316	(I)	**250**	470	
1-Pentyl-1,3-cyclohexadiene	–	2.08	0.208	0.0187	I	*3*	160	
5-Pentylcyclohexa-1,3-diene	–	0.85	0.085	0.0076	I	*3*	395	
β-Elemene	–	0.18	0.018	0.0016	(I)	250	1899	
1,5,8-*p*-menthatriene	–	0.02	0.002	0.0001	I	*3*	20,886	
MOET CG 31, III								91
CG 31, IVe (Benzene hydrocarbons, alkyl)								
Amyl benzene	–	4.15	0.415	0.0372	I	*3*	81	
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
β-Selinene	–	17.14	2.767	0.2484	(I)	222	894	
α-Selinene	–	3.33	0.596	0.0535	(I)	**222**	4150	
β-Caryophyllene	01.007	0.88	0.190	0.0171	(I)	222	13,008	
β-Pinene	01.003	0.58	0.073	0.0065	(I)	222	34,015	
MOET CG 31, V								678
CG 32 (Epoxides)								
β-Caryophyllene epoxide	16.043	0.63	0.063	0.0056	(I)	**109**	19,334	
trans-Limonene epoxide	–	0.09	0.009	0.0008	I	*3*	3555	
Limonene 8,9-oxide	–	0.06	0.006	0.0005	III	*0.15*	278	
cis-Limonene epoxide	–	0.03	0.003	0.0003	I	*3*	9829	
MOET CG 32								248

[a]Intake calculations for the individual components are based on the use level of 10 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (NOAEL) to the intake. The combined margin of exposure (MOET) is calculated for each assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

[b]When a NOAEL value is available or read-across is applied, the allocation to the Cramer class is put into parentheses.

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

[d]An uncertainty factor of 2 was applied to the NOAEL of 250 mg/kg bw per day for terpineol (short duration of the study).

As shown in Table 5, for several assessment groups, the MOE(T) was < 100 at the proposed use levels of the additive. The lowest MOET was calculated for CG 11. From the lowest MOET of 16 in chickens for fattening, the MOET for CG 11 was calculated for the other target species considering the respective daily feed intake/kg bw and conditions of use. The results are summarised in Table 6.

TABLE 6 Combined margin of exposure (MOET) for the assessment group CG 11 (Phthalides) calculated for the different target animal categories at the proposed use level and maximum safe use levels in feed calculated to ensure a MOET ≥ 100 (500 for cats).

Animal category	Daily feed intake (g DM/kg bw)	Proposed use level (mg/kg complete feed)[a]	Lowest MOET CG 11	Maximum safe use level (mg/kg complete feed)[a,b]
Chickens for fattening	79	10	16	1.6
Laying hens	53	10	23	2.3
Turkeys for fattening	59	10	21	2.1
Piglets	44	20	14	2.8
Pigs for fattening	37	20	17	3.3
Sows lactating	30	20	21	4.1
Veal calves (milk replacer)	19	15	43	6.5
Cattle for fattening	20	15	41	6.2
Dairy cows	31	15	27	4.0
Sheep/goats	20	15	41	6.2
Horses	20	20	31	6.2
Rabbits	50	10	25	2.5
Salmon	18	10	68	6.8
Dogs	17	20	36	7.2
Cats[b]	20	20	31	1.2
Ornamental fish	5	10	246	–[c]

[a]Complete feed containing 88% DM, milk replacer 94.5% DM.

[b]The MOET for cats is increased to 500 because of the reduced capacity of glucuronidation.

[c]For the species for which the MOET is > 100, the proposed use level is considered safe.

At the proposed use levels, the MOET was below the value of 100 for all species except ornamental fish. For the other species, the maximum safe use levels in feed were calculated in order to ensure an MOET ≥ 100. Because glucuronidation is an important metabolic pathway to facilitate the excretion of the components of the essential oil and considering that cats have a low capacity for glucuronidation (Court & Greenblatt, 1997; Lautz et al., 2021), the use of celery seed oil as additive in cat feed needs a wider margin of exposure. An MOET of 500 is considered adequate.

The maximum use level proposed by the applicant of 10 mg/kg complete feed for ornamental fish is considered safe (without considering the presence of perillaldehyde and bergapten). For the other species, the calculated maximum safe levels in feed (without considering the presence of perillaldehyde and bergapten) are shown in Table 6. These levels are extrapolated to physiologically related minor species. For the other species not considered, the lowest value of 1.2 mg/kg complete feed is applied.

3.4.3.2 | *Perillaldehyde*

Low concentrations of perillaldehyde were detected in four out of seven batches of the additive under assessment (average: 0.021%, range: 0.016%–0.025%). The highest intake estimates of perillaldehyde for the target species were calculated considering the highest analysed value of perillaldehyde in the essential oil (0.025%) and the maximum safe use levels for the different target species (without considering perillaldehyde and bergapten, Table 6). The results are reported in Table 7.

TABLE 7 Target animal intake of perillaldehyde (as µg/kg bw per day) at the maximum safe use levels for the different target species calculated considering the highest analysed value of perillaldehyde in celery seed oil (0.025%).

