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Safety and efficacy of a feed additive consisting of an essential oil from the seeds of *Myristica fragrans* Houtt. (nutmeg 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 from the seeds of *Myristica fragrans* Houtt. (nutmeg oil), when used as a sensory additive in feed and water for drinking for all animal species. The additive contains myristicin (up to 12%), safrole (2.30%), elemicin (0.40%) and methyleugenol (0.33%). For long-living and reproductive animals, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered of low concern the use of the additive in complete feed at 0.2 mg/kg for laying hens and rabbits, 0.3 mg/kg for sows and dairy cows, 0.5 mg/kg for sheep/goats, horses and cats, 0.6 mg/kg for dogs and 2.5 mg/kg for ornamental fish. For short-living animals, the Panel had no safety concern when the additive is used at the maximum proposed use level of 10 mg/kg for veal calves, cattle for fattening, sheep/goats, horses for meat production, and salmon and for the other species, at 3.3 mg/kg for turkeys for fattening, 2.8 mg/kg chickens for fattening, 5.0 mg/kg for piglets, 6.0 mg/kg for pigs for fattening and 4.4 mg/kg for rabbits for meat production. These conclusions were extrapolated to other physiologically related species. For any other species, the additive was considered of low concern at 0.2 mg/kg. The use of nutmeg oil in animal feed was expected to be of no concern for consumers and the environment. The additive should be considered as irritant to skin and eyes and as a skin and respiratory sensitiser. Based on the presence of safrole, nutmeg oil is classified as carcinogen (category 1B) and handled accordingly. Since nutmeg oil was recognised to flavour food and its function in feed would be the same, no further demonstration of efficacy was considered necessary.

Keywords: sensory additives, flavouring compounds, *Myristica fragrans* Houtt., nutmeg oil, myristicin, safrole, elemicin

Requestor: European Commission

Question number: EFSA-Q-2010-01296 (old EFSA-Q remains associated to the last opinion of the group, no new EFSA-Q to be generated)

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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 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 18 preparations (cassia oil, cassia bark extract (solvent-based, sb), camphor oil, cinnamon oil, cinnamon bark oleoresin, cinnamon tincture, laurel leaves oil, laurel leaves extract/oleoresin, litsea berry oil, boldo extract (water-based, wb), boldo tincture, ylang-ylang oil, mace oil, nutmeg oil, nutmeg oleoresin, kawakawa tincture, pepper oil and pepper oleoresin) belonging to botanically defined group (BDG) 6 – Laurales, Magnoliales, Piperales, when used as a feed additive for all animal species (category: sensory additives; functional group: flavouring compounds). During the assessment, the applicant withdrew the applications for eight preparations.[3] These preparations 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 18 preparations under application: an essential oil from the nuts of *Myristica fragrans* Houtt. (nutmeg oil) for all animal species.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 3 January 2011.

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 nutmeg oil (*Myristica fragrans* Houtt.), when used under the proposed conditions of use (see Section **3.2.4**).

1.2. Additional information

Nutmeg oil from *Myristica fragrans* Houtt. 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) and foreseen for re-evaluation. It has not been assessed as a feed additive in the EU.

There is no specific EU authorisation for any *M. fragrans* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008[5] flavouring preparations produced from food or food ingredients with flavouring properties, 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."

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] On 13/3/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1050 Brussels, Belgium.
[3] On 8 October 2020, EFSA was informed about the withdrawal of the applications on cassia bark extract (sb), cinnamon bark oleoresin, laurel leaves extract/oleoresin, mace oil, nutmeg oleoresin, boldo extract (wb), boldo tincture and kawakawa tincture.
[4] Register of feed additives, Annex II, withdrawn by OJ L162, 10.5.2021, p. 5.
[5] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

The Committee for Veterinary Medicinal Products of the European Medicines Agency (EMA) issued a summary report for veterinary use on '*Myristicae aetheroleum*', described as the 'volatile oil' obtained by steam distillation of the seed of *Myristica fragrans* Houtt. (EMA, 1998).

The European Medicines Agency (EMA) published a public statement on the use of medicinal products containing methyleugenol (EMA, 2005), which mentions *Myristica fragrans* L. (with a methyleugenol content in the seed in the range 20–900 mg/kg).

M.fragrans Houtt. is described in a monograph prepared by the International Programme on Chemical Safety (IPCS, 1991) from the World Health Organization (WHO).

Nutmeg oil (Myristicae fragrantis aetheroleum) is described in a monograph of the European Pharmacopoeia 11.0 (PhEur, 2022). It is defined as the oil obtained by steam distillation of the dried and crushed kernels of *M. fragrans* Houtt.

Many of the individual components of nutmeg 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), the EFSA Panel on Food Additives and Flavourings (FAF) and/or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The flavouring compounds currently authorised for feed[6] and/or food[7] use, together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[8], and the corresponding EFSA opinion are listed 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

	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	Ethanol	02.078	2013
03	α, ß-Unsaturated (alkene or alkyne) straight-chain and branched-chain aliphatic primary alcohols/aldehydes/acids, acetals and esters with esters containing α, β-unsaturated alcohol and acetal containing α, β-unsaturated alcohols or aldehydes	Geranyl acetate	09.011	2016a
04	Non-conjugated and accumulated unsaturated straight-chain and branched-chain aliphatic primary alcohols, aldehydes, acids, acetals and esters	Citronellol	02.011	2016b
		Citronellyl acetate	09.012	
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		2-(4-Methylphenyl) propan-2-ol	02.042	
		4-Terpinenol	02.072	
		α-Terpinyl acetate	09.015	

[6] 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.

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

[8] 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.

	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	*d,l*-Borneol	02.016	2016c
		Fenchyl alcohol	02.038	
		d,l-Bornyl acetate	09.017	
		Sabinene hydrate[(a),(b)] (4-thujanol)	02.085	WHO (2000) (JECFA)
		Pinocarveol[(a)]	02.100	CEF (2011a) CEF (2012)
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012b, 2021a
17	Propenylhydroxybenzenes	Isoeugenol[(c)]	04.004	2012c
18	Allylhydroxybenzenes	Eugenol	04.003	2011
		4-Allyl-2,6-dimethoxyphenol	04.051	
26	Aromatic ethers including anisole derivatives	1,2-Dimethoxy-4-(prop-1-enyl)benzene[(d)] (methyl isoeugenol)	04.013	2012d
31	Aliphatic and aromatic hydrocarbons and acetals containing saturated aldehydes	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	2016d
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		δ-3-Carene	01.029	
		δ-Cadinene[(a),(f)]	01.021	CEF (2011b)
		β-Bisabolene[(a)]	01.028	
		Germacra-1(10),4(14),5-triene (δ-Germacrene)[(a),(f)]	01.042	
		β-Phellandrene[(a),(f)]	01.055	
		1,1,7-Trimethyltricyclo [2.2.1.0.(2.6)]heptane (tricyclene)[(a),(f)]	01.060	
		Limonene[(a),(e)]	01.001	CEF (2015a)
		4(10)-Thujene (sabinene)[(a)]	01.059	
		cis-3,7-Dimethyl-1,3,6-octatriene	01.064	
		3,7-Dimethyl-1,3,6-octatriene[(g)] (β-ocimene)	01.018	CEF (2015b)
		α-Farnesene[(a)]	01.040	

(*): 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): JECFA evaluated sabinene hydrate [02.085] as a mixture of cis- and trans-sabinene hydrate (WHO, 2000).

(c): EFSA evaluated isoeugenol [04.004], a mixture of *(Z)*- and *(E)*-isomers. The composition of stereoisomeric mixture was not specified.
(d): EFSA evaluated 1,2-dimethoxy-4-(prop-1-enyl)benzene [04.013], a mixture of *(Z)*- and *(E)*-isomers. The composition of stereoisomeric mixture was not specified.
(e): JECFA and EFSA evaluated d-limonene [01.045] (EFSA, 2008). *d*-limonene [01.045] and *l*-limonene [01.046] were also evaluated for use in feed (EFSA FEEDAP Panel, 2015).
(f): 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 the additional toxicity data requested (EFSA CEF Panel, 2011b) were not submitted and the CEF Panel was unable to complete its assessment.
(g): EFSA evaluated β-ocimene [01.018], as a mixture of *(E)*- and *(Z)*-isomers, containing 50–70% *(E)*-isomer and 17–17% *(Z)*-isomer (EFSA CEF Panel, 2015b).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[9] in support of the authorisation request for the use of nutmeg oil from *M. fragrans* Houtt. as a feed additive. The dossier was received on 14/12/2020 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2010-01517.

