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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of an essential oil derived from leaves and terminal branchlets of *Melaleuca alternifolia* (Maiden & Betche) Cheel (tea tree oil) for use in 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 tea tree oil obtained from leaves and terminal branchlets of *Melaleuca alternifolia* (Maiden & Betche) Cheel when used as a sensory additive for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that tea tree oil was very unlikely to be of safety concern for long-living and reproductive animals and is of no concern for target species for fattening at the following concentrations in complete feed: 1.1 mg/kg for chickens for fattening, 1.5 mg/kg for turkeys for fattening, 1.7 mg/kg for laying hens, 2.0 mg/kg for piglets, 2.4 mg/kg for pigs for fattening, 3.1 mg/kg for sows, 5.0 mg/kg for veal calves (milk replacer) and salmonids, 4.4 mg/kg for cattle for fattening, sheep/goats and horses, 2.9 mg/kg for dairy cows, 1.8 mg/kg for rabbits, 0.9 mg/kg for cats, 5.3 mg/kg for dogs, 6.6 for crustaceans and 15 mg/kg for ornamental fish. These conclusions were extrapolated to other physiologically related species. For any other species, the additive is very unlikely to be of safety concern at 1.1 mg/kg complete feed. No concerns for consumers and the environment were identified following the use of the additive up to the highest safe use level in feed. Regarding user safety, tea tree oil should be considered as an irritant to skin and eyes and as a dermal and respiratory sensitiser. It is classified as a reprotoxic substance (category 1B) following CLP criteria and should be handled accordingly. Since *M. alternifolia* and its preparations were recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy was considered necessary.

KEYWORDS

efficacy, flavouring compounds, *Melaleuca alternifolia* (Maiden & Betche) Cheel, safety, sensory additives, tea tree oil, terpinen-4-ol

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1 | INTRODUCTION

1.1 | Background and Terms of Reference

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

The European Commission received a request from Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)[2] for authorisation/re-evaluation of 18 additives (namely geranium oil, geranium rose oil, eucalyptus oil, eucalyptus tincture, clove oil, clove tincture, broom tea tree oil, purple loosestrife tincture, tea tree oil, melaleuca cajuputi oil, niaouli oil, allspice oil, bay oil, pomegranate bark extract, bambusa tincture, citronella oil, lemongrass oil and vetiveria oil) belonging to botanically defined group (BDG) 07 – Geraniales, Myrtales, Poales, when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings). During the assessment, the applicant withdrew the application for six additives.[3] These additives were deleted from the register of feed additives.[4] During the course of the assessment, this application was split, and the present opinion covers only one out of the remaining 12 additives under application: tea tree oil from *Melaleuca alternifolia* (Maiden & Betche) Cheel[5] for all animal species.

The remaining 11 additives belonging to botanically defined group (BDG) 07 – Geraniales, Myrtales, Poales under application are assessed in separate opinions.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 21 December 2010.

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 feed additive consisting of tea tree oil from *M. alternifolia*, when used under the proposed conditions of use (see Section 3.3.4).

1.2 | Additional information

Tea tree oil from *M. alternifolia*[6] is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined). It has not been assessed as a feed additive in the EU.

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[7] in support of the authorisation request for the use of tea tree oil from *M. alternifolia* as a feed additive. The dossier was received on 28 February 2024 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2024-00119.[8]

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

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2]On 13/03/2013, EFSA was informed by the applicant that the applicant company changed to FEFANA asbl, Avenue Louise 130 A, Box 1, 1050 Brussels, Belgium.
[3]Broom teatree oil, geranium oil, bay oil and vetiveria oil (27 February 2019); bambusa tincture and allspice oil (18 November 2022).
[4]Register of feed additives, Annex II, withdrawn by OJ L162, 10.05.2021, p. 5.
[5]Accepted name: *Melaleuca alternifolia* Cheel; synonyms: *Melaleuca linariifolia var. alternifolia* Maiden & Betche (also spelled as Maiden and Betch in EMA monograph and EMA assessment report (EMA, 2015a, 2015b).
[6]Synonyms: *Melaleuca linariifolia* var. *alternifolia* Maid and Bet.
[7]Dossier reference: FAD-2010-0219.
[8]The original application EFSA-Q-2010-01282 was split on 28/02/2024 and a new EFSA-Q-2024-00119 was generated.

Many of the components of the essential oil under assessment have been already evaluated by the FEEDAP Panel as chemically defined flavourings (CDGs). The applicant submitted a written agreement to reuse the data submitted for the assessment of chemically defined flavourings (dossiers, publications and unpublished reports) for the risk assessment of preparations belonging to BDG 07, including the current one under assessment.[9]

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phytochemical marker in the additive. The evaluation report is related to the methods of analysis for each feed additive included in the group BDG 07 (Geraniales, Myrtales, Poales). During the assessment, upon request from EFSA, the EURL issued two amendments[10] of the original report.[11] The additive under assessment, tea tree oil, is included in the second amendment. In particular, the EURL recommended a method based on gas chromatography with flame ionisation detection (GC-FID) for the quantification of the phytochemical marker *4-terpinenol* in *tea tree oil*. [12]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of tea tree oil from *M. alternifolia* 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 Scientific Committee, 2009); Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012); Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a); Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b); Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c); Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018); Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019); Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023a); Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a); Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b); Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment (EFSA Scientific Committee, 2019c); and General approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021a).[14]

3 | ASSESSMENT

The additive under assessment, tea tree oil, is an essential oil obtained from the leaves and terminal branchlets from *Melaleuca alternifolia* (Maiden & Betche) Cheel and is 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

Melaleuca alternifolia (Maiden & Betche) Cheel is a small tree belonging to the Myrtaceae family, native to Australia. It is commonly referred to as the tea (ti) tree or the narrow-leaved paper bark tree because of its white papery bark, a characteristic shared with other *Melaleuca* species. Its commercial value resides with the essential oil (tea tree oil) produced from the leaves and terminal branchlets. The antimicrobial properties of the oil were first reported in the 1920s, and initially, it was produced from natural stands of *M. alternifolia* and, probably, also from other *Melaleuca* species. Six chemotypes, of *M. alternifolia* have been described, each producing an oil with a distinct chemical composition, although all seem to possess similar antimicrobial properties. In an effort to ensure consistency of the product, commercial plantations of *M. alternifolia* were established from the 1970s onwards and an internationally recognised standard for 'Oil of *Melaleuca*—terpinen-4-ol type' was agreed. However, the international standard for tea tree oil does not specify the *Melaleuca* species but is based on the oil chemotype.

The raw material for the production of tea tree oil is the leaves (with or without the terminal branchlets) of *M. alternifolia* sourced from China, New Caledonia and Australia. The volatile constituents are extracted by steam distillation, in which steam is passed through the plant material. The steam carries up the volatile constituents which are then condensed. The essential oil is then separated from water by decantation.

[9]Technical dossier/Supplementary information February 2023/Letter dated 31/01/2023.

[10]Preparations included in the first amendment: geranium rose oil, eucalyptus oil, lemongrass oil and clove oil; preparations included in the second amendment: citronella oil, melaleuca cajuputi oil, tea tree oil, clove tincture and eucalyptus tincture.

