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Safety and efficacy of a feed additive consisting of an essential oil from the aerial parts of *Anethum graveolens* L. (dill herb oil) for use in dogs and cats (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 aerial parts of *Anethum graveolens* L. (dill herb oil), when used as a sensory additive (flavouring) in feed for dogs and cats. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that dill herb oil is safe at use levels in complete feed of 7 mg/kg for dogs and 5 mg/kg for cats. The additive under assessment should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser. Since the aerial parts of *A. graveolens* 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 was considered necessary.

Keywords: sensory additives, flavouring compounds, *Anethum graveolens* L., dill herb oil, carvone, estragole, apiole

Requestor: European Commission

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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 seven years after the entry into force of this Regulation.

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 29 preparations (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 preparations (dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract, opoponax oil,[3] parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil[4] and parsley tincture[5]). These preparations were deleted from the register of feed additives.[6] During the course of the assessment, this application was split and the present opinion covers only one out of the 20 remaining preparations under application: dill herb oil from the aerial parts of *Anethum graveolens* L. for all animal species. During the assessment, the applicant requested a change in the species limiting the application for authorisation to dogs and cats.[7]

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 and user and on the efficacy of the product dill herb oil (*A. graveolens*), when used under the proposed conditions of use (see Section 3.2.4).

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

1.2. Additional information

The additive 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.

[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 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1,050 Brussels, Belgium.
[3] On 27 February 2019, EFSA was informed by the applicant about the withdrawal of the applications on dill seed extract, celery seed extract (oleoresin), caraway oleoresin/extract and opoponax oil.
[4] On 2 April 2020, EFSA was informed by the applicant about the withdrawal of the applications on parsley oil, hares ear tincture, taiga root extract (sb), ajowan oil.
[5] On 9 December 2020, the applicant informed EFSA about the withdrawal of the application on celery tincture.
[6] Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, p. 5.
[7] Technical dossier/Supplementary information October 2021/SIn_reply_dill_herb_oil.

There is no specific EU authorisation for any *A. graveolens* L. preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[8], 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'.

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 Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), the EFSA Panel on Food Additives and Flavourings (FAF) and/or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The list of flavouring compounds currently authorised for food[9] and feed[10] uses together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[11] and the corresponding EFSA opinion are given in Table 1.

Table 1: Flavouring compounds already assessed by EFSA as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA opinion

CG	Chemical Group	Product – EU register name (common name)	FLAVIS No	EFSA* or JECFA opinion, Year
08	Secondary alicyclic saturated and unsaturated alcohols, ketones, ketals and esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols	Borneol	02.016	2016a
		Carvone[(a),(b)]	07.012	2014, SC
16	Aliphatic and alicyclic ethers	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran[(a)] (dill ether)	13.198	2008a, AFC WHO, 2012a,b, (JECFA)
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	Limonene[(a),(c)]	01.001	2008b, AFC
		1-Isopropyl-4-methylbenzene (p-cymene)	01.002	2015
		Terpinolene	01.005	
		α-Phellandrene	01.006	
		1-Isopropenyl-4-methylbenzene	01.010	
		α-Terpinene	01.019	
		γ-Terpinene	01.020	
		Pin-2(10)-ene (β-pinene)	01.003	2016b
		Pin-2(3)-ene (α-pinene)	01.004	
		Myrcene	01.008	
		Camphene	01.009	
		Germacra-1(10),4(14),5-triene (δ-Germacrene)[(a),(d)]	01.042	2011a, CEF
		β-Phellandrene[(a),(d)]	01.055	
		Sabinene (4(10)-thujene)[(a)]	01.059	2015a, CEF

[8] 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.

[9] 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.

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

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

CG	Chemical Group	Product – EU register name (common name)	FLAVIS No	EFSA* or JECFA opinion, Year
32	Epoxides	β-Caryophyllene epoxide(a)	16.043	2014, CEF

*: FEEDAP opinion unless otherwise indicated.
(a): Evaluated for use in food. According to Regulation (EC) 1,565/2000, flavourings evaluated by JECFA before 2000 are not required to be re-evaluated by EFSA.
(b): JECFA evaluated (+) and (−)-carvone before 2000. The EFSA CEF Panel assessed the genotoxicity and carcinogenicity of d-carvone [07.146] and concluded that since genotoxic concern could be ruled out, the substance together with the structurally related l-carvone [07.147], as well as carveol and carvyl derivatives could be evaluated through the Procedure (EFSA CEF Panel, 2011b). The EFSA Scientific Committee evaluated carvone and its isomers, d-carvone and l-carvone. D-Carvone [07.146] and l-carvone [07.147] were also evaluated for use in food and feed (EFSA FEEDAP Panel, 2016a).
(c): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008b). D-Limonene [01.045] and l-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015).
(d): Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[12] in support of the authorisation request for the use of dill herb oil from *A. graveolens* as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings (CDGs). The applicant submitted a written agreement to reuse the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 2.[13]