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

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings. The applicant submitted a written agreement to use the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 6, including the current one under assessment.[10]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical markers in botanically defined flavourings from Group 06 – Laurales, Magnoliales, Piperales. During the assessment, upon request from the EC and EFSA, the EURL issued two amendments of the original report.[11] For the additive under assessment, *nutmeg oil*, the evaluation of the method of analysis is included in the second amendment. In particular, for the characterisation of nutmeg oil the EURL recommended methods based on gas chromatography with flame ionisation detector (GC-FID) for the quantification of the phytochemical marker *pin-2(3)-ene* (hereinafter referred to as α-pinene) in *nutmeg oil*.[12]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of nutmeg oil from *M. fragrans* Houtt. is in line with the principles laid down in Regulation (EC) No 429/2008[13] and the relevant guidance documents: 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, 2012e), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012f), 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

[9] FEED dossier reference: FAD-2010-0218.
[10] Technical dossier/Supplementary information/Letter dated 29/4/2021.
[11] Preparations included in the first amendment: ylang ylang oil, camphor white oil and cinnamon tincture; preparations included in the second amendment: nutmeg oil, laurel leaves oil, pepper oil black, cinnamon oil, cassia oil and pepper oleoresin black.
[12] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2010-0218?search&form-return.
[13] 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.

environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA 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, 2019b), General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP, 2021b).[14]

3. Assessment

The additive under assessment, nutmeg oil, is an essential oil obtained from the seeds of *M. fragrans* Houtt., 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

M. fragrans Houtt., known as the nutmeg tree, is a dioecious evergreen tree belonging to the Myristicaceae family. It is native to Indonesia but is now cultivated in other tropical regions. The tree is the source of two spices, nutmeg which is the whole or ground seed, and mace which derives from the aril, a red coloured outgrowth covering the seed. Nutmeg is primarily valued for its culinary properties. It also has found use as an incense, as a folk remedy and as a source of nutmeg butter, a fatty acid-rich exudate of the seed. The seeds of *M. fragrans* are sometimes referred to as 'true nutmeg' to distinguish them from other seeds also locally described as nutmeg (e.g. Japanese nutmeg from *Monodora myristica* (Gaertin.) Dunal or Brazilian nutmeg from *Cryptocarya moschata* Nees & Mart.).

The essential oil is obtained from the dried and crushed seeds by steam distillation. Briefly, steam is passed through the plant material. The steam extracts 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 nutmeg oil

The essential oil under assessment is a colourless to pale yellow clear mobile liquid with a characteristic odour. In seven batches of the additive (all originating from Indonesia), the density (20°C) ranged between 897 and 905 kg/m^3 (specification: 862–922 kg/m^3), the refractive index (20°C) between 1.480 and 1.483 (specification: 1.475–1.488) and the specific optical rotation (at 20°C, five batches) between 12.96° and 14.20° (specification: 8°–18°).[15] Nutmeg oil is identified with the single Chemical Abstracts Service (CAS) number 8008-45-5, the European Inventory of Existing Chemical Substances (EINECS) number 282-013-3, the Flavor Extract Manufacturers Association (FEMA) number 2793 and the Council of Europe (CoE) number 296.

The product specifications used by the applicant are based on those developed by the International Organization for Standardization (ISO) 3215:1998 for Oil of nutmeg, Indonesian type (*M. fragrans* Houtt.),[16] adapted to reflect the concentrations of the main components of the essential oil. Seven compounds contribute to the specifications as shown in Table 2, with α-pinene and myristicin selected as phytochemical markers. Analysis of seven batches of the additive showed compliance with these specifications when analysed by GC-FID and expressed as percentage of gas chromatographic peak area (% GC area).[17]

[14] https://www.efsa.europa.eu/sites/default/files/2021-05/general-approach-assessment-botanical-preparations-containing-genotoxic-carcinogenic-compounds.pdf.
[15] Technical dossier/Supplementary information June 2020/Annex_II_ SIn_Reply_nutmeg_oil_COA_chromatograms.
[16] Technical dossier/Supplementary information June 2020/Annex_III_ SIn_Reply_nutmeg_oil_ISO.
[17] Technical dossier/Supplementary information June 2020/SIn_reply_nutmeg_oil/Table 3.

Table 2: Major constituents of the essential oil from the seeds of *Myristica fragrans* Houtt. as defined by specifications: 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 per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent EU register name	CAS no	FLAVIS no	% GC area	
			Specification[(a)]	Range
Sabinene	3387-41-5	01.059	14–29	19.7–23.8
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	15–28	20.7–22.7
Myristicin	607-91-0	–	5–12	8.3–10.7
β-Pinene (pin-2(10)ene)	127-91-3	01.003	13–18	14.2–16.5
4-Terpinenol	562-74-3	02.072	2–6	3.6–4.4
Limonene	138-86-3	01.001	2–7	4.3–5.8
γ-Terpinene	99-85-4	01.020	2–6	3.0–4.5

EU: European Union; CAS no: Chemical Abstracts Service number; FLAVIS no: EU Flavour Information System numbers.
(a): Specifications defined based on GC–FID analysis.

The applicant provided a full characterisation of the same seven batches obtained by gas chromatography–mass spectrometry (GC–MS).[15] In total, up to 68 peaks were detected in the chromatogram, 61 of which were identified and accounted on average for 99.4% (99.1–100%) of the product (as the GC area). The seven compounds indicated in the product specifications accounted for 79.0% on average (range 78.4–79.5%) of the % GC area (Table 3). Besides these seven compounds, 28 other compounds were detected at individual levels > 0.1% and are also listed in Table 3. These 35 compounds together account on average for 98.9% (98.7–99.3%) of the product. The remaining 26 compounds (ranging between 0.01% and 0.002%) and accounting for 0.5% are listed in the footnote.[18] Based on the available data on the characterisation, nutmeg oil is considered a fully defined mixture (EFSA SC, 2019a).

The FEEDAP Panel notes that the concentration of myristicin determined by GC–MS (and expressed as % GC area, without the use of reference standards for calibration) was outside the range of specification set based on analysis by GC–FID. For quantitative purposes based on GC are percentages, GC–FID is considered more reliable than GC–MS, as the response of the flame ionisation detector (FID) is less influenced by structural differences than the mass spectrometer detector (MS). For this reason, for myristicin, one of the main constituents of nutmeg oil and a substance of concern, the FEEDAP Panel disregarded the values determined by GC–MS and considered the highest value of the specification range (12%) as the highest expected value for myristicin in the additive under assessment.

Table 3: Constituents of the essential oil from the seeds of *Myristica fragrans* Houtt. 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 EU register name	CAS no	FLAVIS no	% GC area	
			Mean	Range
Sabinene	3387-41-5	01.059	20.60	19.05–21.62
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	19.94	18.65–21.31
Myristicin	607-91-0	–	13.08	12.42–13.98

[18] Additional constituents: constituents (n = 9) between ≥0.05 and < 0.1%: *(E)*-α-bergamotene, citronellyl acetate, β-bisabolene, α-cubebene, citronellol, *(E)*-methyl isoeugenol, cis-piperitol, 1-isopropenyl-4-methylbenzene, α-fenchene; constituents (n = 13) between ≥0.01 and < 0.05%: 4-terpinenyl acetate, *trans*-piperitol, α-farnesene, *(Z)*-isoeugenol, *trans*-3,7-dimethyl-1,3,6-octatriene, *d,l*-borneol, tricyclene, β-caryophyllene, *trans*-cadina-1,4-diene, cis-3,7-dimethyl-1,3,6-octatriene, 2-(4-methylphenyl)propan-2-ol, germacra-1(10),4(14),5-triene, pinocarveol; constituents (n = 4) < 0.01%: 1,8-cineole, fenchyl alcohol, pinocarvone, 2,4-thujadiene.

Constituent EU register name	CAS no	FLAVIS no	% GC area	
			Mean	Range
β-Pinene (pin-2(10)ene)	127-91-3	01.003	12.96	12.29–13.76
4-Terpinenol	562-74-3	02.072	4.13	3.97–4.63
Limonene	138-86-3	01.001	4.07	3.77–4.57
γ-Terpinene	99-85-4	01.020	4.03	3.30–4.85
α-Terpinene	99-86-5	01.019	2.82	2.35–3.37
Myrcene	123-35-3	01.008	2.13	1.66–2.32
β-Phellandrene	555-10-2	01.055	2.03	1.61–2.46
Safrole[(a)]	94-59-7	–	2.02	1.69–2.30
α-Thujene	2867-05-2	–	1.75	1.51–1.87
Terpinolene	586-62-9	01.005	1.52	1.42–1.62
δ-3-Carene	13466-78-9	01.029	0.94	0.72–1.12
α-Phellandrene	99-83-2	01.006	0.84	0.73–0.93
p-Cymene (1-isopropyl-4-methylbenzene)	99-87-2	01.002	0.83	0.51–1.16
(E)-isoeugenol	5932-68-3	–	0.75	0.70–0.89
α-Terpineol	98-55-5	02.014	0.67	0.60–0.84
Camphene	79-92-5	01.009	0.35	0.19–0.48
Elemicin	487-11-6	–	0.35	0.31–0.40
trans-Sabinene hydrate	17699-16-0	–	0.31	0.19–0.41
p-Pentylanisole	20056-58-0	–	0.31	0.30–0.31
4-Allyl-2,6-dimethoxyphenol	6627-88-9	04.051	0.30	0.26–0.36
α-Copaene	3856-25-5	–	0.30	0.26–0.32
Eugenol	97-53-0	04.003	0.29	0.26–0.35
Linalool	78-70-6	02.013	0.25	0.18–0.39
α-Terpinyl acetate	80-26-2	09.015	0.25	0.14–0.29
Methyleugenol[(b)]	93-15-2	04.012	0.23	0.18–0.33
cis-Sabinene hydrate	15537-55-0	–	0.19	0.07–0.30
Geranyl acetate	105-87-3	09.011	0.17	0.16–0.19
cis-p-2-menthen-1-ol	29803-82-5	–	0.16	0.09–0.20
d,l-Bornyl acetate	76-49-3	09.017	0.12	0.04–0.15
trans-p-2-menthen-1-ol	29803-81-4	–	0.11	0.05–0.15
δ-Cadinene	29350-73-0	01.021	0.11	0.09–0.12
Ethanol	64-17-5	02.078	0.10	0.09–0.12
Total			98.9	98.7–99.3[(c)]