[11]Preparations included in the second amendment: citronella oil, melaleuca oil, niaouli oil, tea tree oil, eucalyptus tincture, clove tincture.

[12]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0219_en.

[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.

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

3.2 | Uses other than feed flavouring

'Tea tree oil' (*Melaleuca aetheroleum*) is described in monographs of the European Pharmacopoeia 11.0 (PhEur, 2022) and of the European Medicines Agency (EMA, 2015a, 2015b) for medicinal uses.

Tea tree oil is used as a fungicide, for example, on grapes and tomatoes. It is also used as fragrance and in human and animal care products (e.g. mouthwash, toothpaste, shampoo, deodorants, lotions and antifungal treatment) (ECHA, 2024a).

3.3 | Characterisation

3.3.1 | Characterisation of tea tree oil

Tea tree oil is a clear, mobile, colourless or pale-yellow liquid with characteristic odour of turpentine. Tea tree oil is identified with the single Chemical Abstracts Service (CAS) number 68647-73-4, the European Inventory of Existing Commercial Chemical Substances (EINECS) number 285-377-1, the Flavor Extract Manufacturers Association (FEMA) number 3902 and the Council of Europe (CoE) number 275.

For tea tree oil, the specifications used by the applicant are based on those developed by the International Organization for Standardization (ISO) 4730:2017 for oil of *Melaleuca* (Tea tree oil),[15] which were adapted to reflect the concentrations of selected volatile components. Four components are included in the specifications as shown in Table 1, with 4-terpinenol as phytochemical marker. The analysis of one batch of the additive showed compliance with the specification when analysed by GC-FID and expressed as percentage of gas chromatographic peak area (% GC area).[16] Analysis of seven batches of the additive showed compliance with these specifications when analysed by gas chromatography–mass spectrometry (GC–MS) and expressed as percentage of gas chromatographic peak area (% GC area).[17] The applicant provided a full analysis of the volatile constituents in seven batches obtained by GC–MS.[18] The four compounds indicated in the product specifications accounted for about 72.5% on average (range 69.1%–74.6%) of % GC area (Table 2).

TABLE 1 Constituents of tea tree oil, as defined by specifications and batch to batch variation based on the analysis of seven batches by gas chromatography–mass spectrometry (GC–MS). The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

Constituent			% GC area		
EU register name	CAS No	FLAVIS No	Specification[a]	Mean	Range
4-Terpinenol	562-74-3	02.072	30–48	39.43	37.34–41.87
γ-Terpinene	99-85-4	01.020	10–28	20.46	18.15–21.77
α-Terpinene	99-86-5	01.019	5–13	10.24	9.20–10.63
1,8-Cineole	470-82-6	03.001	< 15	2.40	1.55–3.16
Total				72.52	69.05–74.61[b]

Abbreviations: CAS No, chemical abstracts service number; EU, European Union; FLAVIS No, EU flavour information system numbers.

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

[b]The values given for Total are the lowest and the highest values of the sum of the components in the individual batches analysed.

In total, up to 103 peaks were detected in the chromatogram, 95 of which were identified and accounted on average for 98.6% (96.1%–100.0%) of the % GC area. Besides the four compounds indicated in the product specifications, 14 other compounds were detected at individual levels > 0.5% and are listed in Table 2. These 18 compounds account on average for 95.3% (93.2%–97.1%) of the % GC area. The remaining 77 compounds (ranging between 0.01% and 0.48%) and accounting on average for 3.2% of the % GC area are listed in the footnote.[19] Eight unidentified compounds were detected in different batches of tea tree oil. Based on the chromatographic profile and fragmentation patterns, two of them were

[15]Technical dossier/Supplementary information October 2023/Annex_III_SIn_reply_tea_tree_oil_ISO_4730_2017.

[16]Technical dossier/Supplementary information October 2023/GC-FID analysis: 4-terpinenol 39.7%, γ-terpinene 20.1%, 1,8-cineole 2.4% and α-terpinene 10.0%.

[17]Technical dossier/Supplementary information October 2023/Annex_II_SIn_reply_tea_tree_oil_Table 4.

[18]Technical dossier/Supplementary information October 2023/Conf_Annex_II_SIn_reply_tea_tree_oil_CoA.

[19]Additional constituents: constituents ($n = 26$) < 0.1% and ≥ 0.5%: γ-muurolene, β-pinene (pin-2(10)-ene), β-caryophyllene, *(Z)*-anethol, alloaromadendr-9-ene, (–)-globulol, γ-cadinene, 4(10)-thujene (sabinene), α-gurjunene, guaia-6,9-diene, sabina ketone, cubenene, isocitronellol, α-selinene, linalool oxide, *cis*-p-2-menthen-1-ol, *cis*-pinene hydrate, 1-epi-cubenol, cadina-3,5-diene, viridiflorol, laevo-pinocarveol, β-thujene, isoledene, *trans*-p-2-menthen-1-ol, *trans*-piperitol, hinesene; constituents ($n = 18$) < 0.05% and ≥ 0.01%: selina-5,11-diene, bicyclo[3.1.0]hexane, 1-methyl-6-(1-methylethylidene)-, cubenol, anethofuran, 5-guaiene-11-ol, α-maaliene, 3,7,10-humulatriene, 3-cyclohexene-1-methanol, 2-hydroxy- alpha,alpha,4-trimethyl-, α-copaene, *d,l*-isoborneol, linalool, *trans*-sabinene hydrate, α-ylangene, γ-maalinene, 1-isopropenyl-4-methylbenzene, *cis*-p-2,8-menthadien-1-ol, *o*-cymene, α-cubebene; constituents ($n = 33$) < 0.05% and ≥ 0.01%: *d,l*-borneol, β-ocimene, β-terpineol, plinol d, maaliol, *cis*-piperitol, *p*-menth-1-en-3-one, epi-β-caryophyllene, *(E)*-isovalencenol, cyclohexane, 1-methyl-4-(1-methylethylidene)-, camphene, spathulenol, β-dihydroagarofuran, 1(5),11-guaiadiene, 1-aromadendrene, valerena-4,7(11)-diene, (–)-*cis*-carane, hex-3(*cis*)-en-1-ol, epiglobulol, β-gurjenene, β-longipinene, 4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane, benzene, (1,3-dimethyl-2-butenyl)-, isogermacrene D, *cis*-isocarveol, 4-hydroxy-4-methylpentan-2-one, *p*-menthane, selina-3,7(11)-diene, *trans*-p-2-menthene-1,4-diol, cubebol, 1(5),7(11)-guaiadiene, limonene dioxide and methyleugenol.

tentatively identified as sesquiterpenes, whereas the others are likely to be terpenes or their oxygenated derivatives. Based on these data, tea tree oil is considered a fully defined mixture (EFSA Scientific Committee, 2019a).

TABLE 2 Constituents of tea tree oil accounting for > 0.5% of the composition (based on the analysis of seven batches) not included in the specifications. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%.