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 animal feed. The evaluation report is related to the methods of analysis for each feed additive included the group BDG 02 (Apiales and Austrobaileyales). In particular, for the determination of the phytochemical marker *carvone* in *dill herb oil*, the EURL recommended a method based on gas chromatography coupled with flame ionisation detection (GC-FID).[14]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of dill herb oil from *A. graveolens* is in line with the principles laid down in Regulation (EC) No 429/2008[15] and the relevant guidance documents: Opinion of the Scientific Committee on harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic (EFSA, 2005); statement on the applicability of the margin of exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed (EFSA SC, 2012); guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009); compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012); guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a); guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b); 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

[12] FEED dossier reference: FAD-2010-0221.
[13] Technical dossier/Supplementary information/Letter dated 29/04/2021.
[14] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0221_en
[15] 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.

assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018); guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a); statement on the genotoxicity assessment of chemical mixtures (EFSA SC, 2019b); guidance on the use of the threshold of toxicological concern approach in food safety assessment (EFSA SC, 2019c); and general approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021a).

3. Assessment

The additive under assessment, dill herb oil, is obtained from the aerial parts of *Anethum graveolens* L. It is intended for use as a sensory additive (functional group: flavouring compounds) in feed for cats and dogs.

3.1. Origin and extraction

Anethum graveolens L. is an annual herbaceous plant belonging to the family Apiaceae. Commonly referred to as Dill, the plant is characterised by finely divided leaves and small white to yellow flowers produced in umbels. Native to Asia minor and the Mediterranean region it is now found growing throughout the world. The apical parts of the plant may be dried and used as an herb and the fruit (misleadingly called seeds) used as a spice. The plant also has a long history of medicinal use, predominately in the treatment of digestive disorders. *A. graveolens* is a source of essential oils produced either from the fruit alone (Dill seed oil) or from the leaves and stems and sometimes including the fruit (Dill weed oil). Dill seed oil differs from Indian Dill seed oil or Dill seed oil, Indian type which is obtained from another species of *Anethum* (*Anethum sowa* Roxb. Ex Fleming).

The starting material for the production of dill herb oil are the stalks and leaves of the plant harvested prior to ripening of the seeds. The volatile constituents are extracted by steam distillation. Briefly, steam is passed through the plant material. The steam carries up the volatile constituents which are then condensed. The essential oil is then separated from water by decantation.

3.2. Characterisation

3.2.1. Characterisation of dill herb oil

Dill herb oil is a liquid, with a characteristic grass-like aroma. In five batches of the additive (originating from Hungary, Moldova or Russian Federation in 2020), the refractive index (20°C) ranged between 1.4835 and 1.4849 (specification: 1.48–1.59), the density (20°C) between 0.9004 and 0.9017 kg/L (specification: 0.895–0.910 kg/L), the optical rotation (20°C) between 77.47° and 86.20° (specification 75–95°). Dill herb oil is identified with the single Chemical Abstracts Service (CAS) number 8006-75-5,[18] the European Inventory of Existing Chemical Substances (EINECS) number 289-790-8, the Flavor Extract Manufacturers Association (FEMA) 2383 and the Council of Europe (CoE) 42.

No international standard is available for the essential oil obtained by steam distillation of the aerial parts from *A. graveolens*. The product specifications were set based on the concentrations of the main volatile components, analysed by gas chromatography with flame ionisation detection (GC-FID) and expressed as % of gas chromatographic peak area (% GC area) and on the available literature on dill herb oil (Burdock, 2009; Tisserand and Young, 2014; Cohen et al., 2020). Four components contribute to the specification as shown in Table 2, with carvone selected as the phytochemical marker. The applicant provided the full characterisation of the volatile constituents in five batches obtained by gas chromatography–mass spectrometry (GC–MS).[17] The four compounds account for about 91.2% on average (range 90.9–91.8%) of % GC area (Table 2).

[16] https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf
[17] Technical dossier/Supplementary information October 2021/Annex_II_Sin_reply_dill_herb_oil_CoA_chrom.
[18] The FEEDAP Panel notes that The CAS or FEMA numbers do not distinguish between dill seed oil and dill weed oil.