EU: European Union; CAS no: Chemical Abstracts Service number; FLAVIS no: EU Flavour Information System numbers.
(a): Substance which shall not be added as such to food (Annex III), maximum level in food is set by Regulation (EC) No 1334/2008, including meat products (15 mg/kg), fish products (15 mg/kg), soups and sauces (25 mg/kg) and non-alcoholic beverages (1 mg/kg).
(b): Substance which shall not be added as such to food (Annex III), maximum level in food is set by Regulation (EC) No 1334/2008, including dairy products (20 mg/kg), meat products (15 mg/kg), fish products (10 mg/kg), soups and sauces (60 mg/kg), ready-to eat savouries (20 mg/kg) and non-alcoholic beverages (1 mg/kg).
(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 regarding substances of concern and chemical composition of the plant species *M. fragrans* and its preparations.[19] The search identified the presence of four *p*-allylalkoxybenzene derivatives, i.e. myristicin (a characteristic constituent of *M. fragrans*), safrole, elemicin and methyleugenol (Muchtaridi et al., 2010; Ogawa and Ito, 2019). An analysis of the seven batches of the nutmeg oil under assessment (see Tables 2 and 3) confirmed the presence of myristicin (8.3–10.7%, when analysed by GC-FID), safrole (1.69–2.30%), elemicin (0.31–0.40%) and methyleugenol (0.18–0.33%).

[19] Technical dossier/Supplementary information June 2020/Literature search_nutmeg_oil.

3.2.2. Impurities

The applicant referred to the 'periodic testing' of some representative flavourings premixtures for mercury, cadmium and 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. Since nutmeg oil is produced by steam distillation, the likelihood of any measurable carryover of all the above-mentioned elements is considered low, except for mercury.

3.2.3. Shelf life

The typical shelf-life of nutmeg 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).[20] However, no data supporting this statement were provided.

3.2.4. Conditions of use

Nutmeg 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 4. No use level has been proposed by the applicant for the other target species and for the use in water for drinking.

Table 4: Conditions of use for the essential oil from the seeds of *Myristica fragrans* Houtt.: Maximum proposed use levels in complete feed for certain target animal categories

Animal category	Maximum use level (mg/kg complete feed)
Long-living and reproductive animals	
Laying hen	0.2
Sow lactating	0.3
Dairy cow	0.3
Sheep/goat	0.5
Horse	0.5
Rabbit	0.2
Dog	0.6
Cat	0.5
Ornamental fish	2.5
Species for fattening	
Chicken for fattening	6.5
Turkey for fattening	8.7
Piglet	10
Pig for fattening	10
Veal calf (milk replacer)	10
Cattle for fattening	10
Sheep/goat	10
Horse	10
Rabbit	10
Salmon	10

3.3. Safety

The assessment of safety of nutmeg oil is based on the maximum use level proposed by the applicant for the species listed above (see Table 4).

Many of the components of nutmeg oil, accounting for about 80% of the total % GC peak areas, have been previously assessed and considered safe for use as flavourings, and are currently authorised for use in food[7] without limitations and for use in feed[6] at individual use levels higher than those

[20] Technical dossier/Section II.

resulting from the intended use of the essential oil in feed. The list of the compounds already evaluated by the EFSA Panels is given in Table 1 (see Section 1.2).

The oil under assessment contains myristicin, safrole, elemicin and methyleugenol, which together account for up to 15% of the % GC area, when considering the highest proposed specification of 12% for myristicin.

Four compounds listed in Table 1, δ-cadinene [01.021], δ-germacrene [01.042], β-phellandrene [01.055] and tricyclene [01.060] were evaluated in FGE25.Rev2 by applying the Procedure described in the Guidance on the data required for the risk assessment of flavourings to be used in or on foods (EFSA CEF Panel, 2010). For these compounds, for which there is no concern for genotoxicity, EFSA requested additional toxicity data (EFSA CEF Panel, 2011b). In the absence of such toxicological data, the CEF Panel was unable to complete its assessment. As a result, these compounds are no longer authorised for use as flavours in food. For these compounds, in the absence of toxicity data, the FEEDAP Panel applies the threshold of toxicological concern (TTC) approach or read-across from structurally related substances, as recommended 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).

Sixteen components[21] accounting together on average for 3.2% of the GC area have not been previously assessed for use as flavourings. The FEEDAP Panel notes that they are aliphatic mono- or sesqui-terpenes structurally related to flavourings already assessed in CG 6, 8 and 31 and a similar metabolic and toxicological profile is expected. Because of their lipophilic nature, they are expected to be rapidly absorbed from the gastrointestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2012a, 2015, 2016c,d).

The following sections focus on the *p*-allylalkoxybenzenes, mainly on myristicin, based on the evidence provided by the applicant in the form of several literature searches. For the absorption, distribution, metabolism and excretion (ADME) and the toxicology of safrole, reference is made to the safety evaluation made by the FEEDAP Panel in the EFSA opinion on cinnamon bark oil and cinnamon leaf oil (EFSA FEEDAP Panel, 2022a) and for the ADME and toxicology of methyleugenol to the opinion on laurel leaf oil (EFSA FEEDAP Panel, 2023a).

3.3.1. Absorption, distribution, metabolism and excretion

_p_-Allylalkoxybenzenes (myristicin, safrole, elemicin and methyleugenol)

The additive contains four *p*-allylalkoxybenzene derivatives, myristicin, safrole, elemicin and methyleugenol, with myristicin accounting for up to 12%.

In 2009, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the data from experimental and human studies of ADME of safrole, myristicin, estragole and methyleugenol and other *p*-allylalkoxybenzene derivatives and concluded that they are rapidly absorbed after consumption by the oral route. Their distribution, evaluated in animals with labelled compounds, is also rapid. Excretion of low doses is almost complete within 24 h as CO_2 in exhaled air and as hydroxylated metabolites in urine and their conjugated derivatives. At higher doses, excretion in exhaled air decreases while the urinary fraction of metabolites increases.

A comprehensive review of metabolic studies performed in experimental animals was made by JECFA (WHO, 2009), which identified three main biotransformation pathways for the metabolism of *p*-allylalkoxybenzenes (either methylenedioxy- or methoxy-substituted):

1) *O*-Demethylenation of safrole, myristicin (and apiole) and *O*-demethylation of the one or more of the methoxy substituents of estragole, methyleugenol or elemicin followed by excretion of the hydroxylated compounds in the conjugated forms. The *O*-dealkylation pathway is predominant at low doses of the compounds in humans, mice and rats.
2) Epoxidation of the double bond in the allyl side-chain forming the 2′,3′-epoxide which is then hydrolysed by the epoxide hydrolase producing the diol or is conjugated with glutathione.
3) A bioactivation pathway of methylenedioxy- or methoxy-substituted *p*-allylalkoxybenzenes produced by the hydroxylation of the alkene side-chain forming the 1′-hydroxymetabolite which can be conjugated with either glucuronic acid or sulfate or can undergo isomerisation. The sulfate conjugate of the 1′-hydroxymetabolite is considered the metabolite, which is the

[21] *cis*-*p*-2-menthen-1-ol, *trans*-*p*-2-menthen-1-ol, 4-terpinenyl acetate, *trans*-sabinene hydrate, *cis*-sabinene hydrate, *trans*-piperitol, *cis*-piperitol (CG 6); pinocarvone (CG 8); *trans*-3,7-dimethyl-1,3,6-octatriene, α-thujene, α-copaene, α-bergamotene, α-cubebene, α-fenchene, *trans*-cadina-1,4-diene and 2,4-thujadiene (CG 31).

hepatotoxic and hepatocarcinogenic agent of some of these compounds in rodents. The sulfate conjugate is unstable, and hydrolysis generates a reactive electrophilic intermediate which binds to proteins and DNA. The formation of protein and DNA adducts in liver is dose dependent as demonstrated *in vivo*. At low doses the *O*-demethylenation of myristicin and safrole is by far the predominant pathway, giving rise to dihydroxyallylbenzene metabolites that are readily excreted either free or as sulfate or glucuronic acid conjugates. At high doses in rodents, the *O*-demethylenation pathway becomes saturated, and 1′-hydroxylation and epoxidation of the allyl side-chain become more prevalent.

The FEEDAP Panel noted that in 2019 the National Toxicology Program (NTP) published a report on toxicity studies of myristicin, including data on ADME (NTP, 2019). No reference to new studies additional to those described in WHO (2009) were cited in this report. Because myristicin is the predominant *p*-allylalkoxybenzene derivative in the additive, its metabolism is considered in more detail.

The main metabolic step of myristicin is the oxidative removal of the methylene bridge with formation of two free phenolic hydroxy groups, which are subject to conjugation with glucuronic acid or sulfate or methylation. Minor pathways of myristicin metabolism are epoxidation of the double bond in the side chain with subsequent hydrolysation to a diol and hydroxylation in the alpha-position of the side chain. A conjugation of this aliphatic hydroxy group with glucuronic acid leads to excretion, whereas a sulfate conjugation produces a labile intermediate, which is unstable and breaks down to form a highly reactive carbonium ion, which can form covalent adducts with DNA and other macromolecules.