Animal category	Daily feed intake (g DM/kg bw)	Maximum safe use level (mg/kg complete feed)[a,b]	Perillaldehyde intake (µg/kg bw per day)[a]
Chickens for fattening	79	1.6	0.036
Laying hens	53	2.3	0.035
Turkeys for fattening	59	2.1	0.035
Piglets	44	2.8	0.035
Pigs for fattening	37	3.3	0.034
Sows lactating	30	4.1	0.035
Veal calves (milk replacer)	19	6.5	0.033
Cattle for fattening	20	6.2	0.035

TABLE 7 (Continued)

Animal category	Daily feed intake (g DM/kg bw)	Maximum safe use level (mg/kg complete feed)[a,b]	Perillaldehyde intake (μg/kg bw per day)[a]
Dairy cows	31	4.0	0.035
Sheep/goats	20	6.2	0.035
Horses	20	6.2	0.035
Rabbits	50	2.5	0.036
Salmon	18	6.8	0.034
Dogs	17	7.2	0.034
Cats	20	1.2	0.007
Ornamental fish	5	10	0.013

[a]Complete feed containing 88% DM, milk replacer 94.5% DM.
[b]Without considering bergapten and perillaldehyde.

The use of celery seed oil would result in a daily intake of perillaldehyde ranging from 0.007 to 0.036 μg/kg bw.

Perillaldehyde occurs in feed materials rich in *d*-limonene, such as celery seeds and citrus by-products. For citrus by-products, there is evidence that they are used in diets of most of the target species at different concentrations depending on the target species. As comparable evidence is not available for celery seeds, the FEEDAP Panel considers that perillaldehyde intake from feed materials can be estimated based on the data available for citrus by-products. According to the information submitted by the applicant and reviewed by the FEEDAP Panel in the assessment of expressed lemon oil and its fractions and on lime oil, the concentrations of citrus by-products in the diets of target animals range from 5% up to 30% in ruminants (EFSA FEEDAP Panel, 2021b). Taking into account an inclusion level of 10% citrus by-products for poultry and 20% for the other species and considering the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b), the daily intake of citrus by-products has been estimated to be 7.9 g dry matter (DM)/kg bw for poultry, 8.8 g DM/kg bw for pigs, 6.2 g DM/kg bw for ruminants, 4 g DM/kg bw for horses, 10 g DM/kg bw for rabbits and 3.6 g DM/kg bw for fish. For the current assessment, the applicant submitted additional evidence showing that citrus by-products are also used in feed for dogs at about 10% (Brambillasca et al., 2013; Pacheco et al., 2021), resulting in a daily intake of citrus by-products of 1.7 g DM/kg bw for dogs.

Considering the literature data provided by the applicant on the occurrence of perillaldehyde in citrus peel[36] and assuming that citrus peel represents 62.5% of citrus by-product[37] (Bampidis & Robinson, 2006), the FEEDAP Panel estimated an average content of 0.0004% perillaldehyde in citrus by-products[38] (EFSA FEEDAP Panel, 2021b). Based on citrus by-product intake (see above), the daily intake of perillaldehyde via feed was calculated to be 32 μg/kg bw for poultry, 36 μg/kg bw for pigs, 24 μg/kg bw for ruminants, 16 μg/kg bw for horses, 40 μg/kg bw for rabbits and 14 μg/kg bw for fish (EFSA FEEDAP Panel, 2021b). For dogs, the estimated daily intake of perillaldehyde from citrus by-products is 6.8 μg/kg bw.

These concentrations are 250- to 1100-fold higher than those resulting from the use of celery seed oil at the concentrations in feed considered safe (without considering perillaldehyde) for the different target species (ranging from 1.2 to 10 mg/kg complete feed, see Section 3.4.3.1). Therefore, based on this comparison (EFSA FEEDAP Panel, 2021b), for these species, the use of celery seed oil is considered of no concern.

For the remaining species, cats and ornamental fish, the FEEDAP Panel applied the threshold of toxicological concern (TTC) concept. The intake of perillaldehyde arising from the use of the additive was compared with the threshold of the TTC of 0.0025 μg/kg bw per day established for potential DNA-reactive mutagens and/or carcinogens in human risk assessment (EFSA Scientific Committee, 2019c).

At the use levels of 1.2 and 10 mg/kg complete feed for cats and ornamental fish, respectively (see Table 6), the perillaldehyde intake would result in 0.007 and 0.013 μg/kg bw per day. These levels are above the threshold of 0.0025 μg/kg bw per day. Therefore, no conclusion can be drawn on the safety of celery seed oil which contains perillaldehyde for cats and ornamental fish.

3.4.3.3 | *Bergapten*

The highest intake estimates of bergapten for the target species were calculated considering the highest analysed value of bergapten in the essential oil (0.0011%) and the maximum safe use levels for the different target species (without considering perillaldehyde and bergapten, Table 6). The results are reported in Table 8.