Constituent			% GC area	
EU register name	**CAS No**	**FLAVIS No**	**Mean**	**Range**
α-Terpineol	98-55-5	02.014	4.68	3.03–6.91
Terpinolene	586-62-9	01.005	3.31	2.94–3.64
p-Cymene (1-isopropyl-4-methylbenzene)	99-87-6	01.002	3.23	2.48–3.90
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	3.03	2.07–4.71
β-Phellandrene	555-10-2	01.055	1.87	1.71–2.12
d-Limonene[a]	5989-27-5	01.045	1.80	0.61–2.90
Aromadendrene	72747-25-2	–	1.71	1.15–2.60
Viridiflorene	21747-46-6	–	1.69	0.70–2.33
δ-Cadinene	29350-73-0	01.021	1.43	1.29–1.53
α-Thujene	2867-05-2	–	1.02	0.86–1.11
Bicyclogermacrene	67650-90-2	–	0.95	0.95–0.95
Myrcene	123-35-3	01.008	0.67	0.62–0.69
α-Phellandrene	99-83-2	01.006	0.57	0.51–0.68
Alloaromadendrene	25246-27-9	–	0.56	0.47–0.62
Total			22.82	20.98–24.72[b]

Abbreviations: CAS No, chemical abstracts service number; EU, European Union; FLAVIS No, EU flavour information system number.

[a]Stereochemistry not given, however considering that the naturally occurring limonene is typically *d*-limonene, it is assumed that this form also occurs in tea tree oil.

[b]The values given for Total are the lowest and the highest values of the sum of the components in the individual batches analysed.

The applicant performed a literature search (see Section 3.3) for the chemical composition of *M. alternifolia* and its preparations to identify the presence of any recognised substances of concern.[20] Apart from the presence of 1,8-cineole (up to 60%) and 4-terpinenol (up to 45%) in the essential oil from the leaves of *M. alternifolia*, the EFSA Compendium of botanicals also reported methyleugenol as potential substance of concern present in trace amounts in *M. alternifolia* (EFSA, 2012).[21] The levels of methyleugenol analysed in 128 tea tree oil commercial samples ranged from < 0.01% to 0.06% (mean, 0.02%) (Southwell et al., 2011 as reported by EMA, 2015a, 2015b; Tisserand & Young, 2014; CIR, 2021). The natural occurrence of methyleugenol in tea tree oil has been reported to be about 0.01% (Fukushima et al., 2020). Methyleugenol is included in the list of substances which shall not be added as such to food according to Annex III of Regulation (EC) No 1334/2008, and for which maximum levels in food are set by Regulation (EC) No 1334/2008.[22]

Analysis of seven batches of tea tree oil under assessment detected the presence of methyleugenol in one batch at a concentration corresponding to the limit of detection (LOD, 0.01%).

No other substances of concern were identified in the literature provided by the applicant.

3.3.2 | Impurities

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

[20]Technical dossier/Supplementary information October 2023/Literature search_tea_tree_oil.

[21]Online version: https://www.efsa.europa.eu/en/data-report/compendium-botanicals.

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

3.3.3 | Shelf-life

The typical shelf-life of tea tree 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).[23] However, no data supporting this statement were provided.

As described in the literature, inappropriate storage of tea tree oil may lead to photo-oxidation resulting in the formation of degradation products such as ascaridole and 1,2,4-trihydroxymenthane which are considered responsible for the induction of contact allergy observed after exposure to tea tree oil (PhEur Commentary, 2020; EMA, 2015b).

3.3.4 | Conditions of use

Tea tree oil is intended to be added to feed and water for drinking for all animal species without a withdrawal period. The maximum proposed use levels in complete feed for the animal species and categories are listed in Table 3. No use level has been proposed by the applicant for the use in water for drinking.

TABLE 3 Maximum proposed use levels of the essential oil from *Melaleuca alternifolia* (Maiden & Betche) Cheel in complete feed.

Animal category	Maximum use level (mg/kg complete feed)
Chickens for fattening	25
Laying hens	20
Turkeys for fattening	40
Pigs for fattening	20
Piglets	30
Sows lactating	20
Veal calves (milk replacer)	60
Cattle for fattening	30
Dairy cows	20
Sheep/goats	20
Horses	20
Rabbits	20
Salmon and other fin fish	40
Crustaceans (shrimps)	15
Dogs	20
Cats	20
Ornamental fish	15
Other species	15

3.4 | Safety

The assessment of safety of tea tree oil is based on the maximum use levels in complete feed proposed by the applicant (Table 3).

No studies to support the safety for target animals, consumers and users were performed with the additive under assessment. The applicant carried out an extensive database search to identify data related to the chemical composition and the safety of preparations obtained from *M. alternifolia*.[24] Four cumulative databases (LIVIVO, NCBI, OVID and ToxInfo), 13 single databases including PubMed and Web of Science and 12 publishers' search facilities including Elsevier, Ingenta, Springer and Wiley were used. The literature search (no time limits) was conducted in February 2023. The keywords used covered different aspects of safety and the inclusion and exclusion criteria were provided by the applicant.

The FEEDAP Panel notes that an evaluation of tea tree oil is ongoing by EFSA and ECHA in the context of the renewal (re-approval) of tea tree oil as plant protection product according to Regulation (EC) No 1107/2009[25] and of the proposal for

[23]Technical dossier/Section II.

[24]Technical dossier/Supplementary information October 2023/Literature_search_Tea tree_oil.

[25]Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

Harmonised Classification and Labelling (CLH) according to Regulation (EC) No 1272/2008.[26] While the EFSA Pesticides Peer Review is ongoing, the ECHA Committee for Risk Assessment (RAC) already published its opinion in February 2024 (ECHA, 2024a). Based on the available evidence, ECHA classified tea tree oil as Repr 1b (H360Fd: May damage fertility. Suspected of damaging the unborn child).

The information considered relevant by the RAC for the proposed classification is compiled in a background document in the Annex I to the RAC opinion (ECHA, 2024b). This information includes toxicological studies with tea tree oils similar to the additive under assessment. The studies which were considered relevant for the current assessment are briefly described in the next sections.

Many of the individual components of the essential oil have been already assessed as chemically defined flavourings for use in feed and food by the FEEDAP Panel, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC), the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The flavouring compounds currently authorised for feed[27] and food[28] use, together with the EU Flavour Information System (FLAVIS) number, the chemical group as defined in Commission Regulation (EC) No 1565/2000[29] and the corresponding EFSA opinion are listed in Table 4.

TABLE 4 Flavouring compounds already assessed by EFSA and/or by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as chemically defined flavourings, grouped according to the chemical group (CG) as defined in Commission Regulation (EC) No 1565/2000, with indication of the EU Flavour Information System (FLAVIS) number and the corresponding EFSA/JECFA opinion.

CG	Chemical group	Product (EU register name)	FLAVIS No	EFSA*/JECFA opinion, year
04	Non-conjugated and accumulated unsaturated straight-chain and branched chain aliphatic primary alcohols, aldehydes, acids, acetals and esters	Hex-3(cis)-en-1-ol	02.056	2016a
06	Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols and esters with esters containing tertiary alcohols ethers	Linalool	02.013	2012a
		α-Terpineol	02.014	
		4-Terpinenol	02.072	
		β-Terpineol[a] *p*-menth-8-en-1-ol	02.097	WHO, 2000
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	2016b
		d,l-Isoborneol	02.059	
		p-Menth-1-en-3-one[a]	07.175	2011a, CEF
10	Secondary aliphatic saturated or unsaturated alcohols, ketones, ketals and esters with a second secondary or tertiary oxygenated functional group	4-Hydroxy-4-methylpentan-2-one[a]	07.165	2011b, CEF
13	Furanones and tetrahydrofurfuryl derivatives	Linalool oxide[c]	13.140	2012b
16	Aliphatic and alicyclic ethers	1,8-Cineole	03.001	2012c, 2021b
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	
		d-Limonene	01.045	

[26]Regulation (EC) No 1272/2008 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 (Text with EEA relevance). OJ L 353, 31.12.2008, p. 1–1355.