Similar metabolic pathways have been described for safrole (European Commission, 2001; reviewed in EFSA FEEDAP Panel, 2022a) and for methyleugenol (IARC, 2018; reviewed in EFSA FEEDAP Panel, 2023a).

Limited information is available on the metabolism of elemicin. Considering the structural similarity with methyleugenol, it can be inferred that similar metabolic pathways occur. When elemicin was incubated with rat and human microsomes, seven metabolites were identified in this *in vitro* model: 2′,3′-dihydroxyelemicin, 1′-hydroxyelemicin, 3-hydroxyelemicin, elemicin-2′,3′-oxide, 4-hydroxy-3,5-dimethoxyallylbenzene, 3-hydroxy-4,5-dimethoxyallylbenzene and a minor unidentified metabolite. Glucuronidation of 1′-hydroxyelemicin, representing a detoxification pathway, was the most important pathway in rat and in human microsomes; the bioactivation of 1′-hydroxyelemicin by sulfonation showed to represent only a minor pathway in both rat and human microsomes. However, it is expected that in species with a reduced capacity of glucuronidation (i.e. cats) the sulfation will become the predominant conjugation step. The application of physiologically based kinetic (PBK) models predicted that in rat liver the formation of the 1′-sulfoxy metabolite is 11- and 2-fold lower as compared to the formation of the 1′-sulfoxymetabolites of estragole and methyleugenol, respectively (van den Berg et al., 2012).

Both WHO (2009) and NTP (2019) described in detail an *in vivo* study carried out by Beyer et al., 2006. In this study, rats were orally administered 100 mg/kg body weight (bw) of safrole, myristicin or elemicin, either individually or as an aqueous suspension of ground nutmeg at 500 mg/kg bw from two different batches of powdered nutmeg. The urine collected over a 24-h period was analysed by GC–MS after enzymatic hydrolysis. In the same study, the urine of a human nutmeg abuser (an inpatient of a psychiatric hospital) that had consumed powder of about five nutmegs (20–50 g of nutmeg, corresponding to 140–280 mg elemicin or 2.3–4.6 mg elemicin/kg bw, 100–200 mg myristicin or 1.6–3.2 mg myristicin/kg bw, and approximately 20 mg safrole or 0.3 mg safrole/kg bw, as described in WHO Food Additives Series:60, 2009) was also analysed by GC–MS.

In the 24-h urine of rats dosed with safrole, myristicin or elemicin at 100 mg/kg bw metabolites derived from *O*-demethylenation (*O*-demethylation for elemicin), epoxidation and hydrolysis (as a 2,3-dihydroxy derivative) and 1′-hydroxylation were identified. *O*-Demethylenation was the main metabolic step for safrole and myristicin and side-chain hydroxylation in position 2′ and 3′ for elemicin. From the comparison of the relative peak area of the metabolites, it appeared that the formation of the minor metabolite 1′-hydroxymetabolite was higher for elemicin when compared with safrole or myristicin at the same dose level. All metabolites were partly excreted as glucuronides and/or sulfates.

When rats were dosed orally with an aqueous suspension of ground nutmeg at 500 mg/kg bw (approximately 1–2 mg myristicin/kg bw, with lower amounts of safrole and elemicin), metabolites resulting from *O*-demethylenation or *O*-demethylation, and epoxidation followed by hydrolysis were detected in the urine. The major metabolite was 3,4-dihydroxy-5-methoxyallylbenzene, resulting from the *O*-demethylenation of myristicin. The *O*-demethylenation metabolite of safrole, the

O-demethylation metabolite of elemicin and the side-chain hydroxylated metabolites (in position 2′ and 3′) of elemicin and myristicin were also present. No 1′-hydroxymetabolites were detected (limit of detection not given). A similar metabolic profile was observed in the urine of a human patient after ingestion of 20–50 g of nutmeg (corresponding to up to 280 mg elemicin, 200 mg myristicin and 20 mg safrole) (Beyer et al., 2006).

Based on the above, the four *p*-allylakoxybenzene derivatives, myristicin, safrole, elemicin and methyleugenol, are well absorbed, extensively metabolised and rapidly excreted. The detoxication and bioactivation pathways are well established in experimental animals and are the same in humans as shown by some data. The bioactivation pathway is very minor for low doses, however it is dose dependent.

3.3.2. Toxicology

In the previous assessments of essential oils containing *p*-allylalkoxybenzenes, a read-across from methyleugenol to other *p*-allylalkoxybenzenes was applied based on considerations on the structural and metabolic similarity among all the components of the assessment group (EFSA FEEDAP Panel, 2022a,b,c, 2023a,b).

For myristicin, which is present in nutmeg oil in much higher concentrations (up to 12% according to the specification) compared to the essential oils previously evaluated, the specific toxicological information is considered more in detail and summarised in the next sections. In particular, compound-specific data are available for *in vitro* genotoxicity, subchronic oral toxicity and acute toxicity of myristicin. Furthermore, neurotoxic effects (including hallucinogenic effects) have been associated with the acute overdose of nutmeg and related to the presence of myristicin (reviewed by Hallström and Thuvander, 1997; Dolan et al., 2010; Rahman et al., 2015; Götz et al., 2022).

3.3.2.1. Genotoxicity and carcinogenicity

For fully defined mixtures, the EFSA Scientific Committee (EFSA SC) recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential using all available information, including read-across and quantitative structure–activity relationship (QSAR) considerations about their genotoxic potential (EFSA SC, 2019b). Therefore, the potential genotoxicity of identified constituents is first considered. Then, *in vitro* genotoxicity studies performed with nutmeg oil similar to the additive under assessment are described.

p-Allylalkoxybenzenes (myristicin, safrole, elemicin and methyleugenol)

Nutmeg oil contains safrole (range: 1.69–2.30%) and methyleugenol (0.18–0.32%), two compounds with experimentally proven genotoxicity and carcinogenicity in rodents as reviewed by the Scientific Committee on Food (European Commission, 2001) and IARC (2018). The genotoxicity and carcinogenicity studies with safrole and methyleugenol and other structurally related *p*-allylalkoxybenzenes were reviewed by the FEEDAP Panel (EFSA FEEDAP Panel, 2022a,c, 2023a).

In addition, the additive contains myristicin (up to the highest proposed specification of 12%) and elemicin (0.31–0.40%), two compounds which also belong to the class of *p*-allylalkoxybenzenes. They are structurally related to compounds with experimentally proven genotoxicity and carcinogenicity in rodents like safrole and methyleugenol.

For *p*-allylalkoxybenzenes, the FEEDAP Panel identified a reference point for neoplastic endpoints derived from a carcinogenicity study in rat with methyleugenol (NTP, 2000) by applying the benchmark dose (BMD) approach with model averaging. Dose–response modelling using hepatocellular carcinomas in male rats as a response yielded a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) of 22.2 mg/kg bw per day (Suparmi et al., 2019). This $BMDL_{10}$ value was selected as reference point for the assessment group of *p*-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022b) irrespective of their relative potency (EFSA FEEDAP Panel, 2022c).

Myristicin

Myristicin was not mutagenic in *S*. Typhimurium strains TA97, TA98, TA100 or TA1535, when tested with or without metabolic activation. Myristicin was negative for induction of micronuclei in mice. In rats, myristicin induced small but statistically significant increases in micronuclei that were within or just at the limit of the laboratory historical control ranges (mean ± two standard deviations) (NTP, 2019). However, the negative results in *S*. Typhimurium strains may be a consequence of the limited metabolic activation provided by the S9 mix. Therefore, the standard bacterial mutagenicity

tests conducted by NTP may be inadequate to predict the *in vivo* mutagenic potential of these *p*-allylalkoxybenzenes.

The data on the carcinogenicity of myristicin are limited. The possible carcinogenic activity of a variety of alkenylbenzenes, including myristicin, was investigated in newborn male mice, injected intraperitoneally (i.p.) with nine different compounds at days 1, 8, 15 and 22 after birth. Among these, estragole, safrole and methyleugenol induced a significant number of hepatomas at 13 months, whereas anethole, elemicin, myristicin, dillapiole, parsley apiole and eugenol did not under the limited conditions of the study (Miller et al., 1983). Although there is a clearly demonstrated difference between the two groups of substances, the design of the study showed significant limitations due to the dosing regimen, route of administration and duration,[22] which prevented a conclusion to be reached.

Phillips et al. (1984) investigated the formation of DNA adducts induced by those *p*-allylalkoxybenzenes already studied by Miller et al. (1983). It was demonstrated that myristicin induced DNA-adducts in the liver of newborn mice after i.p. injection, although the yield was 10 times lower compared to methyleugenol at the same dose. These data were confirmed by Randerath et al. (1984) in female adult CD-1 mice, receiving the compounds by i.p. injection and by Zhou et al. (2007), in cultured human HepG2-cells.

The data on the genotoxicity and carcinogenicity of myristicin have been recently reviewed (Götz et al., 2022). The authors concluded that further and more adequate studies are needed to allow for a conclusive evaluation of the mutagenic potential of myristicin. In order to evaluate the relative potency of myristicin compared to other *p*-allylakoxybenzenes, such as safrole, comparative studies would be needed. In addition, the *in vivo* mutagenic potential of myristicin has not been assessed using approaches such as transgenic rodent models.