Table 2: Major constituents of the essential oil from the aerial parts of *Anethum graveolens* L.: specifications and batch to batch variation based on the analysis of five batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent	CAS No	FLAVIS No	% GC area		
EU register name			Specification	Mean[(a)]	Range
Carvone	99–49-0	07.012	28–45	42.2	41.1–43.1
Limonene	138–86-3	01.001	16–35	21.5	21.0–22.5
α-Phellandrene	99–83-2	01.006	16–31	20.7	19.8–21.0
Dill ether (3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran)	70786–44-6	13.198	4–9	6.89	6.64–7.18
Total				91.2	90.9–91.8

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): Mean calculated on five batches.

In total, up to 24 constituents were identified and accounted on average for 99.6% (99.5–99.9%) of the GC area. Besides the four compounds indicated in the product specifications, 20 other compounds were detected and are listed in Table 3. Based on the available data on the characterisation, dill herb oil is considered a fully defined mixture.

Table 3: Other constituents of the essential oil from the aerial parts of *Anethum graveolens* L. (based on the analysis of five batches) not included in the specification. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent	CAS No	FLAVIS No	% GC area	
EU register name			Mean[(a)]	Range
β-Phellandrene	555-10-2	01.055	2.27	2.08–2.48
cis-Dihydrocarvone	3792-53-8	–	1.81	1.70–1.89
1-Isopropyl-4-methylbenzene	99-87-6	01.002	1.55	1.20–1.86
Pin-2(3)-ene (α-pinene)	80-56-8	01.004	0.77	0.76–0.81
trans-Dihydrocarvone		–	0.68	0.65–0.69
Myrcene	123-35-3	01.008	0.36	0.34–0.37
Germacra-1(10),4(14),5-triene	23986-74-5	01.042	0.18	0.16–0.20
Pin-2(10)-ene (β-pinene)	127-91-3	01.003	0.16	0.15–0.16
α-Thujene	2867-05-2	–	0.14	0.14–0.15
1-Isopropenyl-4-methylbenzene	1195-32-0	01.010	0.09	0.08–0.09
4(10)-Thujene (sabinene)	3387-41-5	01.059	0.08	0.08–0.08
Isopiperitenone	529-01-1	–	0.07	0.06–0.07
Terpinolene	586-62-9	01.005	0.07	0.06–0.07
Borneol	507-70-0	02.016	0.04	0.04–0.05
cis-Limonene oxide	13837-75-7	–	0.03	0.00–0.04
Camphene	79-92-5	01.009	0.03	0.02–0.03
γ-Terpinene	99-85-4	01.020	0.02	0.02–0.03
trans-3,7-Dimethyl-1,3,6-octatriene	3779-61-1	–	0.02	0.02–0.02
α-Terpinene	99-86-5	01.019	0.02	0.02–0.02
2,3-Dehydro-1,8-cineole	92760-25-3	–	0.01	0.01–0.02
Total			8.38	8.07–8.64

EU: European Union; CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System numbers.
(a): Mean calculated on five batches.

The applicant performed a literature search regarding substances of concern and chemical composition of the plant species *A. graveolens* and its preparations.[19] The presence of estragole in an essential oil from the 'live plant' *A. graveolens* has been reported (but not quantified) in the EFSA Compendium based on one reference (EFSA, 2012).[20] However, several publications (Burdock, 2009; Tisserand and Young, 2014; Cohen et al., 2020) and the database on volatile compound in food (VCF) did not describe estragole as a constituent of dill herb oils. In addition, myristicin and dillapiole (and/or a structurally related substance apiole) have been identified in dill herb oil as substances of concern (Burdock, 2009; Rana and Blazquez, 2014; Weisany et al., 2015; Behbahani et al., 2017; Sousa et al., 2017; Madandoust and Fooladchang, 2018; Rostaei et al., 2018) (references identified in the above-mentioned literature search).

Estragole, myristicin, dillapiole and apiole were not detected in the five batches of dill herb oil with the GC–MS method used for the characterisation of the additive (limit of detection, LOD 0.0003% for estragole and apiole, myristicin 0.001%). Further analysis on two batches showed dillapiole (injected without dilution and estimated using myristicin as standard) was below the limit of detection (LOD, 0.01%) and that myristicin analysed by HPLC with UV detection was below the limit of quantification (LOQ, 0.005%).[21]

3.2.2. Impurities

The applicant makes reference to the 'periodic testing' of some representative flavourings premixtures for mercury, cadmium, lead, arsenic, fluoride, dioxins and polychlorinated biphenyls (PCBs), organo-chloride pesticides, organo-phosphorous pesticides, aflatoxins B1, B2, G1, G2 and ochratoxin A. However, no data have been provided. Since dill herb oil is produced by steam distillation, the likelihood of any measurable carry-over of all the above-mentioned elements is low except for mercury.