The use of celery seed oil at the concentrations in feed considered safe (without considering bergapten and perillaldehyde) would result in an intake of bergapten ranging from 0.0003 and 0.0016 μg/kg bw per day.

[36]Calculated considering the occurrence of perillaldehyde in citrus peel (e.g. 0.0004% for mandarins and lemons, and 0.001% for oranges according to Qadir et al., 2018, Kamal et al., 2011, Bourgou et al., 2012, as referenced in EFSA FEEDAP Panel, 2021b).
[37]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.
[38]Occurrence of perillaldehyde in citrus by-products calculated considering the composition of citrus by-products as 60% oranges, 20% lemon and lime, 30% mandarin: 0.0006% × 0.6 + 0.0002 × 0.3 + 0.0002 × 0.1 = 0.0004%.

TABLE 8 Target animal intake of bergapten (as µg/kg bw per day) at the maximum safe use levels for the different target species calculated considering the highest analysed value of bergapten in celery seed oil (0.0011%).

Animal category	Daily feed intake (g DM/kg bw)	Maximum safe use level (mg/kg complete feed)[a,b]	Bergapten intake (µg/kg bw per day)[a]
Chickens for fattening	79	1.6	0.0016
Laying hens	53	2.3	0.0015
Turkeys for fattening	59	2.1	0.0015
Piglets	44	2.8	0.0015
Pigs for fattening	37	3.3	0.0015
Sows lactating	30	4.1	0.0015
Veal calves (milk replacer)	19	6.5	0.0014
Cattle for fattening	20	6.2	0.0016
Dairy cows	31	4.0	0.0015
Sheep/goats	20	6.2	0.0016
Horses	20	6.2	0.0016
Rabbits	50	2.5	0.0016
Salmon	18	6.8	0.0015
Dogs	17	7.2	0.0015
Cats	20	1.2	0.0003
Ornamental fish	5	10	0.0006

[a]Complete feed containing 88% DM, milk replacer 94.5% DM.
[b]Without considering bergapten and perillaldehyde.

Furocoumarins and methoxycoumarins occur in citrus by-products. The literature data provided by the applicant on the occurrence of furocoumarins and methoxycoumarins in citrus peel, citrus pulp and citrus seeds[39] was reviewed in the assessment of expressed lemon oil and its fractions and on lime oil (EFSA FEEDAP Panel, 2021b). Based on the data provided, the occurrence of furocoumarins and methoxycoumarins in lemon by-products was estimated to be 0.0018%.[40] 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 rabbits, 3.6 g DM/kg bw for fish and 1.7 g DM/kg bw for dogs), 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, 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 (EFSA FEEDAP Panel, 2021b). For dogs, the estimated daily intake of bergapten from citrus by-products is 30.6 µg/kg bw.

These intakes are several orders of magnitude higher than those resulting from the use of celery seed oil at the concentrations in feed considered safe for the different target species (ranging from 1.2 to 10 mg/kg complete feed, see Section 3.4.3.1). The FEEDAP Panel also notes that these intakes are below the phototoxicity threshold dose (0.25 mg/kg bw) established from human studies.

For cats and ornamental fish, which are not normally exposed to citrus by-products, no conclusion can be drawn.

Use in water for drinking

No specific proposals have been made by the applicant for the use level in water for drinking. The FEEDAP Panel considers that the use in water for drinking is of no concern provided that the total daily intake of the additive does not exceed the daily amount that is considered of no concern when consumed via feed.

3.4.3.4 | *Conclusions on safety for the target species*

The FEEDAP Panel concludes that the levels of celery seed oil summarised in Table 9 are considered of no concern.

For cats, ornamental fish and other species not mentioned in the table, no conclusion can be drawn.

[39]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 0012% 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) (for details, see EFSA FEEDAP Panel, 2021b).

[40]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% × 0.625 + 0 × 32.5 + 0.0001 × 0.05).

TABLE 9 Concentrations of celery seed oil in complete feed (mg/kg) considered of no concern for the respective target species and categories.

Animal categories	Feed concentration of no concern (mg/kg complete feed)[a]
Turkeys for fattening	2.1
Chickens for fattening, other poultry for fattening or reared for laying/reproduction and ornamental birds	1.6
Laying hens and other laying/reproductive birds	2.3
Pigs for fattening	3.3
Piglets and other porcine species for meat production or reared for reproduction	2.8
Sows and other porcine species for reproduction	4.1
Veal calves (milk replacer)	6.5
Sheep/goats	6.2
Cattle for fattening, other bovines for fattening or reared for milk production/reproduction, cervids and camelids at the same physiological stage	6.2
Dairy cows and other bovines, cervids and camelids for milk production or reproduction	4.0
Sheep/goats	6.2
Horses and other equines	6.2
Rabbits and other leporids	2.5
Salmonids and minor fin fish	6.8
Dogs	7.2

[a]Complete feed containing 88% DM, milk replacer 94.5% DM.

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered of no concern when consumed via feed alone for all animal species.