[27]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.

[28]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.

[29]Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 1. 80, 19.7.2000, p. 8.

TABLE 4 (Continued)

CG	Chemical group	Product (EU register name)	FLAVIS No	EFSA*/JECFA opinion, year
		Pin-2(10)-ene (β-pinene)	01.003	2016c
		Pin-2(3)-ene (α-pinene)	01.004	
		β-Caryophyllene	01.007	
		Myrcene	01.008	
		Camphene	01.009	
		3,7-Dimethyl-1,3,6-octatriene (β-ocimene)[d]	01.018	
		δ-Cadinene[a,b]	01.021	2011c, CEF
		1(5),11-Guaiadiene[a,b]	01.023	
		3,7,10-Humulatriene[a,b]	01.043	
		β-Phellandrene[a,b]	01.055	
		4(10)-Thujene (sabinene)[a]	01.059	2015, CEF

*FEEDAP opinion unless otherwise indicated.

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

[b]Evaluated applying the 'Procedure' described in the Guidance on the data required for the risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010). No longer authorised for use as a flavouring in food, as the additional toxicity data requested (EFSA CEF Panel, 2011c) were not submitted and the CEF Panel was unable to complete its assessment.

[c]Linalool oxide [13.140]: A mixture of *cis*- and *trans*-linalool oxide (5-ring) was evaluated [13.140] (EFSA FEEDAP Panel, 2012b).

[d]β-Ocimene [01.018]: as a mixture of *(E)*- and *(Z)*-isomers, containing 50%–70% *(E)*-isomer and 17%–17% *(Z)*-isomer, was evaluated.

As shown in Table 4, a number of components of tea tree oil, accounting for about 92.1% of the GC peak areas, have been previously assessed and considered safe for use as flavourings. They are currently authorised for use in food[30] without limitations and for use in feed[31] at individual use levels higher than those resulting from the intended use in feed of the essential oil under assessment.

Four compounds listed in Table 4, δ-cadinene [01.021], 1(5),11-guaiadiene [01.023], 3,7,10-humulatriene [01.043] and β-phellandrene [01.055] have been evaluated in Flavouring Group Evaluation 25 Revision 2 (FGE.25Rev2) 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 subchronic toxicity data (EFSA CEF Panel, 2011c). In the absence of such toxicological data, the CEF Panel was unable to complete its assessment (EFSA CEF Panel, 2015). 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 Scientific Committee, 2019a).

Sixty-five compounds have not been previously assessed for use as flavourings. The FEEDAP Panel notes that 37 of them[32] accounting on average for 5.4% of the GC peak areas are aliphatic monoterpenes or sesquiterpenes structurally related to flavourings already assessed in CG 31 and for which a similar metabolic and toxicological profile is expected. Because of their lipophilic nature, they are expected to be rapidly absorbed from the gastro-intestinal tract, oxidised to polar oxygenated metabolites, conjugated and excreted (EFSA FEEDAP Panel, 2015, 2016c). Sixteen additional compounds[33] accounting together for 2.1% of the GC peak areas are structurally related to compounds assessed in chemical groups (CGs) 6, 8, 16 and 18 (EFSA FEEDAP Panel, 2011, 2012a, 2012c, 2016b). The eight unidentified compounds are likely to be terpenes or sesquiterpenes and are expected to behave in a similar way.

One batch of the additive contains trace concentrations of methyleugenol (0.01%).

The next section focusses on methyleugenol and on the 12 compounds[34] not previously assessed or not structurally related to flavourings previously assessed, based on the evidence provided by the applicant in the form of literature

[30]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.

[31]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.

[32]1-Methyl-4-(1-methylethylidene)-cyclohexane, *p*-menthane, *o*-cymene, (1,3-dimethyl-2-butenyl)-benzene, aromadendrene, viridiflorene, (–)-*cis*-carane, β-thujene, 1-methyl-6-(1-methylethylidene)-bicyclo[3.1.0]hexane, guaia-6,9-diene, α-gurjunene, β-gurjenene, selina-3,7(11)-diene, 1-aromadendrene, β-longipinene, epi-β-caryophyllene, 1(5),7(11)-guaiadiene, α-selinene, 4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane, α-thujene, α-cubebene, α-copaene, isoledene, α-ylangene, γ-maalinene, α-maaliene, cadina-3,5-diene, valerena-4,7(11)-diene, alloaromadendrene, γ-muurolene, hinesene, alloaromadendr-9-ene, bicyclogermacrene, γ-cadinene, cubenene, selina-5,11-diene, isogermacrene D.

[33](–)-Globulol, viridiflorol, *cis*-pinene hydrate, isocitronellol, cubebol, *trans*-sabinene hydrate, *cis*-p-2-menthen-1-ol, 1-epi-cubenol, cubenol (CG 6); laevo-pinocarveol, *cis*-piperitol, *trans*-piperitol, sabina ketone (CG 8); anethofuran, β-dihydroagarofuran (CG 16); *(Z)*-anethol (CG 18).

[34]*cis-p*-2,8-menthadien-1-ol, plinol D, *trans-p*-2-menthene-1,4-diol, spathulenol, *trans-p*-2-menthen-1-ol, epiglobulol, maaliol, 5-guaiene-11-ol, *(E)*-isovalencenol, *cis*-isocarveol, 3-cyclohexene-1-methanol, 2-hydroxy- alpha,alpha,4-trimethyl- and limonene dioxide.

searches and quantitative structure–activity relationship (QSAR) analysis. For the absorption, distribution, metabolism and excretion (ADME) and the toxicology of methyleugenol, reference is made to the safety evaluation made by the FEEDAP Panel in the opinion on laurel leaf oil (EFSA FEEDAP Panel, 2023b).

3.4.1 | Toxicology

3.4.1.1 | *Genotoxicity and carcinogenicity*

For fully defined mixtures, the EFSA Scientific Committee recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential using all available information, including read-across and QSAR considerations about their genotoxic potential (EFSA Scientific Committee, 2019b). Therefore, the potential genotoxicity of identified constituents is first considered. Then, in vitro genotoxicity studies performed with tea tree oils similar to the additive under assessment are taken into account, if deemed relevant.