The specific data on myristicin reviewed by the FEEDAP Panel do not change the conclusions on the genotoxicity and carcinogenicity of myristicin reached in the previous assessments, where myristicin was considered of concern based on its structural similarity to safrole and methyleugenol, two compounds with experimentally proven genotoxicity and carcinogenicity in rodents.

Genotoxicity studies with the nutmeg oil

The applicant performed a literature search and retrieved several genotoxicity studies. However, most of these studies were considered not relevant, as they were performed with a different test item, i.e. nutmeg seed soaked in distilled water (Alabi et al., 2016), methanolic leaf extract (Akinboro et al., 2014), ethanolic nutmeg seed extract (Mahmoud et al., 1992). Only one study investigated the genotoxicity of nutmeg oil (commercial sample, further details not mentioned) *in vitro* in an Ames test and a chromosomal aberration test. The Ames test was conducted using *S*. Typhimurium strains TA92, TA1535, TA100, TA1537, TA94 and TA98; the chromosomal aberration tests were carried out using a Chinese hamster fibroblast cell line. Both tests gave negative results (Ishidate et al., 1984).

3.3.2.2. Subchronic oral toxicity studies

p-Allylalkoxybenzenes

The FEEDAP Panel identified a no observed adverse effect level (NOAEL) of 10 mg/kg bw per day for non-neoplastic lesions (effect on liver and the glandular stomach) from a 90-day study in mice with methyleugenol (NTP, 2000). Considering the structural similarity and the similar mode of action of *p*-allylalkoxybenzenes, the FEEDAP Panel selected the NOAEL of 10 mg/kg bw per day as reference point for the assessment group *p*-allylalkoxybenzenes for non-neoplastic endpoints (EFSA FEEDAP Panel, 2023a).

Myristicin

Myristicin was tested in a repeated dose toxicity assay over a period of 13 weeks in rats and mice dosed with 10, 30, 100, 300 or 600 mg/kg bw by gavage for 5 days per week (NTP, 2019). Rats and mice of both sexes showed increased relative liver weight at doses of 100 mg/kg bw and above ($p < 0.01$); however, smaller but significant differences were seen at the 30 mg/kg bw in male rats ($p < 0.05$) and at 10 mg/kg bw in female mice ($p < 0.01$). This weight difference is associated in both rats and mice with histopathological changes in the liver (fatty change, centrilobular hepatocellular

[22] Although more susceptible new-born mice were used, the limited administration on a few days via intraperitoneal injection is not comparable with lifetime exposure; only the cumulative dose was available in the report.

hypertrophy, hepatocellular necrosis, and oval cell hyperplasia) at doses of 600 mg/kg bw in both rats and mice, with fatty change present in male mice at 100 mg/kg bw. There are no histopathological changes observed in the liver at the lowest two doses in either mice or rats.

This study showed treatment-related differences at the lowest dose tested (increased cholesterol and alanine aminotransferase in female rats and increased relative liver weight in female mice). The FEEDAP Panel considered 10 mg/kg bw per day as the lowest observed adverse effect level (LOAEL) for female mice and rats and applied an additional uncertainty factor (UF) of 3 to extrapolate from the LOAEL to a NOAEL of 3.3 mg/kg bw per day.

The magnitude of the UF was selected considering that (i) no effects were seen in male rats and in male mice at 10 mg/kg bw per day; (ii) in all the subchronic studies with *p*-allylalkoxybenzenes, the liver was the most sensitive organ; (iii) the magnitude of the NOAEL derived for methyleugenol is very close to the LOAEL for myristicin in female mice and rats.

The FEEDAP Panel considers that this NOAEL would also be protective for possible neurotoxic effects (see Section 3.3.2.3).

3.3.2.3. Other toxicological effects

The applicant provided a literature search to address the neurotoxic effect (including hallucinogenic effects) associated with the acute overdose of nutmeg which have been observed in humans and their relevance for the target species at the proposed use levels of nutmeg oil in feed. The applicant carried out a structured database search using four meta-search sites (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. The literature search (no time limits) was conducted in August 2022 and was aimed at identifying publications referring to 'nutmeg', 'myristicin' and/or 'myristica fragrans' (CAS No. 607-91-0, 8008-45-5, 84082-68-8, 84082-68-8) in relation to neurotoxic*, hallucinogen*, psychotrop*, narcotic* effects. A detailed description of the iterations used, and the inclusion/exclusion criteria applied for the selection were provided.[23] The search identified 15 relevant publications, 23 additional publications were identified by a complementary search via Google Scholar, and seven more publications from the list of references of the review papers. In total 45 references were selected and submitted.

Several reports of nutmeg intoxications and cases of abuse associated with hallucination have been published after 1960s. The doses ingested ranged generally between 5–15 g of nutmeg. Symptoms appear usually 2–6 h after ingestion and may persist for 9 h up to several days. The available information has been reviewed by several authors (Hallström and Thuvander, 1997; Dolan et al., 2010; Rahman et al., 2015; Götz et al., 2022). Neurotoxic effects of myristicin have been reported to occur in a dose-dependent manner. In the study conducted by Hallström and Thuvander (1997) a single oral dose of 400 mg myristicin (corresponding to 6–7 mg/kg bw) produced mild cerebral stimulation in 4 out of 10 human subjects. However, the minimum dosage that has been reported from intoxication cases to cause psychogenic effects in humans is 5 g ground nutmeg/person corresponding to 1 to 2 mg myristicin/kg bw. The authors see the reason for the lower effect dose of myristicin in nutmeg poisonings compared to the experimental administration of pure myristicin in the combined action of myristicin with other components of nutmeg (Hallström and Thuvander, 1997; Rahman et al., 2015).

The formation of the metabolite 3-methoxy-4,5-methylendioxyamphetamin (MMDA) has been hypothesised as responsible for the psychotropic effect of myristicin (Shulgin, 1966; Kalbhen, 1971), however, the metabolite could not be detected in rat or human urine following exposure to nutmeg (Beyer et al., 2006) and the hypothesis could not be confirmed.

In laboratory animals, limited evidence indicated that no toxic effects were observed in rats (n = 25) administered myristicin perorally at a dose of 10 mg/kg bw for 26 days (Truitt et al., 1960 as referenced in Hallström and Thuvander, 1997).

No information was retrieved on neurotoxic effects in the target species.

3.3.2.4. Conclusions on toxicology

In the absence of specific carcinogenicity data for myristicin, the FEEDAP Panel retains the $BMDL_{10}$ of 22.2 mg/kg bw per day derived from a carcinogenicity study in rat with methyleugenol, as reference point for the assessment group of *p*-allylalkoxybenzenes for neoplastic lesions.

For non-neoplastic endpoints, the FEEDAP Panel identified a NOAEL of 3.3 mg/kg bw per day from a 90-day study with myristicin, which is applied in the current assessment as compound-specific

[23] Technical dossier/Supplementary information March 2023/Annex_II_literature search_nutmeg_neurotox.

reference point to myristicin. For the other *p*-allylalkoxybenzene derivatives present in the additive (safrole, elemicin and methyleugenol), the FEEDAP Panel retains the NOAEL of 10 mg/kg bw per day derived from the mice study with methyleugenol.

3.3.3. Safety for the target species

Tolerance studies in 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 the individual components can be grouped into assessment groups, based on structural and metabolic similarity. The combined toxicity can be predicted using the dose addition assumption within an assessment group, taking into account the relative toxic potency of each component.

As the additive under assessment is a fully defined mixture (the identified components represent > 99% of the % GC area, see Section 3.2.1), the FEEDAP Panel applied a component-based approach to assess the safety for target species of the essential oil. Substances for which a concern for genotoxicity has been identified (myristicin, safrole and methyleugenol) are assessed separately.

Components other than myristicin, safrole, elemicin and methyleugenol

Based on considerations related to structural and metabolic similarities, the components were allocated to eight assessment groups, corresponding to the chemical groups (CGs) 1, 3, 6, 8, 17, 18, 26 and 31, as defined in Annex I of Regulation (EC) No 1565/2000. For chemical group 31 ('aliphatic and aromatic hydrocarbons'), sub-assessment groups as defined in Flavouring Group Evaluation 25 (FGE.25) and FGE.78 were established (EFSA CEF Panel, 2015a,b). 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 of target animals was estimated considering the use levels in feed, the percentage of the component in the oil and the default values for feed intake according to the guidance on the safety of feed additives for target species (EFSA FEEDAP Panel, 2017b). Default values on body weight are used to express exposure in terms of mg/kg bw per day. The intake levels of the individual components calculated for chickens for fattening, the species with the highest ratio of feed intake/bw 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 NOAEL values. Structural and metabolic similarity among the components in the assessment groups were evaluated to explore the application of read-across allowing extrapolation from a known NOAEL of a component of an assessment group to the other components of the group with no available NOAEL or, if sufficient evidence were available for members of a (sub-)assessment group, to derive a (sub-)assessment group NOAEL.