3.4.4 | Safety for the consumer

Celery seed oil 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 2.28 mg/kg bw per day for celery seed (FEMA 2268) and 0.021 mg/kg bw per day for celery seed oil (FEMA 2271).

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 4, Section 3.4).

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 celery seed oil are expected to be extensively metabolised and excreted in the target species. Also for perillaldehyde and bergapten, the available data indicate that they are absorbed, metabolised and rapidly excreted and at the concentrations in feed resulting from the use of the additive they are not expected to accumulate in animal tissues and products (see Section 3.4.1). Considering the above and the reported human exposure due to the direct use of celery seed oil in food (Burdock, 2009), it is unlikely that the consumption of products from animals given celery seed oil would substantially increase human background exposure. The use of celery seed oil in animal nutrition under the proposed conditions of use is safe for the consumers.

3.4.5 | Safety for the user

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

No studies relevant to user safety of essential oils obtained from fruit of *Apium graveolens* L. were found in the literature review.[41]

The applicant provided a safety data sheet[42] for celery seed oil where hazards for users have been identified.

The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser.

When handling the essential oil, exposure of unprotected users to perillaldehyde and bergapten may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

[41]Technical dossier/ Supplementary Information April 2022/2022-04-08_SIn_Reply_celery_seed_oil.

[42]Technical dossier/Supplementary Information April 2022/Annex_VII_SIn reply_celery_seed_oil_MSDS. Inhalation hazard (H304, may be fatal if swallowed and enters airways), hazards for skin irritation (H315, category 2), skin sensitisation (H317, category 1).

3.4.6 | Safety for the environment

A. graveolens is native to Europe and is grown and consumed worldwide. The use of celery seed oil in animal feed under the proposed conditions of use is not expected to pose a risk to the environment.

3.5 | Efficacy

A. graveolens, its seed and their preparations are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2268 (celery, seed), 2269 (celery seed extract), 2270 (celery seed extract solid) and 2271 (celery seed oil).

Since celery seed oil and its 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.

4 | CONCLUSIONS

Celery seed oil from *Apium graveolens* L. may be produced from plants of different geographical origins and by various processes resulting in preparations with different composition and toxicological profiles. Therefore, the following conclusions apply only to celery seed oil which contains ≤ 0.025% perillaldehyde and ≤ 0.001% bergapten and is produced by steam distillation of fruit of *A. graveolens*.

The conclusions of the FEEDAP Panel on the concentrations of celery seed oil in complete feed (mg/kg), which are considered of no concern for the respective target species are summarised as follow:

Animal categories	Feed concentration of no concern (mg/kg complete feed)[a]
Turkeys for fattening	2.1
Chickens for fattening, other poultry for fattening or reared for laying/reproduction and ornamental birds	1.6
Laying hens and other laying/reproductive birds	2.3
Pigs for fattening	3.3
Piglets and other porcine species for meat production or reared for reproduction	2.8
Sows and other porcine species for reproduction	4.1
Veal calves (milk replacer)	6.5
Sheep/goats	6.2
Cattle for fattening, other bovines for fattening or reared for milk production/reproduction, cervids and camelids at the same physiological stage	6.2
Dairy cows and other bovines, cervids and camelids for milk production or reproduction	4.0
Sheep/goats	6.2
Horses and other equines	6.2
Rabbits and other leporids	2.5
Salmonids and minor fin fish	6.8
Dogs	7.2

[a] Complete feed containing 88% DM, milk replacer 94.5% DM.

For cats, ornamental fish and other species, no conclusion can be drawn. The FEEDAP Panel considers that the use in water for drinking is of no concern provided that the total daily intake of the additive does not exceed the daily amount that is considered of no concern when consumed via feed.

No concerns for consumers were identified following the use of the additive up to the highest use level in feed considered of no concern for the target animals.

The additive under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser. When handling the essential oil, exposure of unprotected users to perillaldehyde and bergapten may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

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

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

5 | RECOMMENDATIONS

The specification should ensure that the concentration of perillaldehyde and bergapten in the additive should not exceed 0.025% and 0.001%, respectively.

6 | DOCUMENTATION PROVIDED TO EFSA/CHRONOLOGY

Date	Event
28/10/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 02 - Apiales and Austrobaileyales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
09/11/2010	Reception mandate from the European Commission
26/02/2013	EFSA informed the applicant (EFSA ref. 7,150,727) 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
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, and opoponax oil
24/06/2019	Application validated by EFSA – Start of the scientific assessment
03/07/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for the target species, safety for the consumer, safety for the user, safety for the environment*
30/09/2019	Comments received from Member States
08/04/2022	Reception of supplementary information from the applicant (partial dataset on celery seed oil) - Scientific assessment remains suspended
31/10/2022	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives – partial report related to nine additives *(dill herb oil, dill tincture, dong quai tincture, cumin oil, fennel tincture, parsley tincture, anise tincture, star anise tincture and ferula assa-foetida oil)*
16/12/2022	Reception of an addendum of the Evaluation report of the European Union Reference Laboratory for Feed Additives – final report related to 11 additives *(celery seed oil, caraway seed oil, coriander oil, taiga root tincture, fennel oil, common ivy extract (sb), ginseng tincture, anise oil, anise star oil, anise star terpenes and omicha tincture)*
02/02/2024	The application was split and a new EFSA-Q-2024-00060 was assigned to the additive included in the present assessment. Scientific assessment re-started for the additive included in the present assessment
27/06/2024	Opinion adopted by the FEEDAP Panel on celery seed oil (EFSA-Q-2024-00060). End of the Scientific assessment for the additive included in the present assessment. The assessment of other additives belonging to BDG 02 is still ongoing