The genotoxic potential of 12 compounds (*cis-p*-2,8-menthadien-1-ol, plinol D, *trans-p*-2-menthene-1,4-diol, spathulenol, *trans-p*-2-menthen-1-ol, epiglobulol, maaliol, 5-guaiene-11-ol, *(E)*-isovalencenol, *cis*-isocarveol, 3-cyclohexene-1-methanol, 2-hydroxy-alpha,alpha,4-trimethyl- and limonene dioxide) was predicted by the applicant using the Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox. No structural alerts were identified for spathulenol, plinol D, epiglobulol, maaliol and 5-guaiene-11-ol. For six compounds, i.e. *cis-p*-2,8-menthadien-1-ol, *trans-p*-2-menthene-1,4-diol, *trans-p*-2-menthen-1-ol, *(E)*-isovalencenol, *cis*-isocarveol, and 3-cyclohexene-1-methanol, 2-hydroxy- alpha,alpha,4-trimethyl- structural alerts were due to the presence of a vinyl/allyl alcohol group and for limonene dioxide to the presence of an epoxide. In all cases, predictions of mutagenicity by Ames test (with and without S9) were made by 'read-across' analyses of data available for similar substances to the target compounds (i.e. analogues obtained by categorisation). Categories were defined using general mechanistic and endpoint profilers as well as empirical profilers. Subcategorisation was performed in order to exclude analogues less similar to the target compounds. For all compounds mutagenicity read-across-based predictions were found negative.[35] On this basis, the alerts raised were discounted.

The genotoxicity of *d*-limonene epoxide (stereochemistry not specified), investigated in the Ames test and the SOS Chromotest, gave negative results (Basler et al., 1989 as referenced in EFSA CEF Panel, 2014). When V79 Chinese hamster cells were incubated with *d*-limonene epoxide, no increase in sister chromatid exchange was observed (von der Hude et al., 1991, as referenced in EFSA CEF Panel, 2014).

Methyleugenol

One batch of the additive contained methyleugenol (0.01%), a compound with experimentally proven genotoxicity and carcinogenicity in rodents (IARC, 2018; EFSA FEEDAP Panel, 2023b).

For methyleugenol, the FEEDAP Panel identified a reference point for neoplastic endpoints derived from a carcinogenicity study in rats (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).

Genotoxicity studies with tea tree oil

A literature search performed by the applicant retrieved the following studies on the essential tea tree oil.

Essential oil from *M. alternifolia* (tea tree oil) was not mutagenic in the bacterial reverse mutation assay performed in Salmonella Typhimurium TA98 and TA100 strains and in *Escherichia coli* WP2 uvrA strain, with and without an extrinsic metabolic activation system (Evandri et al., 2005). The mutagenic potential of commercially available tea tree oils from *M. alternifolia* (composition not given) was also examined by the Ames Test performed in Salmonella Typhimurium strains TA102, TA100 and TA98 with and without S9 mixture. No mutagenic effect was induced by any of the brands of tea tree oil (Fletcher et al., 2005). The crude essential oil from *M. alternifolia* (tea tree oil) did not induce chromosomal damage when tested in vitro by the micronucleus and the chromosome aberration tests performed in human peripheral blood lymphocytes (Pereira et al., 2014). Results obtained by an in vitro Comet assay in Hela cells (Rajkowska et al., 2016) were not further considered in the assessment, because this assay is not internationally validated. The EMA assessment report (EMA, 2015b) cites the same studies described above. In addition, EMA describes an in vivo micronucleus test in rodent, which gave negative results. However, the original report was not available, and the study cannot be further considered in the assessment.

The FEEDAP Panel notes that additional mutagenicity/genotoxicity studies with tea tree oil similar to the additive under assessment (test item composition: 4-terpinenol 40.5%–42.8%, γ-terpinene 17.8%–20.4%, α-terpinene 7.9%–9.7%, 1,8-cineole 1.5%–3.0%) have been described in Annex I to the RAC opinion (ECHA, 2024b). The data set included in vitro

[35]Technical dossier/Supplementary information October 2023/Annex VII_SIn_reply_tea_tree_oil_QSAR.

tests (bacterial reverse mutation test, mammalian cell gene mutation test, mammalian micronucleus test, mammalian chromosomal aberration test) and an in vivo test for DNA damage (mouse micronucleus test). All the studies were negative.

Carcinogenicity studies with tea tree oil are not available (ECHA, 2024b).

Conclusions on genotoxicity and carcinogenicity

Based on the data set described in the RAC opinion, tea tree oil can be considered as non-genotoxic. However, as methyleugenol has been detected in one batch of the oil under assessment, the FEEDAP Panel considers that the additive may raise concerns with regard to genotoxicity. The consequences of the possible presence of methyleugenol in tea tree oil is, therefore, considered separately in Sections 3.4.2 and 3.4.3.

3.4.1.2 | *Repeated dose oral toxicity studies with tea tree oil*

No toxicity studies with the additive under assessment were provided by the applicant.

However, the FEEDAP Panel notes that repeated dose oral toxicity studies with tea tree oils similar to the additive under assessment have been described in Annex I to the RAC opinion[36] (ECHA, 2024b). The toxicological data set includes repeated dose toxicity studies in rats (two 28-day and two 90-day studies) and dogs (one 90-day study) and reproductive toxicity studies in rats (a two-generation study and two developmental toxicity studies) and rabbits (a developmental toxicity study).

An overview of the studies is presented in Table 5.

TABLE 5 Summary of animal studies with tea tree oil as described in Annex I to the opinion of the ECHA Committee for Risk Assessment (RAC) proposing harmonised classification and labelling of tea tree oil (adapted from ECHA, 2024b).

Type of study[a]	Test item description (tea tree oil)	Dose levels (mg/kg bw per day)	NOAEL[b] (mg/kg bw per day)	Rel.[c] score	Reference in ECHA opinion
28-day study, rats, oral gavage OECD 407, GLP	Compliant with ISO 4730:2017	5, 15, 45	45 (the highest dose tested)	1	ECHA dissemination 2017b
28-day feeding study, rats (Wistar rat) non-GLP	4-Terpinenol 37.98% γ-Terpinene 21.04% α-Terpinene 9.45% 1,8-Cineole 5.67% (compliant with ISO)	0, 62.5, 125, 250	62.5 (based on effects observed on testes, epididymis, sperms and liver starting at 125 mg/kg bw per day)	2	Anonymous 2010b
90-day feeding study, rats (Wistar rat) OECD 408, GLP	4-Terpinenol 37.98% γ-Terpinene 21.04% α-Terpinene 9.45% 1,8-Cineole 5.67% (compliant with ISO)	0, 30, 60, 120	Males: 30 (based on effects on sperm counts at 60 mg/kg bw per day) Females: 60 (based on minimal spleen vacuolation and minimal tubular dilatation in kidneys at 120 mg/kg bw per day)	1	Anonymous 2011b
90-day feeding study, rats (Wistar rat) OECD 408, GLP	4-Terpinenol 42.36% γ-Terpinene 20.90% α-Terpinene 10.30% 1,8-Cineole 1.53% (compliant with ISO)	0, 60	60 (LOAEL, effects on sperm reversible after recovery period)	1	Anonymous 2016a
90-day study, Beagle dog oral gavage OECD 409, GLP	4-Terpinenol 41.92% γ-Terpinene 20.35% α-Terpinene 9.95% 1,8-Cineole 4.42%	0, 30, 75/60, 180/120 (dose reduction on day 27)	30 (based on effects on viability and motility of the canine spermatids at 75/60 mg/kg bw)	1	Anonymous 2018a
Two generation study, rat, oral (gavage) OECD 416, GLP	4-Terpinenol 42.36% γ-Terpinene 20.90% α-Terpinene 10.30% 1,8-Cineole 1.53% (compliant with ISO)	Gen P: 0, 10, 25, 50 Gen F1: 0, 10, 25 38	Reproduction: 10[d] (based on overall effects on sperm parameters in both the P and F1 generations) Parental/Offspring: 25[d] (based on effects on body weight in Gen P and on the litter at 50 mg/kg bw per day)	1	Anonymous 2017a

(Continues)

[36]The full reports of these studies were not available to FEEDAP.