Toxicological data of subchronic studies, from which NOAEL values could be derived, were available for ethanol [02.078] in CG 1 (EFSA FEEDAP Panel, 2013), citral [05.020] the reference compound in CG 3 (EFSA FEEDAP Panel, 2016a), citronellol [02.011] (EFSA FEEDAP Panel, 2016b), terpineol [02.230][24] and linalool [02.013] in CG 6 (EFSA FEEDAP Panel, 2012a), 1,8-cineole [03.001] (EFSA FEEDAP Panel, 2012b, 2021a), eugenol [04.003] and *trans*-anethole [04.051] in CG 18 (EFSA FEEDAP Panel, 2011), methyl isoeugenol [04.013] in CG 26 (EFSA FEEDAP Panel, 2012d), myrcene [01.008], *d*-limonene [01.045], *p*-cymene [01.002] and β-caryophyllene [01.007] in CG 31 (EFSA FEEDAP Panel, 2015, 2016d).

Considering the structural and metabolic similarities, read-across was applied using the NOAEL of 345 mg/kg bw per day for citral [05.020] to extrapolate to geranyl acetate [09.011] in CG 3.

For the subgroup of terpinyl derivatives in CG 6, i.e. α-terpineol [02.072], 4-terpinenol [02.072] and α-terpinyl acetate [09.015], the reference point was selected based on the NOAEL of 250 mg/kg bw per day available for terpineol [02.230] and d-limonene [01.045].

The NOAELs of 250 and 222 mg/kg bw for the representative compounds of CG 31, d-limonene [01.045] and β-caryophyllene [01.007] were applied, respectively, using read-across to the compounds

[24] 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.

within sub-assessment group III (γ-terpinene [01.020], α-terpinene [01.019], β-phellandrene [01.055], terpinolene [01.005] and α-phellandrene [01.006]) and group V (sabinene [01.059], α-pinene [01.004], β-pinene [01.003], δ-carene [01.029], camphene [01.009] and δ-cadinene [01.021])[25] (EFSA CEF Panel, 2015a,b). The FEEDAP Panel applied the same NOAEL value from sabinene [01.059] to trans-sabinene hydrate [02.085] and cis-sabinene hydrate in CG 8.

For the remaining compounds,[26] 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).

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 SC, 2019a). A 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.[27]

The approach to the safety assessment of nutmeg oil for the chickens for fattening is summarised in Table 5. The calculations were done for chickens for fattening, the species with the highest ratio of feed intake/bw and represent the worst-case scenario at the use level of 6.5 mg/kg in feed.

Table 5: Compositional data, intake values (calculated for chickens for fattening at 6.5 mg/kg complete feed), reference points and margin of exposure (MOE) for the individual components of nutmeg oil classified according to 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[(c)]**	**MOE**	**MOET**
Constituent	–	**%**	**mg/kg**	**mg/kg bw per day**	–	**mg/kg bw per day**	–	–
CG 6								
4-Terpineol	02.072	4.63	0.463	0.0270	(I)	250	9,249	
cis-p-2-Menthen-1-ol	–	0.20	0.020	0.0012	I	*3*	2,558	
trans-p-2-Menthen-1-ol	–	0.15	0.015	0.0009	I	*3*	3,382	
4-Terpinenyl acetate	–	0.04	0.004	0.0002	I	3	12,241	
2-(4-Methylphenyl) propan-2-ol	02.042	0.01	0.001	0.0001	I	3	42,843	
MOET CG 6								1,111

[25] Some of these compounds are not listed in Table 5 because their individual margin of exposure (MOE) was > 50,000.

[26] cis-para-2-menthen-1-ol, trans-para-2-menthen-1-ol, 4-terpinenyl acetate and 2-(4-methylphenyl)propan-2-ol (CG 6); *d,l*-bornyl acetate [09.017], *cis*-piperitol, trans-piperitol, *d,l*-borneol [02.016], pinocarveol [02.100], fenchyl alcohol [02.038] and pinocarvone (CG 8); *(E)*-isoeugenol and *(Z)*-isoeugenol (CG 17); p-pentylanisole (CG 26); 1-isopropenyl-4-methylbenzene (CG 31, IVe); α-thujene, α-copaene, (E)-α-bergamotene, α-cubebene, α-fenchene, tricyclene [01.060], *trans*-cadina-1,4-diene and 2,4-thujadiene (CG 31, V); germacra-1(10),4(14),5-triene [01.042] (CG 31, VI).

[27] Compounds included in the assessment groups but not reported in the table: ethanol (CG 1); geranyl acetate (CG 3); citronellol and citronellyl acetate (CG 4); α-terpineol, linalool and α-terpinyl acetate (CG 6); trans-sabinene hydrate, cis-sabinene hydrate and fenchyl alcohol (CG 8); 1,8-cineole (CG 16); 4-allyl-2,6-dimethoxyphenol and eugenol (CG 18); α-farnesene, trans-3,7-dimethyl-1,3,6-octatriene and cis-3,7-dimethyl-1,3,6-octatriene (CG 31, II); β-bisabolene (CG 31, III); camphene, δ-cadinene and β-caryophyllene (CG 31, V); germacra-1(10),4(14),5-triene (CG 31, VI).

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								
d,l-Bornyl acetate	09.017	0.15	0.015	0.0009	I	*3*	3,427	
cis-Piperitol	–	0.09	0.009	0.0005	I	*3*	5,588	
trans-Piperitol	–	0.03	0.003	0.0002	I	*3*	15,57,9	
d,l-Borneol	02.016	0.02	0.002	0.0001	I	*3*	27,059	
Pinocarveol	02.100	0.02	0.002	0.0001	I	*3*	30,242	
Pinocarvone	–	0.01	0.001	0.0000	III	*0.15*	4,284	
MOET CG 8								1,193
CG 17								
(*E*)-Isoeugenol	–	0.89	0.089	0.0052	I	*3*	581	
(*Z*)-Isoeugenol	–	0.03	0.003	0.0002	I	*3*	15,121	
MOET CG 17								559
CG 26								
p-Pentylanisole	–	0.31	0.031	0.0018	I	*3*	1,637	
CG 31, II (Acyclic alkanes)								
Myrcene	01.008	2.32	0.232	0.0136	(I)	**44**	3,246	
CG 31, III (Cyclohexene hydrocarbons)								
d-Limonene	01.045	4.57	0.457	0.0267	(I)	**250**	9,375	
γ-Terpinene	01.020	4.85	0.485	0.0283	(I)	250	8,830	
α-Terpinene	01.019	3.37	0.337	0.0197	(I)	250	12,721	
β-Phellandrene	01.055	2.46	0.246	0.0144	(I)	250	17,416	
Terpinolene	01.005	1.62	0.162	0.0095	(I)	250	26,398	
α-Phellandrene	01.006	0.93	0.093	0.0054	(I)	250	46,019	
MOET CG 31, III								2,406
CG 31, IVe (Benzene hydrocarbons, alkyl)								
p-Cymene	01.002	1.16	0.116	0.0068	(I)	**154**	22,732	
1-Isopropenyl-4-methylbenzene	01.010	0.06	0.006	0.0004	I	*3*	8,161	
MOET CG 31, IVe								6,005
CG 31, V (Bi-, tricyclic, non-aromatic hydrocarbons)								
Sabinene	01.059	21.62	2.162	0.1262	(I)	222	1,760	
α-Pinene	01.004	21.31	2.131	0.1244	(I)	222	1,785	
β-Pinene	01.003	13.76	1.376	0.0803	(I)	222	2,765	
α-Thujene	–	1.87	0.187	0.0109	I	*3*	275	
δ-3-Carene	01.029	1.12	0.112	0.0066	(I)	222	33,848	
α-Copaene	–	0.32	0.032	0.0019	I	*3*	1,597	
(*E*)-α-bergamotene	–	0.11	0.011	0.0007	I	3	4,590	
α-Cubebene	–	0.07	0.007	0.0004	I	*3*	7,790	
α-Fenchene	–	0.11	0.011	0.0006	I	*3*	4,760	
Tricyclene	01.060	0.03	0.003	0.0002	I	*3*	19,774	
trans-Cadina-1,4-diene	–	0.01	0.001	0.0001	I	*3*	36,723	

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**	–	–
2,4-Thujadiene	–	0.002	0.0002	0.0000	III	*0.15*	12,853	
MOET CG 31, V								154

(a): Intake calculations for the individual components are based on the use level of 6.5 mg/kg in feed for chickens for fattening, the species with the highest ratio of feed intake/body weight. The MOE for each component is calculated as the ratio of the reference point (no observed adverse effect level, 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.

As shown in Table 5, for all the assessment groups, the MOET was ≥ 100. Therefore, no safety concern was identified for the nutmeg oil when used as a feed additive for chickens for fattening at the proposed use levels (6.5 mg/kg) without considering myristicin, safrole, elemicin and methyleugenol.

From the lowest MOET of 154 for chickens for fattening, the MOET was calculated for the other target species considering the respective daily feed intake and conditions of use. The results are summarised in Table 6.