ABBREVIATIONS

AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
BDG	Botanically defined group
BMD	Benchmark dose
$BMDL_{10}$	Benchmark dose (BMD) lower confidence limit for a benchmark response of 10%
BW	Body Weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CDG	Chemically Defined Group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	Chemical Group
CoE	Council of Europe
DM	Dry Matter
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
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
GLP	Good Laboratory Practice
ISO	International Standard Organization
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MF	mutation frequency
MOE	Margin of Exposure
MOET	Combined Margin of Exposure (Total)
NOAEL	No Observed Adverse Effect Level
OECD	Organization for Economic Co-operation and Development
QSAR	Quantitative Structure–Activity Relationship
SC	EFSA Scientific Committee
TG	Test Guideline
TGR	transgenic Muta™ Mouse (assay)
TTC	Threshold of Toxicological Concern
WHO	World Health Organization

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2010-01286 (new EFSA-Q-2024-00060)

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REFERENCES

Bampidis, V., & Robinson, P. H. (2006). Citrus by-products as ruminant feeds: A review. *Animal Feed Science and Technology*, *128*, 175–217. https://doi.org/10.1016/j.anifeedsci.2005.12.002

Basler, A., von der Hude, W., & Seelbach, A. (1989). Genotoxicity of epoxides. I. Investigations with the SOS chromotest and Salmonella/mammalian microsome test. *Mutagenesis*, 4, 313–314.

Bourgou, S., Rahali, F. Z., Ourghemmi, I., & Saïdani Tounsi, M. (2012). Changes of peel essential oil composition of four Tunisian citrus during fruit maturation. *The Scientific World Journal*, *2012*, 528593. https://doi.org/10.1100/2012/528593

Brambillasca, S., Britos, A., Deluca, C., Fraga, M., & Cajarville, C. (2013). Addition of citrus pulp and apple pomace in diets for dogs: Influence on fermentation kinetics, digestion, faecal characteristics and bacterial populations. *Archives of Animal Nutrition*, *67*, 492–502. https://doi.org/10.1080/1745039X.2013.857079

Burdock, G. A. (2009). *Fenaroli's handbook of flavor ingredients* (6th ed., pp. 278–280). CRC Press, Taylor & Francis Group. https://doi.org/10.1201/9781439847503

Chebrolu, K. K., Jifon, J., & Patil, B. S. (2016). Modulation of flavanone and furocoumarin levels in grapefruits (*Citrus paradisi* Macfad.) by production and storage conditions. *Food Chemistry*, *196*, 374–380. https://doi.org/10.1016/j.foodchem.2015.09.028

Court, M. H., & Greenblatt, D. J. (1997). Molecular basis for deficient acetaminophen glucuronidation in cats. An interspecies comparison of enzyme kinetics in liver Microsomes. *Biochemical Pharmacology*, *53*, 1041–1047. https://doi.org/10.1016/s0006-2952(97)00072-5

Cramer, G. M., Ford, R. A., & Hall, R. L. (1978). Estimation of toxic hazard – A decision tree approach. *Food and Cosmetics Toxicology*, *16*, 255–276. https://doi.org/10.1016/s0015-6264(76)80522-6

EFSA (European Food Safety Authority). (2012). Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements. *EFSA Journal*, 10(5), 2663. https://doi.org/10.2903/j.efsa.2012.2663

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2009). Scientific Opinion on Flavouring Group Evaluation 80, Revision 1 (FGE.80Rev1): Consideration of alicyclic, alicyclic-fused and aromatic-fused ring lactones evaluated by JECFA (61st meeting) structurally related to a aromatic lactone evaluated by EFSA in FGE.27 (2008). *EFSA Journal*, *7*(9), 1169. https://doi.org/10.2903/j.efsa.2009.1169

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2010). Guidance on the data required for the risk assessment of flavourings. *EFSA Journal*, *8*(6), 1623. https://doi.org/10.2093/j.efsa.2010.1623

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2011a). Scientific Opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8. *EFSA Journal*, *9*(5), 1847. https://doi.org/10.2903/j.efsa.2011.1847

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2011b). Scientific opinion on Flavouring group evaluation 11, revision 2 (FGE.11Rev2): Aliphatic dialcohols, diketones, and hydroxyketones from chemical groups 8 and 10. *EFSA Journal*, *9*(2), 1170. https://doi.org/10.2903/j.efsa.2011.1170