TABLE 5 (Continued)

Type of study[a]	Test item description (tea tree oil)	Dose levels (mg/kg bw per day)	NOAEL[b] (mg/kg bw per day)	Rel.[c] score	Reference in ECHA opinion
Prenatal developmental study, rats (Wistar rat), females, oral gavage OECD 414, GLP	4-Terpinenol 41.73% γ-Terpinene 14.23% α-Terpinene 8.18% 1,8-Cineole 1.80%	0, 75/30, 150/60, 300/120 (dose reduction on GD 8)	Maternal toxicity: 30 (based on effects on maternal body weight, food intake at 60 mg/kg bw) Fetal toxicity: 60 (based on effects on fetal weight at 120 mg/kg bw)	1	Anonymous 2012a
Prenatal developmental study, oral gavage, rat OECD 414, GLP	4-Terpinenol 37%	0, 20, 100, 250	Maternal toxicity: 20 (based on clinical signs, reduced food consumption and reduced maternal body weight gain at 100 and 250 mg/kg bw per day) Fetal toxicity: 20 (secondary to maternal toxicity)	1	ECHA dissemination 2011
Prenatal developmental study, rabbits (New Zealand white rabbits), oral gavage OECD 414, GLP	4-Terpinenol 41.92% γ-Terpinene 20.35% α-Terpinene 9.95% 1,8-Cineole 4.42%	0, 15, 30, 75	Maternal toxicity: 75 (no effects observed) Fetal toxicity: 30 (based on post implantation loss at 75 mg/kg bw per day) Teratogenicity: 75 (no effects observed)	1	Anonymous 2018b

[a]The full reports of the studies were not available to the FEEDAP Panel.

[b]According to the Annex I to RAC opinion (ECHA, 2024b), unless otherwise indicated.

[c]Reliability score as reported in Annex I to the RAC opinion (ECHA, 2024b). Reliability score 1: reliable; Reliability score 2: reliable with restrictions.

[d]According to the minutes of the Pesticides Peer Review meeting (meeting held on 11 March 2024). The Pesticide Peer Review is ongoing.

The FEEDAP Panel notes that most of the studies consistently indicated effects on the reproductive system, particularly in males.

The effect of tea tree oil on sexual function and fertility has been investigated in a two-generation reproduction study performed according to OECD Test Guideline (TG) 416 (Anonymous 2017a, as referenced in ECHA, 2024b). Wistar rats were exposed to tea tree oil by oral gavage at 0, 10, 25 or 50 mg/kg bw per day in the parental generation, and 0, 10, 25 or 38 mg/kg bw per day in the F1 generation (doses reduced because of alterations in reproductive performance in the parental generation). As reported in the minutes of Pesticides Peer Review meeting (working group on mammalian toxicology),[37] from this study a no observed adverse effect level (NOAEL) of 10 mg kg bw per day for reproductive toxicity was identified based on fertility (based on overall effects on sperm parameters in both the P and F1 generations). In addition, the adverse effects of tea tree oil on testes and/or sperm count, and motility were observed in the repeated dose toxicity studies (in a 28-day study in rats, two 90-day studies in rats and in a 90-day study in dogs). These effects were not observed at the dose of 30 mg/kg bw per day, which can be considered as the NOAEL of the 90-day studies in rats and dogs.

Two studies in rats and one in rabbits investigated the potential of tea tree oil to adversely affect development. A NOAEL for maternal and developmental toxicity of 30 mg/kg bw per day was identified from the first rat study based on effects observed on mortality (at 300 mg/kg bw per day) and reduced body weight and food consumption (at 60 and 120 mg/kg bw per day) (Anonymous 2012a, as referenced in ECHA, 2024b). In the second rat study, several maternal toxicity effects were seen in dams in the mid- and high-dose groups (100 and 250 mg/kg bw per day), as evidenced primarily by clinical signs, reduced food consumption and reduced weight gain suggesting a NOAEL of 20 mg/kg bw per day (ECHA dissemination onsite, study report 2011, as referenced in ECHA 2024b). The NOAEL for maternal toxicity in rabbits was 75 mg/kg bw per day (Anonymous 2018b, as referenced in ECHA 2024b). The effects on fetal toxicity in the rat studies (delayed ossification, skeletal malformations) and in the rabbit study (post implantation losses) were discounted as they were considered secondary to maternal effects.

The FEEDAP Panel adopts the lowest NOAEL of 10 mg/kg bw per day for effects on sperm parameters from the two-generation study,[38] as a reference point to assess the safety of tea tree oil under assessment.

3.4.2 | Safety for the target species

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

[37]https://www.efsa.europa.eu/sites/default/files/2023-12/minutes-ppr-mammalian-toxicity.pdf, meeting held on 11 March 2024. The Pesticide Peer Review is ongoing.

[38]Study report not available to FEEDAP.

In the absence of these data, the approach to the safety assessment of a mixture whose individual components are known is based on the safety assessment of each individual component (component-based approach). This approach requires that the mixture is sufficiently characterised and that the individual components can be grouped into assessment groups, based on structural and metabolic similarity. Alternatively, the approach to the safety assessment of the whole mixture can be based on read-across from a sufficiently similar mixture (whole mixture approach) (EFSA Scientific Committee, 2019a).

Relevant toxicological studies with tea tree oils of similar composition have been identified (see Section 3.4.1).

The FEEDAP Panel notes that toxicological data are not available for the two major components of the tea tree oil, 4-terpinenol [02.072] and γ-terpinene [01.020], which together account for about 60% of the total % GC area (up to 76% by specification). Therefore, the outcome of a component-based approach based on the extrapolation of reference points from the representative compounds terpineol [02.230] and *d*-limonene [01.045], which are minor constituents of tea tree oil, is expected to introduce a high degree of uncertainty. The FEEDAP Panel also notes that the toxicological data set available for a tea tree oil similar to the additive under assessment indicates NOAEL values in the range 10–75 mg/kg bw per day, which are far lower than the NOAEL of 250 mg/kg bw per day for the representative components terpineol [02.230] and *d*-limonene [01.045].

Therefore, the FEEDAP Panel considers that the whole mixture approach based on read-across from a sufficiently similar mixture is the most suitable approach to the assessment of the safety for the target species for tea tree oil. Methyleugenol, a genotoxic compound, is assessed separately.