Table 6: Combined margin of exposure (MOET) for the assessment group 'bi-, tricyclic, non-aromatic hydrocarbons' (CG 31, V) calculated for the target species at the proposed use levels in feed

Animal category	Body weight (kg)	Feed intake (g DM/day)	Proposed use levels (mg/kg feed)[(1)]	Lowest MOET
Long-living and reproductive animals				
Laying hens	2	106	0.2	7,460
Sow lactating	175	5,280	0.3	8,787
Dairy cow	650	20,000	0.3	8,503
Sheep/goat	60	1,200	0.5	7,908
Horse	400	8,000	0.5	7,908
Rabbit	2	100	0.2	7,908
Dog	15	250	0.6	7,753
Cat	3	60	0.5	7,908[(2)]
Ornamental fish	0.012	0.054	2.5	6,326
Target species for fattening				
Chicken for fattening	2	158	6.5	154
Turkey for fattening	3	176	8.7	154
Piglet	20	880	10	180
Pig for fattening	60	2,200	10	214
Veal calf (milk replacer)	100	1,890	10	447
Cattle for fattening	400	8,000	10	395
Sheep/goat	60	1,200	10	395
Horse	400	8,000	10	395
Rabbit	2	100	10	158
Salmon	0.12	2.1	10	439

(1): Complete feed containing 88% DM, milk replacer 94.5% DM.
(2): The MOET for cats is increased to 500 because of the reduced capacity of glucuronidation.

Table 6 shows that when the additive is used at the proposed use levels in complete feed the MOET is above the value of 100 for all species/categories. Because glucuronidation is an important metabolic reaction to facilitate the excretion of the components of the essential oil and considering that cats have a low capacity for glucuronidation (Court and Greenblatt, 1997; Lautz et al., 2021), the use of nutmeg oil as additive in cat feed needs a wider margin of exposure. A MOET of 500 is considered adequate. Therefore, for all species listed in Table 6, no safety concern (without considering the presence of myristicin, safrole, elemicin and methyleugenol) was identified for nutmeg oil, when used as a feed additive at the proposed use levels.

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 (EFSA FEEDAP Panel, 2010).

p-*Allylalkoxybenzenes: myristicin, safrole, elemicin and methyleugenol*

Myristicin (up to the highest proposed specification of 12%) and safrole (up to 2.3%) were detected in all batches of the oil under assessment. At the proposed use levels in feed (ranging from 0.2 to 10 mg/kg complete feed), they would result in a concentration of 0.024–1.2 mg myristicin/kg complete feed and 0.005–0.23 mg safrole/kg complete feed.

Lower concentrations of elemicin (up to 0.40%) and methyleugenol (up to 0.33%) were detected in all batches of the oil under assessment. The use of nutmeg oil at the proposed use level in feed, would result in concentrations of 0.8–40 μg elemicin/kg complete feed and 0.66–33 μg methyleugenol/kg complete feed.

Myristicin, safrole, elemicin and methyleugenol share the same structural features, the same metabolic pathways, particularly the formation of the reactive 1′-sulfoxymetabolite (see Section 3.3.1) and the same mode of action. 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). 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, 2021b), different reference points and a different magnitude of the MOET are applied for long-living and reproductive animals (including those animals reared for laying/breeding/reproduction) and for short-living animals. Short-living animals are defined as those animals raised for fattening whose lifespan under farming conditions makes it very unlikely that they develop cancer as a result of the exposure to genotoxic and/or carcinogenic substances in the diet.

For long-living animals and reproductive animals, a MOE(T) with a magnitude > 10,000 when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study is considered indicative of low concern. The FEEDAP Panel identified the $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol (NTP, 2000; Suparmi et al., 2019), as the reference point for the entire group of *p*-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022b). In the current assessment this reference point is applied to the sum of myristicin, safrole, elemicin and methyleugenol. The assessment of the combined exposure to myristicin, safrole, elemicin and methyleugenol for long-living animals is reported in Table 7.

Table 7: Combined exposure and combined margin of exposure (MOET) for the assessment group *p*-allylalkoxybenzenes calculated at the maximum proposed use level of the additive in feed for long-living and reproductive animals based on $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol

Animal category long-living and reproductive animals	Daily feed intake	Body weight	Use level in feed	Combined intake[(a)]	MOET
	kg DM/day	kg	mg/kg feed	μg/kg bw per day	
Laying hen	0.106	2	0.2	1.81	12,146
Sow lactating	5.28	175	0.3	1.55	14,224
Dairy cow	20	650	0.3	1.58	13,948
Sheep/goat	1.2	60	0.5	1.71	12,875

Animal category long-living and reproductive animals	Daily feed intake	Body weight	Use level in feed	Combined intake[(a)]	MOET
	kg DM/day	kg	mg/kg feed	µg/kg bw per day	
Horse	8	400	0.5	1.71	12,875
Rabbit	0.1	2	0.2	1.71	12,875
Dog	0.25	15	0.6	1.71	12,875
Cat	0.06	3	0.5	1.71	12,875
Ornamental fish	0.000054	0.012	2.5	1.92	11,444

(a): Combined intake of myristicin, safrole, methyleugenol and elemicin.

When the estimated exposures for long-living and reproductive animals are compared to the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day (Suparmi et al., 2019), a MOET > 10,000 which is considered of low concern, is obtained for all target animal species.

For short-living animals, genotoxicity and carcinogenicity endpoints are not considered relevant, therefore a lower magnitude of the MOET (> 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP, 2021b). The FEEDAP Panel identified a NOAEL of 3.3 mg/kg bw per day extrapolated from the LOAEL in the subchronic rat study with myristicin (NTP, 2019). In the current assessment this NOAEL is applied to myristicin, whereas a NOAEL of 10 mg/kg bw per day is applied to the other *p*-allylakoxybenzenes (safrole, elemicin and methyleugenol). The assessment of the combined exposure to myristicin, safrole, elemicin and methyleugenol for short-living animals is reported in Table 8.

Table 8: Combined exposure and combined margin of exposure (MOET) for the assessment group *p*-allylalkoxybenzenes calculated at the maximum proposed use level of the additive for target species for fattening based on a NOAEL of 3.3 mg/kg bw per day for myristicin and of a NOAEL of 10 mg/kg bw per day for the other compounds

Animal category: Target species for fattening	Daily feed intake	Body weight	Proposed use level in feed	Combined intake[(a)]	MOET	Use level of no concern in feed
	kg DM/ day	kg	mg/kg feed	µg/kg bw per day		mg/kg feed
Chicken for fattening	0.158	2	6.5	88	43	2.8
Turkey for fattening	0.176	3	8.7	87	43	3.7
Piglet	0.88	20	10	75	50	5.0
Pig for fattening	2.2	60	10	63	60	6.0
Veal calf (milk replacer)	1.89	100	10	30	125	10
Cattle for fattening	8	400	10	34	110	10
Sheep/goat	1.2	60	10	34	110	10
Horse	8	400	10	34	110	10
Rabbit	0.1	2	10	85	44	4.4
Salmon	0.0021	0.12	10	30	123	10

(a): Combined intake of myristicin, safrole, methyeugenol and elemicin.

When comparing the exposure of short-living animals to the reference point based on non-neoplastic endpoints, a magnitude of the MOET > 100, which is considered of no safety concern, is obtained for veal calves, cattle for fattening, sheep/goats, horses and salmon. For the other species, the calculated concentrations in complete feed which are considered of no safety concern are 2.8 mg/kg chickens for fattening, 3.7 mg/kg for turkeys for fattening, 5.0 mg/kg for piglets, 6.0 mg/kg for pigs for fattening, and 4.4 mg/kg for rabbits.

3.3.3.1. Conclusions on safety for the target species

Based on the MOET calculated considering the presence of myristicin, safrole, elemicin and methyleugenol in the product at 12%, 2.30% 0.40% and 0.33% and the conditions of use in the different species, the FEEDAP Panel concludes that:

For all long-living and reproductive animals (including those animals reared for laying/ reproduction) the use of the additive is considered of low concern (MOET > 10,000) at the proposed level in complete feed of 0.2 mg/kg for laying hens and other laying/reproductive birds including animals reared for laying/reproduction, ornamental birds, and rabbits; 0.3 mg/kg for sows and all pigs (Suidae) for reproduction including animals reared for reproduction, dairy cows, other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction; 0.5 mg/kg for sheep/goats, horses and other Equidae and cats; 0.6 mg/kg for dogs and 2.5 mg/kg for ornamental fish.

The Panel considers that the use in water for drinking in long-living and reproductive animals is of low concern provided that the total daily intake of the additive does not exceed the daily amount that is considered of low concern when consumed via feed.

For short-living animals,[28] the Panel has no safety concern when the additive is used at the maximum proposed use level of 10 mg/kg for veal calves (milk replacer), cattle for fattening, other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production, salmonids and minor fin fish. For the other species, the calculated concentrations in complete feed which are considered of no safety concern are 2.8 mg/kg chickens for fattening and minor poultry for fattening, 3.7 mg/kg for turkeys for fattening, 6.0 mg/kg for pigs for fattening, 5.0 mg/kg for piglets and all pigs (Suidae) for meat production, and 4.4 mg/kg for rabbits for meat production.

For any other species not covered above, the value considered of no concern is 0.2 mg/kg complete feed.

For short-living animals, the Panel has no safety concern for the use in water for drinking 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.

3.3.4. Safety for the consumer

Nutmeg and nutmeg oil (mace oil) are added to a wide range of food categories for flavouring purposes. Although individual consumption figures are not available, the Fenaroli's handbook of flavour ingredients (Burdock, 2009) cites values of 2.0 mg/kg bw per day for nutmeg (FEMA 2792) and of 0.2245 mg/kg bw per day for nutmeg oil (FEMA 2793). Fenaroli also reports use levels in food and beverages in the range of 3.8 mg/kg up to 1,480 mg/kg for nutmeg oil.