3.2.3. Shelf-life

The typical shelf-life of dill herb oil is stated to be at least 12 months, when stored in tightly closed containers under standard conditions (in a cool, dry place protected from light).[22] However, no data supporting this statement were provided.

3.2.4. Conditions of use

Dill herb oil is intended to be added to feed for cats and dogs. The maximum proposed use level in complete feed is 20 mg/kg for dogs and 10 mg/kg for cats.

3.3. Safety

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

Many of the components of dill herb oil, accounting for about 97% of the GC peak areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food[9] without limitations and for use in feed[10] at individual use levels higher than those resulting from the intended use of the essential oil in feed. The list of the compounds already evaluated by the EFSA Panels and JECFA is given in Table 1 (see Section 1.2).

Two compounds, germacra-1(10),4(14),5-triene [01.042] 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, 2011a). In the absence of such data, the EFSA CEF Panel was unable to complete its assessment. As a result, these compounds are not authorised for use as flavours in food. For these compounds, the FEEDAP Panel applies the approach recommended in the Guidance document on harmonised methodologies for human health,

[19] Technical dossier/Supplementary information October 2021/Literature search_dill_herb_oil.
[20] Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.
[21] Technical dossier/Supplementary information October 2021/Annex_VII_Sin_reply_dill_herb_oil_Myristicin_check.
[22] Technical dossier/Section II.

animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

Few volatile components (trans-dihydrocarvone, cis-dihydrocarvone, isopiperitenone, 2,3-dehydro-1,8-cineole, trans-3,7-dimethyl-1,3,6-octatriene and α-thujene) have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesquiterpenes structurally related to flavourings already assessed in CG 8, 16 and 31 and a similar metabolic and toxicological profile is expected. These lipophilic compounds, accounting for about 3% of the GC area, are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2012c, 2015, 2016a, 2016b).

The genotoxic potential of isopiperitenone was assessed using the Organization for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox (V 4.4.1). For isopiperitenone, the alerts due to the presence of ketones and α,β-unsaturated carbonyls were discounted by the FEEDAP Panel based on read-across analysis.[23]

3.3.1. Safety for the target species

Tolerance studies with target species and/or toxicological studies in laboratory animals made with the essential oil under application have not been submitted.

In the absence of these data, the approach to the safety assessment of a mixture whose individual components are known is based on the safety assessment of each individual component (component-based approach). This approach requires that the mixture is sufficiently characterised. The individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component (EFSA SC, 2019a).

As the additive under assessment is a fully defined mixture (> 99.5% of the components were identified, see Section 3.2.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the essential oil. Although not detected in the oil under assessment, the potential presence of substances for which a concern for genotoxicity has been identified (estragole, myristicin, apiole and dillapiole) is assessed separately.

Components other than p-allylalkoxybenzenes (estragole, myristicin, apiole and dillapiole)

Based on considerations related to structural and metabolic similarities, the components were allocated to four assessment groups, corresponding to the chemical groups (CGs) 8, 16, 31 and 32, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), the application of subassessment groups as defined in FGE.25 and FGE.78 is applied (EFSA CEF Panel, 2015a,b). The allocation of the components to the (sub-)assessment groups is shown in Tables 4 and 5 and in the corresponding footnotes.

For each component in the assessment group, exposure in target animals was estimated considering the use levels in feed, the percentage of the component in the oil and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b). Default values on body weight are used to express exposure in terms of mg/kg body weight (bw) per day. The intake levels of the individual components calculated for dog and cat are shown in Tables 4 and 5.

For hazard characterisation, each component of an assessment group was first assigned to the structural class according to Cramer classification. For some components in the assessment group, toxicological data were available to derive a no observed adverse effect level (NOAEL) value or a 95% lower confidence limit for the benchmark dose response of 10% ($BMDL_{10}$). Structural and metabolic similarity among the components in the assessment groups was assessed to explore the application of read-across. If justified, extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL was made. If sufficient evidence was available for the members of a (sub-)assessment group, a (sub-)assessment group NOAEL was derived.

[23] Technical dossier/Supplementary information October 2021/Annex V_SIn_reply_dill_herb_oil_QSAR_isopiperitenone. For isopiperitenone structural alerts were due to the presence of ketones and α,β-unsaturated carbonyls. The mutagenicity (Ames test) prediction was made by "read-across" analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers as well as empirical profilers. Mutagenicity (with and without S9) read-across based predictions were found negative for all categories of analogues.