Whole mixture approach (without considering the presence of methyleugenol)

Because of the similarity of the tea tree oil tested in the two-generation study (Anonymous 2017a, as referenced in ECHA, 2024b) (see Section 3.4.1.2) with the additive under assessment, the FEEDAP Panel applied the NOAEL of 10 mg/kg bw per day for fertility as the reference point to derive maximum safe feed concentrations of the additive for the target species. Following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), an uncertainty factor (UF) of 100 was applied to the reference point and the maximum safe feed concentrations of tea tree oil (without considering the presence of methyleugenol) was calculated (Table 6). Generally, for cats, an additional UF of 5 is applied, considering their unusually low capacity for glucuronidation, particularly of aromatic compounds (Court & Greenblatt, 1997; Lautz et al., 2021).

TABLE 6 Maximum safe concentrations of tea tree oil in feed for the target animal species and categories calculated using the reference point of 10 mg/kg bw per day derived from a two-generation study in rats (Anonymous 2017a, as referenced in ECHA, 2023b) and applying an uncertainty factor of 100 (500 for cats).

Animal category	Daily feed intake (g DM/kg bw)	Proposed use level (mg/kg complete feed)[a]	Maximum safe concentration (mg/kg complete feed)[a]
Chickens for fattening	79	25	1.1
Laying hens	53	20	1.7
Turkeys for fattening	59	40	1.5
Piglets	44	20	2.0
Pigs for fattening	37	30	2.4
Sows	30	20	3.1
Veal calves (milk replacer)	19	60	5.0
Cattle for fattening	20	30	4.4
Dairy cows	31	20	2.9
Sheep/goats	20	20	4.4
Horses	20	20	4.4
Rabbits	50	20	1.8
Salmonids	18	40	5.0
Crustaceans (shrimps)	13.3[b]	15	6.6
Dogs	17	20	5.3
Cats[c]	20	20	0.9
Ornamental fish	5	15	19.6[d]

[a]Complete feed containing 88% dry matter (DM), milk replacer 94.5% DM.

[b]Based on an estimated body weight of 0.015 kg and a daily feed intake of 0.2 g DM/day for the white shrimp *Litopenaeus vannamei* (Hosseini Aghuzbeni et al., 2017; Lee & Lee, 2018; Toyes-Vargas et al., 2017).

[c]For cats, an additional uncertainty factor of 5 is applied because of the reduced capacity of glucuronidation.

[d]For ornamental fish, the proposed use level is considered safe (without considering the presence of methyleugenol).

The maximum use level in complete feed proposed by the applicant of 15 mg/kg is safe for ornamental fish. For the other species, the resulting maximum safe levels in complete feed are shown in Table 6 (without considering the presence of methyleugenol). These levels are extrapolated to physiologically related minor species. For the other species not considered, the lowest value of 1.1 mg/kg complete feed is applied.

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

Methyleugenol

Methyleugenol belongs to the group of *p*-allylalkoxybenzenes and is a genotoxic carcinogen. According to the general approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021a), different reference points and a different magnitude of the margin of exposure (MOE) 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 and reproductive animals, an MOE 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 carcinogenicity studies in rodents (rats) with methyleugenol (NTP, 2000; Suparmi et al., 2019), as the reference point for the entire group of *p*-allylalkoxybenzenes (EFSA FEEDAP Panel, 2022).

For short-living animals, genotoxicity and carcinogenicity endpoints are not considered biologically relevant; therefore, a lower magnitude of the MOE (> 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP Panel, 2021b). The FEEDAP Panel identified a NOAEL of 10 mg/kg bw per day for non-neoplastic lesions (changes in organ weight[39] and function, including effects on liver[40] and the glandular stomach[41]) from a 90-day study in mice with methyleugenol (NTP, 2000; EFSA FEEDAP Panel, 2023b).

Methyleugenol was detected at the level of the LOD (0.01% corresponding to 100 mg/kg) in one batch of the additive under assessment. The use of tea tree oil at the levels in feed which were considered safe for the different target species, without considering the presence of methyleugenol, ranges from 1.1 to 15.0 mg/kg complete feed (see Table 6). These levels correspond to methyleugenol concentrations ranging from 0.0001 to 0.0015 mg/kg complete feed. The highest daily intake of methyleugenol in µg/kg bw was calculated for the different target animal categories considering the analysed value in the additive (0.01%). The calculated intake values range between 0.004 µg/kg bw per day (in cats) and 0.011 µg/kg bw per day (in sows) (see Appendix A).

When the estimated exposures for long-living and reproductive animals are compared to the $BMDL_{10}$ of 22.2 mg/kg bw per day, derived by Suparmi et al. (2019) from a rodent carcinogenicity study (NTP, 2000), an MOE ranging between 2,088,710 and 5,426,667 is calculated for long-living animals. When comparing the exposure of short-living animals to the reference point based on non-neoplastic endpoints, an MOE ranging between 933,333 and 1,005,714, is obtained for all species (see Appendix A).

The magnitude of the MOE indicated that the presence of methyleugenol in tea tree oil is very unlikely to be of safety concern for long-living and reproductive animals and of no concern for species for fattening.

3.4.2.1 | *Conclusions on safety for the target species*

The FEEDAP Panel concludes that the levels of tea tree oil summarised in Table 7 are considered very unlikely to be of safety concern for long-living and reproductive animals and of no concern for species for fattening.

[39]Increases in absolute liver weights of rats (at doses of 100 mg/kg of higher in males and at doses of 300 mg/kg of higher in females) and mice (at 30, 100 and 300 mg/kg in males and at 300 mg/kg in females) and the increase in testis weight of rats administered 1000 mg/kg.

[40]Cytologic alteration, cytomegaly, Kupffer cell pigmentation, bile duct hyperplasia and foci of cellular alteration.

[41]Incidences of atrophy and chronic inflammation of the mucosa of the glandular stomach were significantly increased in rats administered 300 or 1000 mg/kg; the incidences of lesions of the glandular stomach were increased in one or more groups administered 30 mg/kg or greater.

TABLE 7 Concentrations of tea tree oil in complete feed (mg/kg) which are considered very unlikely to be of safety concern for long-living and reproductive animals and of no concern for species for fattening.

Animal categories	Feed concentration (mg/kg complete feed)[a]
Turkeys for fattening	1.5
Chickens for fattening, other poultry for fattening or reared for laying/reproduction, ornamental birds	1.1
Laying hens and other laying/reproductive birds	1.7
Pigs for fattening	2.4
Piglets and other porcine species for meat production or reared for reproduction	2.0
Sows and other porcine species for reproduction	3.1
Veal calves (milk replacer)	5.0
Cattle for fattening, other bovines for fattening or reared for milk production/reproduction, cervids and camelids at the same physiological stage	4.4
Dairy cows and other bovines, cervids and camelids for milk production or reproduction	2.9
Sheep/goats	4.4
Horses and other equines	4.4
Rabbits and other leporids	1.8
Salmonids and minor fin fish	5.0
Crustaceans (shrimps)	6.6
Dogs	5.3
Cats	0.9
Ornamental fish	15
Other species	1.1

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

The FEEDAP Panel considers that the use in water for drinking is very unlikely to be of safety concern for long-living and reproductive animals and of no concern for species for fattening provided that the total daily intake of the additive does not exceed the daily amount that is considered safe when consumed via feed.

3.4.3 | Safety for the consumer

Tea tree oil is added to food of different categories for flavouring purposes. Although individual consumption figures are not available, Fenaroli's handbook reports use levels ranging from 10 to 50 mg/kg in several food categories (Burdock, 2009).