Many of the individual constituents of the essential oil under assessment are currently authorised as food flavourings without limitations and have been already assessed for consumer safety when used as feed additives in animal production (see Table 1, Section 1.2).

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 nutmeg oil are expected to be extensively metabolised and excreted in the target species. Also, for myristicin, safrole, elemicin and methyleugenol, the available data indicate that they are absorbed, metabolised and rapidly excreted and are not expected to accumulate in animal tissues and products (see Section 3.3.1).

Considering the above and the reported human exposure due to direct use of nutmeg and nutmeg oil in food (Burdock, 2009) it is unlikely that consumption of products from animals given nutmeg oil at the proposed maximum use level would cause a meaningful increase of human background exposure.

No safety concern would be expected for the consumer from the use of nutmeg oil up to the highest safe use level in feed.

3.3.5. Safety for the user

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

The applicant made a literature search aimed at retrieving studies related to the safety of preparations obtained from *M. fragrans* for the users.[29] None of the studies identified during the literature search provided data on endpoints relevant to user safety. However, several reports (Opdyke, 1976 as referenced by Tisserand and Young, 2014; Futrell and Rietschel, 1993) suggest that nutmeg oil is a mild skin irritant and is likely to be a dermal sensitiser.

[28] Short-living animals are defined as those animals raised for fattening whose lifespan under farming conditions makes it very unlikely that they develop cancer as a result of the exposure to genotoxic and/or carcinogenic substances in the diet

[29] Technical dossier/Supplementary information June 2020/Literature_search_nutmeg_oil.

The applicant produced a safety data sheet[30] for nutmeg 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.

Based on the presence of safrole[31] in nutmeg oil in a typical concentration $\geq$ 0.1%, nutmeg oil is classified as carcinogenic (category 1B) in accordance with the classification criteria in Annex I of the CLP Regulation (1272/2008/EC),[32] and should be handled accordingly.[33] This precaution would also cover the risk associated with the exposure to the other *p*-allylalkoxybenzenes.

3.3.6. Safety for the environment

M. fragrans is not a native species to Europe. Therefore, the safety for the environment is assessed based on the individual components of the essential oil.

The major components (α-pinene, β-pinene, 4-terpineol, limonene and γ-terpinene) and additional 17 components accounting for > 0.1% of the composition of the additive (α-terpinene, myrcene, β-phellandrene, terpinolene, δ-3-carene, α-phellandrene, *p*-cymene, α-terpineol, camphene, 4-allyl-2,6-dimethoxyphenol, eugenol, linalool, α-terpinyl acetate, geranyl acetate, bornyl acetate, δ-cadinene and ethanol), accounting together for 95% of the composition of the oil, have been evaluated by EFSA as sensory additives for animal feed (see Table 1, Section 1.2). They were considered to be safe for the environment at individual use levels higher than those resulting from the use of the essential oil in feed.

The remaining identified constituents of the essential oil are mainly aliphatic mono or sesquiterpenes partially substituted with functional groups; they are chemically related to the substances evaluated by EFSA as CG 31 for use in animal feed (EFSA FEEDAP Panel, 2015, 2016d) for which EFSA concluded that they were extensively metabolised by the target species (see Section 3.3.1) and excreted as innocuous metabolites or carbon dioxide. Therefore, no risk for the safety of the environment is foreseen. Average feed levels of constituents of the essential oil are much lower than the use levels for CG 31 substances.

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

3.4. Efficacy

Nutmeg (*M. fragrans* Houtt.) and its oil are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 2792 (nutmeg), 2793 (nutmeg oil).

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

4. Conclusions

Nutmeg oil from *M. fragrans* Houtt. may be produced from plants of different geographical origins and by various processes resulting in preparations with different composition and toxicological profiles. Thus, the following conclusions apply only to nutmeg oil which contains $\leq$ 12% myristicin, $\leq$ 2.30% safrole, $\leq$ 0.40% elemicin and $\leq$ 0.33% methyleugenol, and is produced by steam distillation of the seeds of *M. fragrans*.

[30] Technical dossier/Supplementary Information March 2023/Annex_III_SIn reply_nutmeg_oil_MSDS. Aspiration hazard (H304, category 1), Hazards for skin corrosion/irritation (H315, category 2), skin sensitisation (H317, category 1), may cause allergic skin reactions (H317A, category 1), suspected of causing genetic defects (H341, Muta 2), suspected of causing cancer (H351, Carc. 1B).

[31] Safrole is considered to be a carcinogen category 2B (the agent is possibly carcinogenic to humans; the exposure circumstance entails exposures that are possibly carcinogenic to humans) by the International Agency for the Research on Cancer (IARC) from the World Health Organisation (WHO) (IARC Monograph Volume 10). Under the European Dangerous Substance Directive, safrole is considered to be a carcinogen category 2 (substance which should be regarded as if they are carcinogenic to humans). According to Regulation 1272/2008/EC (CLP), safrole is considered to be a carcinogen category 1B (may cause cancer).

[32] Regulation (EC) No 1271/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

[33] Directive 2004/37/EC of the European Parliament and of the Council of 29 April 2004 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (Sixth individual Directive within the meaning of Article 16(1) of Council Directive 89/391/EEC). OJ L 158, 30.4.2004, p. 50.

For all long-living and reproductive animals (including those animals reared for laying/ reproduction) the use of the additive is considered of low concern (MOET > 10,000) at the proposed level in complete feed of 0.2 mg/kg for laying hens and other laying/reproductive birds including animals reared for laying/reproduction, ornamental birds, and rabbits; 0.3 mg/kg for sows and all pigs (Suidae) for reproduction including animals reared for reproduction, dairy cows, other ruminants and camelids for milk production and reproduction including animals reared for milk production/reproduction; 0.5 mg/kg for sheep/goats, horses and other Equidae and cats; 0.6 mg/kg for dogs and 2.5 mg/kg for ornamental fish. The Panel considers that the use in water for drinking in long-living and reproductive animals is of low concern provided that the total daily intake of the additive does not exceed the daily amount that is considered of low concern when consumed via feed.

For short-living animals, the Panel has no safety concern when the additive is used at the maximum proposed use level of 10 mg/kg for veal calves (milk replacer), cattle for fattening, other ruminants for fattening and camelids at the same physiological stage, horses and other Equidae for meat production, salmonids and minor fin fish. For the other species, the calculated concentrations in complete feed which are considered of no safety concern are 2.8 mg/kg chickens for fattening and minor poultry for fattening, 3.7 mg/kg for turkeys for fattening, 6.0 mg/kg for pigs for fattening, 5.0 mg/kg for piglets and all pigs (Suidae) for meat production, and 4.4 mg/kg for rabbits for meat production. For short-living animals, the Panel has no safety concern for the use in water for drinking 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.

For any other species not covered above, the value considered of no concern is 0.2 mg/kg complete feed.

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

The additive under assessment should be considered as irritant to skin and eyes, and as skin and respiratory sensitiser. Based on the presence of safrole ≥ 0.1%, nutmeg oil is classified as carcinogen (category 1B)[34] and should be handled accordingly.

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

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

5. Recommendations

In line with the principles of the General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic when used as feed additives (EFSA FEEDAP Panel, 2021a), that 'manufacturing processes of botanical feed additives should avoid selective extraction and enrichment of genotoxic and/or carcinogenic substances and should aim at the reduction of these substances', the FEEDAP Panel recommends that nutmeg oil intended to be used as feed additive should contain the lowest possible concentrations of myristicin, safrole, elemicin and methyleugenol.

The specification should ensure that the concentration of myristicin, safrole, elemicin and methyleugenol in the additive should not exceed 12%, 2.3%, 0.4% and 0.33%, respectively.

6. Documentation provided to EFSA/chronology

Date	Event
28/10/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 06 – Laurales, Magnoliales, Piperales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
11/11/2010	Reception mandate from the European Commission
03/01/2011	Application validated by EFSA – Start of the scientific assessment
01/04/2011	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: analytical methods*
20/04/2012	Reception of supplementary information from the applicant
05/04/2011	Comments received from Member States

[34] In accordance with the classification criteria in Annex I of the CLP Regulation (1272/2008/EC).

Date	Event
26/02/2013	EFSA informed the applicant (EFSA ref. 7150727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission
02/08/2013	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
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
18/12/2018	EFSA informed the applicant that the evaluation process restarted
07/02/2019	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 target species, safety for the consumer, safety for the user and environment*
24/06/2020	Reception of supplementary information from the applicant (partial submission: nutmeg oil)
16/02/2022	The application was split and the original EFSA-Q-2010-01296 remained associated to the preparation included in the present assessment.
22/06/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for target species*
08/03/2023	Reception of supplementary information from the applicant. Scientific assessment re-started for the preparation included in the present assessment
12/05/2023	Opinion adopted by the FEEDAP Panel on nutmeg oil. End of the Scientific assessment for BDG 06 (EFSA-Q-2010-01296)

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Abbreviations

BDG	botanically defined group
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CG	chemical group
CLP	Classification, labelling and packaging
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
GC area	gas chromatographic peak area
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry

IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
PBK	physiologically based kinetic (models)
SB	solvent-based
SCF	Scientific Committee on Food
TTC	threshold of toxicological concern
UF	uncertainty factor
WB	water-based
WHO	World Health Organization