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2011c). Scientific Opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic hydrocarbons from chemical group 31. *EFSA Journal*, *9*(6), 2177. https://doi.org/10.2903/j.efsa.2011.2177

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2014). Scientific Opinion on Flavouring Group Evaluation 82, Revision 1 (FGE.82Rev1): Consideration of Epoxides evaluated by the JECFA (65th meeting). *EFSA Journal*, *12*(6), 3708. https://doi.org/10.2903/j.efsa.2014.3708

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2015a). Scientific Opinion on Flavouring Group evaluation 18, revision 3 (FGE.18Rev3): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8. *EFSA Journal*, *13*(5), 4118. https://doi.org/10.2903/j.efsa.2015.4118

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2015b). Scientific Opinion on Flavouring Group evaluation 25, revision 3 (FGE.25Rev3): Aliphatic hydrocarbons from chemical group 31. *EFSA Journal*, *13*(4), 4069. https://doi.org/10.2903/j.efsa.2015.4069

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2015c). Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic hydrocarbons evaluated by EFSA in FGE.25Rev3. *EFSA Journal*, *13*(4), 4067. https://doi.org/10.2903/j.efsa.2015.4067

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2015d). Scientific opinion on Flavouring group evaluation 208 revision 1 (FGE.208Rev1): Consideration of genotoxicity data on representatives for 10 alicyclic aldehydes with the a,b-unsaturation in ring/side-chain and precursors from chemical subgroup 2.2 of FGE.19. *EFSA Journal*, *13*(7), 4173. https://doi.org/10.2903/j.efsa.2015.4173

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano, V., Bolognesi, C., Castle, L., Cravedi, J.-P., Engel, K.-H., Fowler, P., Franz, R., Grob, K., Husøy, T., Kärenlampi, S., Mennes, W., Milana, M. R., Penninks, A., Smith, A., de Fátima Tavares Poças, M., Tlustos, C., Wölfle, D., Zorn, H., … Gürtler, R. (2017). Scientific Opinion on Flavouring Group Evaluation 208 Revision 2 (FGE.208Rev2): Consideration of genotoxicity data on alicyclic aldehydes with α,β-unsaturation in ring/side-chain and precursors from chemical subgroup 2.2 of FGE.19. *EFSA Journal*, *15*(5), 4766. https://doi.org/10.2903/j.efsa.2017.4766

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012a). Scientific opinion on the safety and efficacy of aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers (chemical group 6) when used as flavourings for all animal species. *EFSA Journal*, *10*(11), 2966. https://doi.org/10.2903/j.efsa.2012.2966

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012b). Opinion on the safety and efficacy of furanones and tetrahydrofurfuryl derivatives: 4-hydroxy-2,5-dimethylfuran-3(2H)-one, 4,5-dihydro-2-methylfuran-3(2H)-one, 4-acetoxy-2,5-dimethylfuran -3(2H)-one and linalool oxide (chemical Group 13) when used as flavourings for all animal species. *EFSA Journal*, *10*(7), 2786. https://doi.org/10.2903/j.efsa.2012.2786

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012c). Scientific Opinion on the safety and efficacy of aliphatic and alicyclic ethers (chemical group 16) when used as flavourings for all animal species. *EFSA Journal*, *10*(11), 2967. https://doi.org/10.2903/j.efsa.2012.2967

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012d). Scientific opinion on the safety and efficacy of phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group (chemical group 25) when used as flavourings for all species. *EFSA Journal*, *10*(2), 2573. https://doi.org/10.2903/j.efsa.2012.2573

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012e). Scientific Opinion on the safety and efficacy of alicyclic and aromatic lactones (chemical group 11) when used as flavourings for all animal species. *EFSA Journal*, *10*(3), 2622. https://doi.org/10.2903/j.efsa.2012.2622

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2013). Scientific Opinion on the safety and efficacy of straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes (chemical group 01) when used as flavourings for all animal species. *EFSA Journal*, *11*(4), 3169. https://doi.org/10.2903/j.efsa.2013.3169

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2015). Scientific Opinion on the safety and efficacy of aliphatic and aromatic hydrocarbons (chemical group 31) when used as flavourings for all animal species. *EFSA Journal*, *13*(3), 4053. https://doi.org/10.2903/j.efsa.2015.4053

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2016a). Scientific opinion on the safety and efficacy of secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols from chemical group 8 when used as flavourings for all animal species. *EFSA Journal*, *14*(6), 4475. https://doi.org/10.2903/j.efsa.2016.4475

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2016b). Scientific opinion on the safety and efficacy of aliphatic and aromatic hydrocarbons (chemical group 31) when used as flavourings for all animal species and categories. *EFSA Journal*, *14*(1), 4339. https://doi.org/10.2903/j.efsa.2016.4339

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017a). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, *15*(10), 5023. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2017b). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, *15*(10), 5021. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Innocenti, M. L. (2017c). Guidance on the assessment of the safety of feed additives for the consumer. *EFSA Journal*, *15*(10), 5022. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Martino, L. (2018). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, *16*(5), 5274. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Bastos, M., Christensen, H., Dusemund, B., Kouba, M., Kos Durjava, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Brock, T., de Knecht, J., … Azimonti, G. (2019). Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal*, *17*(4), 5648. https://doi.org/10.2903/j.efsa.2019.5648