Toxicological data for subchronic studies, from which a $BMDL_{10}$ or an NOAEL value could be derived were available for carvone [07.012] (EFSA SC, 2014), 1,8-cineole [03.001] in CG 16 (EFSA FEEDAP Panel, 2021b), myrcene [01.008], limonene [01.001], 1-isopropyl-4-benzene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016b).

The $BMDL_{10}$ for carvone [07.012] was applied to the structurally related compounds *cis*- and *trans*-dihydrocarvone in CG 8.

The additive contains 0.1% 2,3-dehydro-1,8-cineole (DHC), which was also detected as a minor metabolite of 1,8-cineole in human breast milk (Kirsch and Buettner, 2013) but not in rats (Pass et al., 2001). Toxicity studies with DHC were not available. According to the study of Kirsch and Buettner (2013), DHC is further metabolised to 2,3-epoxy-1,8-cineole and 2-oxo-1,8-cineole and 3-oxo-1,8-cineole. Read-across from 1,8-cineole to DHC will probably underestimate the toxicity of DHC because it is expected that the epoxide and the oxo-derivatives generated from DHC have a higher toxicity than the hydroxylated derivatives of 1,8-cineole at various C-positions which make up the majority of the metabolite spectrum of 1,8-cineole. The FEEDAP Panel is therefore of the opinion that read-across from 1,8-cineole to DHC is not possible and applies the TTC approach to DHC, which is allocated to Cramer class II.

Dill ether [13.198] which is present in the additive at approximately 7% shares some structural similarities with DHC. It is expected that it will also be transformed to an epoxide with further generation of keto derivatives or diols after hydrolysis of the epoxide. The FEEDAP Panel is of the opinion that read-across from 1,8-cineole, as proposed by the applicant, will probably underestimate the toxicity of dill ether and considers the application of the TTC more appropriate. The FEEDAP Panel notes that dill ether has been allocated to Cramer class III by EFSA (EFSA, 2008a) and to Cramer class II by JECFA (WHO, 2012a). The difference in the allocation was due to the different interpretation of the complexity of the substituent on the heterocyclic ring (question 11 of Cramer decision tree). Dill ether is a five-member heterocyclic compound, substituted with a cyclic hydrocarbon with an unsaturation in the six-member ring, a structure common to many terpenes (e.g. α-pinene, valencene, δ-3-carene, all compounds allocated to Cramer class I). For this reason, the FEEDAP Panel agrees with the allocation of dill ether to Cramer class II, as the molecule does not have a heterocyclic ring with complex substituent (question 11 of the decision tree) and the substance is a common component of food (question 22 of the decision tree).[24]

Considering the structural and metabolic similarities, the NOAELs for the representative compounds of CG 31, myrcene [01.008], limonene [01.001] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds within subassessment group II, III and V (EFSA CEF Panel, 2015a, 2015b).

For the remaining compounds, isopiperitenone, borneol [02.016], germacra-1(10),4(14),5-triene [01.042] and cis-limonene oxide, toxicity studies and NOAEL values performed with the compounds under assessment were not available and read-across was not possible. Therefore, the threshold of toxicological concern (TTC) approach was applied (EFSA FEEDAP Panel, 2017b).

As the result of the hazard characterisation, a reference point was identified for each component in the assessment group based on the toxicity data available (NOAEL from *in vivo* toxicity study or read-across) or from the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class (i.e. 3, 0.91 and 0.15 mg/kg bw per day, respectively, for Cramer Class I, II and III compounds). Reference points selected for each compound are shown in Tables 4 and 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 SC, 2019a). An MOET $>$ 100 allowed for interspecies and intra-individual variability (as in the default 10×10 uncertainty factor). The compounds resulting individually in an MOE $>$ 50,000 were not further considered in the assessment group as their contribution to the MOE(T) is negligible. They are listed in the footnote.[25]

The approach to the safety assessment of dill herb oil for dogs is summarised in Table 4.

[24] "For this decision tree, *common component of food* denotes a substance that has been reported in the recognised literature as occurring in sufficient quantity (approximately 50 ppm or more) in at least one major food, or in trace quantities at the ppm level or less in several foods, including minor or less frequently consumed foods. The latter include spices, herbs and ethnic specialities. The definition excludes natural or man-made contaminants, and hormones" (Cramer et al., 1978)

[25] Compounds included in the assessment groups but not reported in the table: trans-3,7-dimethyl-1,3,6-octatriene (CG 31, II); terpinolene, γ-terpinene and α-terpinene (CG 31, III); α-pinene, β-pinene, α-thujene, camphene and sabinene (CG 31, V).