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Kouba, M., Kos Durjava, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Dusemund, B. (2020). Scientific opinion on the safety and efficacy of oct-1-en-3-ol, pent-1-en-3-ol, oct-1-en-3-one, oct-1-en-3-yl acetate, isopulegol and 5-methylhept-2-en-4-one, belonging to chemical group 5 and of isopulegone and a-damascone belonging to chemical group 8 when used as flavourings for all animal species. *EFSA Journal*, *18*(2), 6002. https://doi.org/10.2903/j.efsa.2020.6002

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2021a). General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic. https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Kouba, M., Fašmon Durjava, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Dusemund, B. (2021b). Scientific Opinion on the 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. *EFSA Journal*, *19*(4), 6548. https://doi.org/10.2903/j.efsa.2021.6548

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Fašmon Durjava, M., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Sanz, Y., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Dusemund, B. (2022). Scientific opinion on the safety and efficacy of a feed additive consisting of a tincture derived from the roots of *Angelica sinensis* (Oliv.) Diels (dong quai tincture) for use in poultry, horses, dogs and cats (FEFANA asbl). *EFSA Journal*, *20*(12), 7692. https://doi.org/10.2903/j.efsa.2022.7692

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Durjava, M., Dusemund, B., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Galobart, J. (2023a). Guidance on the assessment of the safety of feed additives for the users. *EFSA Journal*, *21*(12), e8469. https://doi.org/10.2903/j.efsa.2023.8469

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis, V., Azimonti, G., Bastos, M. L., Christensen, H., Durjava, M., Kouba, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A., Petkova, M., Ramos, F., Villa, R. E., Woutersen, R., Brantom, P., Chesson, A., … Dusemund, B. (2023b). Safety and efficacy of feed additives consisting of essential oils derived from the flower buds or the leaves of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (clove bud oil and clove leaf oils) for all animal species (FEFANA asbl). *EFSA Journal*, *21*(7), 8183. https://doi.org/10.2903/j.efsa.2023.8183

EFSA Scientific Committee. (2009). Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements, on request of EFSA. *EFSA Journal*, *7*(9), 1249. https://doi.org/10.2903/j.efsa.2009.1249

EFSA Scientific Committee. (2014). Scientific Opinion on the safety assessment of carvone, considering all sources of exposure. *EFSA Journal*, 12(7), 3806, 74 pp. https://doi.org/10.2903/j.efsa.2014.3806

EFSA Scientific Committee, More, S. J., Hardy, A., Bampidis, V., Benford, D., Bennekou, S. H., Bragard, C., Boesten, J., Halldorsson, T. I., Hernandez-Jerez, A. F., Jeger, M. J., Knutsen, H. K., Koutsoumanis, K. P., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., … Hogstrand, C. (2019a). Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. *EFSA Journal*, *17*(3), 5634. https://doi.org/10.2903/j.efsa.2019.5634

EFSA Scientific Committee, More, S., Bampidis, V., Benford, D., Boesten, J., Bragard, C., Halldorsson, T., Hernandez-Jerez, A., Hougaard-Bennekou, S., Koutsoumanis, K., Naegeli, H., Nielsen, S. S., Schrenk, D., Silano, V., Turck, D., Younes, M., Aquilina, G., Crebelli, R., Gürtler, R., … Schlatter, J. (2019b). Statement on the genotoxicity assessment of chemical mixtures. *EFSA Journal*, *17*(1), 5519. https://doi.org/10.2903/j.efsa.2019.5519

EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T. I., Hernandez-Jerez, A. F., Hougaard, B. S., Koutsoumanis, K. P., Machera, K., Naegeli, H., Nielsen, S. S., Schlatter, J. R., Schrenk, D., Silano, V., Turck, D., Younes, M., Gundert-Remy, U., Kass, G. E. N., … Wallace, H. M. (2019c). Guidance on the use of the threshold of toxicological concern approach in food safety assessment. *EFSA Journal*, *17*(6), 5708. https://doi.org/10.2903/j.efsa.2019.5708

Garg, S. K. (1978). Apiumetin-a new furanocoumarin from the seeds of *Apium graveolens*. *Phytochemistry*, 17, 2135–2136.

Garg, S. K. (1979). Coumarins from *Apium graveolens* seeds. *Phytochemistry*, 18, 1580–1581.

Honma, M., Yamada, M., Yasui, M., Horibata, K., Sugiyama, K. I., & Masumura, K. (2021). In vivo and in vitro mutagenicity of perillaldehyde and cinnamaldehyde. *Genes and Environment*, *43*(1), 30. https://doi.org/10.1186/s41021-021-00204-3

Kamal, G. M., Anwar, F., Hussain, A. I., Sarri, N., & Ashraf, M. Y. (2011). Yield and chemical composition of citrus essential oils as affected by drying pretreatment of peels. *International Food Research Journal*, *18*, 1275.

Kubesckka, K. H., & Formacek, V. (2002). Celery seed oil. In *Essential oils analysis by capillary gas chromatography and carbon* ^{13}C *NMR spectroscopy* (pp. 43–47). John Wiley & Sons. ISBN 0.471-96314-3.

Lautz, L. S., Jeddi, M. Z., Girolami, F., Nebbia, C., & Dorne, J. L. C. M. (2021). Metabolism and pharmacokinetics of pharmaceuticals in cats (*Felix sylvestris* catus) and implications for the risk assessment of feed additives and contaminants. *Toxicology Letters*, *338*, 114–127. https://doi.org/10.1016/j.toxlet.2020.11.014

Mercolini, L., Mandrioli, R., Ferranti, A., Sorella, V., Protti, M., Epifano, F., Curinbi, M., & Raggi, M. A. (2013). Quantitative evaluation of Auraptene and Umbelliferone, Chemopreventive Coumarins in citrus fruits, by HPLC-UV-FL-MS. *Journal of Agricultural and Food Chemistry*, *61*, 1694–1701.

Miyake, Y., Murakami, A., Sugiyama, Y., Isobe, M., & Koshimizu, K. (1999). Identification of Coumarins from lemon fruit (*Citrus limon*) as inhibitors of in vitro tumor promotion and superoxide and nitric oxide generation. *Journal of Agricultural and Food Chemistry*, *47*, 3151–3157. https://doi.org/10.1021/jf980999y

Munro, I. C., Ford, R. A., Kennepohl, E., & Sprenger, J. G. (1996). Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. *Food and Chemical Toxicology*, *34*, 829–867. https://doi.org/10.1016/s0278-6915(96)00049-x

OECD. (1997). *OECD Guideline for Testing of Chemicals Test Guideline No. 471: Bacterial reverse mutation test*. Paris, OECD.

OECD. (2020). *OECD Guideline for Testing of Chemicals Test Guideline No: 488: Transgenic rodent somatic and germ cell gene mutation assays*. Paris, OECD.

Pacheco, P. D. G., Baller, M. A., Peres, F. M., Ribeiro, É. D. M., Putarov, T. C., & Carciofi, A. C. (2021). Citrus pulp and orange fiber as dietary fiber sources for dogs. *Animal Feed Science and Technology*, *282*, 115123. https://doi.org/10.1016/j.anifeedsci.2021.115123

PDR for herbal medicines. (2004). *Thomson ed*. ISBN: 1-56363-5125

Qadir, R., Farooq Anwar, T. M., Shahid, M., & Zahoor, S. (2018). Variations in chemical composition, antimicrobial and hemolytic activities of peel essential oils from three local citrus cultivars. *Pure and Applied Biology (PAB)*, *7*, 282–291. https://doi.org/10.19045/bspab.2018.70034

Ramirez-Pelayo, C., Martínez-Quiñones, J., Gil, J., & Durango, D. (2019). Coumarins from the peel of citrus grown in Colombia: Composition, elicitation and antifungal activity. *Heliyon*, *5*, e01937. https://doi.org/10.1016/j.heliyon.2019.e01937

Russo, M., Bonaccorsi, I., Torre, G., Sarò, M., Dugo, P., & Mondello, L. (2014). Underestimated sources of flavonoids, limonoids and dietary fibre: Availability in lemon's by-products. *Journal of Functional Foods*, *9*, 18–26. https://doi.org/10.1016/j.jff.2014.11.008

Scordino, M., Sabatino, L., Belligno, A., & Gagliano, G. (2011). Characterization of polyphenolic compounds in unripe Chinotto (*Citrus myrtifolia*) fruit by HPLC/PDA/ESI/MS-MS. *Natural Product Communications*, *6*, 1857–1862.

WHO (World Health Organization). (2000). Evaluation of certain food additives. WHO Technical Report Series (TRS) 891, Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva. https://apps.who.int/iris/bitstream/10665/42245/1/WHO_TRS_891.pdf

WHO (World Health Organization). (2018). Evaluation of certain food additives. WHO Technical Report Series (TRS) 1014, Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). pp. 71–78. https://apps.who.int/iris/bitstream/handle/10665/279832/9789241210232-eng.pdf#page=83%22%3E

Yan, R., Ko, N. L., Li, S. L., Tam, Y. K., & Lin, G. (2008). Pharmacokinetics and metabolism of ligustilide, a major bioactive component in Rhizoma chuanxiong, in the rat. *Drug Metabolism and Disposition*, *36*, 400–408. https://doi.org/10.1124/dmd.107.017707

Yan, R., Lin, G., Ko, N. L., & Tam, Y. K. (2007). Low oral bioavailability and pharmacokinetics of senkyunolide A, a major bioactive component in Rhizoma Chuanxiong, in the rat. *Therapeutic Drug Monitoring*, *29*, 49–56. https://doi.org/10.1097/ftd.0b013e31802c5862

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