Table 4: Compositional data, intake values (calculated for dogs at 20 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of dill herb oil classified according to assessment groups

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[(c)]**	**MOE**	**MOET**
Constituent	–	**%**	**mg/kg**	**mg/kg bw per day**	–	**mg/kg bw per day**	–	–
CG 8								
Carvone	07.012	43.07	8.614	0.1631	(II)	60	368	
cis-Dihydrocarvone	–	1.89	0.378	0.0072	(II)	60	8,390	
Isopiperitenone	–	0.07	0.015	0.0003	II	*0.91*	3,246	
trans-Dihydrocarvone	–	0.69	0.138	0.0026	(II)	60	22,957	
Borneol	02.016	0.05	0.010	0.0002	I	*3*	16,500	
								302
CG 16								
Dill ether	13.198	7.18	1.437	0.0272	II	0.91	33	
2,3-Dehydro-1,8-cineole	–	0.02	0.003	0.00006	II	0.91	16,016	
								33
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	0.37	0.073	0.0014	(I)	**44**	31,651	
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.001	22.47	4.494	0.0851	(I)	**250**	2,937	
α-Phellandrene	01.006	20.97	4.195	0.0794	(I)	250	3,147	
β-Phellandrene	01.055	2.48	0.496	0.0094	(I)	250	26,613	
MOET CG 31, III								1,437
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	1.86	0.372	0.0070	(I)	**154**	21,858	
1-Isopropenyl-4-methylbenzene	01.010	0.09	0.018	0.0003	I	*3*	8,703	
								6,225
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
Germacra-1(10),4(14),5-triene	01.042	0.20	0.039	0.0007	I	*3*	4,062	
CG 32								
cis-Limonene epoxide	–	0.04	0.008	0.0002	I	*3*	18,857	

(a): Intake calculations for the individual components are based on the use level of 20 mg/kg in feed for dog. 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 an NOAEL value is available or read-across is applied, the allocation to the Cramer class is put into parentheses.
(c): Values **in bold** refer to those components for which the NOAEL value was available, values *in italics* are the 5th percentile of the distribution of NOAELs of the corresponding Cramer Class, other values (plain text) are NOAELs extrapolated by using read-across.

As shown in Table 4, the MOET was >100 for all the assessment groups, except for CG 16, for which the MOET was 33. In order to ensure a MOET ≥100 for CG 16, the concentration in feed should be reduced to 7 mg/kg complete feed. For cat, the corresponding calculations are shown in Table 5.[26]

[26] Compounds included in the assessment groups but not reported in the table: myrcene and trans-3,7-dimethyl-1,3,6-octatriene (CG 31, II); terpinolene, γ-terpinene and α-terpinene (CG 31, III); α-pinene, β-pinene, α-thujene, camphene and sabinene (CG 31, V).

Table 5: Compositional data, intake values (calculated for cats at 10 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of dill herb oil classified according to assessment groups

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[c]**	**MOE**	**MOET**
Constituent	–	**%**	**mg/kg**	**mg/kg bw per day**	–	**mg/kg bw per day**	–	–
CG 8								
Carvone	07.012	43.07	4.307	0.0979	(II)	60	613	
cis-Dihydrocarvone	–	1.89	0.189	0.0043	(II)	60	13,983	
Isopiperitenone	–	0.07	0.007	0.0002	II	*0.91*	5,411	
trans-Dihydrocarvone	–	0.69	0.069	0.0016	(II)	60	38,261	
Borneol	02.016	0.05	0.005	0.0001	I	*3*	27,500	
								503
CG 16								
Dill ether	13.198	7.18	0.718	0.0163	II	*0.91*	56	
2,3-Dehydro-1,8-cineole	–	0.02	0.002	0.00003	II	*0.91*	26,693	
								56
CG 31, III (Cyclohexene hydrocarbons)								
Limonene	01.001	22.47	2.247	0.0511	(I)	**250**	4,896	
α-Phellandrene	01.006	20.97	2.097	0.0477	(I)	250	5,245	
β-Phellandrene	01.055	2.48	0.248	0.0056	(I)	250	44,355	
MOET CG 31, III								2,395
CG 31, Ive (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	1.86	0.186	0.0042	(I)	**154**	36,430	
1-Isopropenyl-4-methylbenzene	01.010	0.09	0.009	0.0002	I	*3*	14,505	
								10,375
CG 31, VI (macrocyclic non-aromatic hydrocarbons)								
Germacra-1(10),4(14),5-triene	01.042	0.20	0.020	0.0004	I	*3*	6,769	
CG 32								
cis-Limonene epoxide	–	0.04	0.004	0.0001	I	*3*	31,429	

(a): Intake calculations for the individual components are based on the use level of 0.2 mg/kg in feed for cat. 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 an 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.

Generally, for cats, an MOET >500 is considered adequate, considering their unusually low capacity for glucuronidation of compounds (Court and Greenblatt, 1997; Lautz et al., 2021). As shown in Table 5, the MOET was >500 for all the assessment groups, except for CG 16 for which the MOE was 56. Because this MOET was derived from a Cramer class, which is already very conservative, a value of 100 seems appropriate. In order to ensure an MOET ≥100 for CG 16, the concentration in feed should be reduced to 5 mg/kg complete feed.

p-Allylalkoxybenzenes (Estragole, myristicin, apiole and dillapiole)

Estragole, dillapiole, apiole and myristicin were below the corresponding LODs (see Section 3.2.1). In a worst-case scenario, it is assumed that they are all present in the additive at a concentration corresponding to the respective LODs. The maximum daily intake of estragole, dillapiole, apiole and myristicin in μg/kg bw per day was calculated at the use level of the additive in feed considered safe for dogs and cats. The calculated intake values for the sum of the four compounds were 0.0207 μg/kg bw per day for dogs and 0.0177 μg/kg bw per day for cats (see Appendix A).

Since estragole, dillapiole, apiole and myristicin share the same mode of action, they are allocated to the same assessment group (*p*-allylalkoxybenzenes) (EFSA SC, 2019a) and an assessment of the combined exposure is performed. When the estimated exposures for the dogs and cats are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, calculated from rodent carcinogenicity studies with methyleugenol (NTP, 2000; Suparmi et al., 2019) and selected by the FEEDAP Panel as reference point for the assessment group p-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022), a combined margin of exposure (MOET) > 1,000,000 is calculated for dogs and cats (Appendix A). The magnitude of this MOET is indicative of a low concern for the target species.

3.3.1.1. Conclusions on safety for the target species

Dill herb oil is safe at use levels in complete feed of 7 mg/kg for dogs and 5 mg/kg for cats.

3.3.2. Safety for the user

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

The applicant produced a safety data sheet[27] for dill herb oil, where hazards for users have been identified. The essential oil under assessment should be considered as irritant to skin and eyes, and as a skin and respiratory sensitiser.

3.4. Efficacy

The herb and the seeds of *A. graveolens* and their oils (including also dill seed oil and dill weed oil) are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009). FEMA allocates the reference number 2383 to 'dill oil'.

Since the herb and the seeds of *A. graveolens* and their oils are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

Dill herb oil from *Anethum graveolens* L. may be produced from plants of different origins and by various processes resulting in preparations with different composition and toxicological profiles. Thus, the following conclusions apply only to dill herb oil free from estragole, apiole, myristicin and dillapiole (see recommendations) produced by steam distillation of the aerial parts of *A. graveolens*.

Dill herb oil is safe at use levels of 7 mg/kg for dogs and 5 mg/kg for cats in complete feed.

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

Since the herb and the seeds of *A. graveolens* and their oil are recognised to flavour food and their function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. Recommendation

The specification should ensure that the concentration of estragole, myristicin, apiole and dillapiole should be as low as possible and should not exceed 0.0003% estragole and apiole, 0.005% myristicin and 0.01% dillapiole.

[27] Technical dossier/Supplementary Information October 2021/Annex_VI_SIn reply_dill_herb oil_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1).

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 character by the European Commission with EFSA and the applicant FEFANA on the assessment of botanical flavourings: characterization, 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: characterization, 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
29/10/2021	Reception of supplementary information from the applicant (partial dataset on dill herb oil) – Scientific assessment remains suspended
24/06/2022	The application was split and a new EFSA-Q-2022-00405 was assigned to the preparation included in the present assessment
31/10/2022	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives – Scientific assessment re-started
22/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment for the preparation included in the present assessment. The assessment of other preparations is still ongoing

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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 Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CoE	Council of Europe
DHC	2,3-dehydro-1,8-cineole
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food Additives and Flavourings
FAO	Food Agricultural Organisation
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of FEFANA (EU Association of Specialty Feed Ingredients and their Mixtures)
FGE	food group evaluation
FLAVIS	The EU Flavour Information System
FL-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography-flame ionisation detection
GC–MS	gas chromatography–mass spectrometry
HPLC	High performance liquid chromatography
JECFA	The Joint FAO/WHO Expert Committee on Food Additives

LOD	limit of detection
LOQ	limit of quantification
MOE	margin of exposure
MOET	combined margin of exposure
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCBs	polychlorinated biphenyls
QSAR	Quantitative Structure–Activity Relationship
sb	solvent-based
SC	EFSA Scientific Committee
TTC	threshold of toxicological concern
UF	uncertainty factor
UV	ultraviolet
VCF	(database on) volatile compound in food
WHO	World Health Organization

Appendix A – Estragole, dillapiole, apiole and myristicin: maximum daily intake and combined margin of exposure for dogs and cats

The maximum daily intake of estragole, dillapiole, apiole and myristicin was calculated for dogs and cats, based on

- the default values for body weight and feed intake (EFSA FEEDAP Panel, 2017b)
- the use level of the additive in feed considered safe (7 mg/kg for dogs and 5 mg/kg for cats) and
- assuming that estragole, dillapiole, apiole and myristicin are all present at concentrations equal to the corresponding limit of detection (0.0003% for estragole and apiole, 0.005% for myristicin and 0.01% for dillapiole).

According to the 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),[16] 'for substances for which carcinogenicity studies in rodents are available, from which a $BMDL_{10}$ can be derived, the MOE approach (EFSA, 2005; EFSA SC, 2012) can be applied. Similarly to human risk assessment, a combined (total) margin of exposure (MOET) with a magnitude of $\geq$ 10,000, when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, would be indicative of a low concern for the target species (EFSA SC, 2019a)'.

Since all the compounds share the same structural features and the same mode of action, although with different potency, they are allocated to the same assessment group (p-allylalkoxybenzenes) and an assessment of the combined exposure is performed as described in the Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA SC, 2019a).

The MOE for each component is calculated as the ratio of the reference point (the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day which has been identified by the FEEDAP Panel as the reference point for the group p-allylalkoxybenzenes, EFSA FEEDAP Panel, 2022) to the intake. The combined margin of exposure (MOET) is calculated for the assessment group as the reciprocal of the sum of the reciprocals of the MOE of the individual substances.

The assessment of the combined exposure to estragole, dillapiole, apiole and myristicin for dogs and cats is reported in Tables A.1 and A.2.

Table A.1: Compositional data, intake values (calculated for dogs at 7 mg/kg complete feed), reference points and margin of exposure (MOE) for estragole, dillapiole, apiole and myristicin (if present in the additive at the corresponding limit of detection), and combined margin of exposure (MOET) for the assessment group p-allylalkoxybenzenes

Composition		Exposure		Hazard characterisation	Risk characterisation	
Assessment group	**Max conc. in the oil**	**Max Feed conc.**	**Intake[(a)]**	**$BMDL_{10}$**	**MOE**	**MOET**
Constituent	**%**	**µg/kg**	**µg/kg bw per day**	**mg/kg bw per day**	–	–
p-Allylalkoxybenzenes						
Estragole	0.0003	0.070	0.0004	22.2	55,817,143	
Dillapiole	0.01	0.700	0.0133	22.2	1,674,514	
Apiole	0.0003	0.070	0.0004	22.2	55,817,143	
Myristicin	0.005	0.350	0.0066	22.2	3,349,029	
MOET			0.0207			1,073,407

(a): Intake calculations for the individual components are based on the use level of 7 mg/kg in feed for dogs. The MOE for each component is calculated as the ratio of the reference point ($BMDL_{10}$) 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.

From the MOET for chickens for fattening, the MOET for *p*-allylalkoxybenzenes was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table A.2.

Table A.2: Compositional data, intake values (calculated for cats at 5 mg/kg complete feed), reference points and margin of exposure (MOE) for estragole, dillapiole, apiole and myristicin (if present in the additive at the corresponding limit of detection), and combined margin of exposure (MOET) for the assessment group p-allylalkoxybenzenes

Composition		Exposure		Hazard characterisation	Risk characterisation	
Assessment group	**Max conc. in the oil**	**Max Feed conc.**	**Intake[(a)]**	**$BMDL_{10}$**	**MOE**	**MOET**
Constituent	**%**	**µg/kg**	**µg/kg bw per day**	**mg/kg bw per day**	–	–
p-Allylalkoxybenzenes						
Estragole	0.0003	0.050	0.0003	22.2	65,120,000	
Dillapiole	0.010	0.500	0.0114	22.2	1,953,600	
Apiole	0.0003	0.050	0.0003	22.2	65,120,000	
Myristicin	0.005	0.250	0.0057	22.2	3,907,200	
MOET			0.0177			1,252,308

(a): Intake calculations for the individual components are based on the use level of 5 mg/kg in feed for cats. The MOE for each component is calculated as the ratio of the reference point ($BMDL_{10}$) 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.