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 4, Section 3.4).

No data on residues in products of animal origin were made available for any of the constituents of the essential oil under assessment. However, the Panel recognises that the constituents of tea tree oil are expected to be extensively metabolised and excreted in the target species (EFSA FEEDAP Panel, 2012a, 2015).

For methyleugenol, the available data indicate that it is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products at the levels present in the additive (EFSA FEEDAP Panel, 2023b).

Considering the above and the reported human exposure due to the direct use of tea tree oil in food (Burdock, 2009), it is unlikely that the consumption of products from animals given tea tree oil at the proposed maximum use level would substantially increase human background exposure. The use of tea tree oil in animal nutrition under the proposed conditions of use is considered safe for human consumers of animal products.

3.4.4 | 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. alternifolia* for users.[42] Of the papers retrieved, 29 were proposed to contain data potentially relevant to user safety. Mostly these references related to beneficial effects or lack of adverse effects of low concentrations of tea tree oil, thus are not relevant to assessment of user safety. One paper, reviewing adverse drug reactions (Bekhof et al., 2022), identified that irritant reactions and contact allergy are known to be caused by undiluted tea tree oil, particularly if it is stored under conditions allowing photo-oxidation. These potential effects are supported by the review of Tisserand and Young (2014) and have been described in the EMA report (2015b) (see Section 3.3.3).

For tea tree oil, hazards for the user have been identified in the RAC opinion (ECHA, 2024a).[43] The FEEDAP Panel notes that, in the RAC opinion (ECHA, 2024a), tea tree oil is classified as reprotoxic category 1B (hazard statement code H360Fd: May damage fertility. Suspected of damaging the unborn child) in accordance with the classification criteria in Annex I of the CLP Regulation (1272/2008/EC)[44] and should be handled accordingly.[45]

The FEEDAP Panel concludes that tea tree oil should be considered as irritant to skin and eyes, and as a dermal and respiratory sensitiser and as a reprotoxic substance.

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

3.4.5 | Safety for the environment

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

The major component(s) (terpinen-4-ol, γ-terpinene, α-terpinene and 1,8-cineole) and additional 26 components (see Table 4, Section 3.4) accounting together for about 92% of the % GC area, have been evaluated by EFSA as sensory additives for animal feed. For 4-terpinenol and 1,8-cineole, the applicant provided evidence that they are present at high concentrations in plants native to Europe (EFSA FEEDAP Panel, 2012a, 2019). For γ-terpinene and α-terpinene, the need of a Phase II assessment is excluded based on (i) their natural occurrence in European plants, (ii) considerations on their extensive metabolism in the target animals and (iii) read across from structurally related compounds included in tolerance studies with a mixture of flavourings ('Herbal mixture') (EFSA FEEDAP Panel, 2023c). Concerning the other components evaluated as feed additives, 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 (see Table 4, Section 3.4).

The remaining identified constituents of the essential oil, which were not evaluated for use in feed, are chemically related to the substances evaluated by EFSA in CG 6, 7, 8, 10, 16, 18, 31 and 32 (EFSA FEEDAP Panel, 2012a, 2015, 2016b, 2016c), for which EFSA concluded that they are extensively metabolised by the target species and excreted as metabolites or carbon dioxide. Therefore, no risk for the safety for the environment is foreseen from these constituents.

The use of tea tree oil from *M. alternifolia* in animal feed under the proposed conditions of use is not expected to pose a risk to the environment.

3.5 | Efficacy

Tea tree oil is listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference number 3902.

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

4 | CONCLUSIONS

Tea tree oil from the leaves and terminal branchlets of *Melaleuca alternifolia* (Maiden & Betche) Cheel may be produced from plants of different geographical origins, resulting in preparations with different composition and toxicological profiles. Thus, the following conclusions apply only to tea tree oil which is produced from the leaves and terminal branchlets of *M. alternifolia* and contains ≤ 0.01% methyleugenol.

[42]Technical dossier/Supplementary information October 2023/Literature search_tea_tree oil.

[43]Aspiration hazard (H304, Category 1), Hazard for skin irritation (H315, Category 2), skin sensitisation (H317, Category 1B), serious eye damage/eye irritation (H319, category 2), Reproductive toxicity (H360Fd, category 1B), in accordance with the criteria outlined in Annex I of 1272/2008/EC (CLP/EU-GHS).

[44]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.

[45]Consolidated text: 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 or reprotoxic substances 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. https://eur-lex.europa.eu/eli/dir/2004/37/2024-04-08.

The conclusions of the FEEDAP Panel on the concentrations in complete feed of tea tree oil, which are considered very unlikely to be of safety concern for long-living and reproductive animals and of no concern for species for fattening are summarised as follows:

Animal categories	Feed concentration (mg/kg complete feed)[a]
Turkeys for fattening	1.5
Chickens for fattening, other poultry for fattening or reared for laying/reproduction, ornamental birds	1.1
Laying hens and other laying/reproductive birds	1.7
Pigs for fattening	2.4
Piglets and other porcine species for meat production or reared for reproduction	2.0
Sows and other porcine species for reproduction	3.1
Veal calves (milk replacer)	5.0
Cattle for fattening, other bovines for fattening or reared for milk production/reproduction, cervids and camelids at the same physiological stage	4.4
Dairy cows and other bovines, cervids and camelids for milk production or reproduction	2.9
Sheep/goats	4.4
Horses and other equines	4.4
Rabbits and other leporids	1.8
Salmonids and minor fin fish	5.0
Crustaceans (shrimps)	6.6
Dogs	5.3
Cats	0.9
Ornamental fish	15
Other species	1.1

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

The FEEDAP Panel considers that the use in water for drinking alone or in combination with use in feed should not exceed the daily amount that is considered very unlikely to be of safety concern when consumed via feed alone.

No concerns for consumers were identified following the use of the additive at the maximum proposed use level in feed.

Tea tree oil is classified as reprotoxic (category 1B) and should be handled accordingly. It should be considered as irritant to skin and eyes, as a dermal and respiratory sensitiser. When handling the additive, exposure of unprotected users to methyleugenol may occur. Therefore, to reduce the risk, the exposure of the users should be minimised.

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

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

5 | RECOMMENDATION

The specification should ensure that tea tree oil from *Melaleuca alternifolia* (Maiden & Betche) Cheel contains ≤ 0.01% methyleugenol.

6 | DOCUMENTATION PROVIDED TO EFSA/CHRONOLOGY

Date	Event
28/10/2010	Dossier received by EFSA. Botanically defined flavourings from Botanical Group 07 – Geraniale, Myrtales, Poales for all animal species and categories. Submitted by Feed Flavourings Authorisation Consortium European Economic Interest Grouping (FFAC EEIG)
09/11/2010	Reception mandate from the European Commission
21/12/2010	Application validated by EFSA – Start of the scientific assessment
22/03/2011	Comments received from Member States

(Continues)

(Continued)

Date	Event
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*
08/01/2013	Reception of supplementary information from the applicant—Scientific assessment remains suspended
26/02/2013	EFSA informed the applicant (EFSA ref. 7,150,727) that, in view of the workload, the evaluation of applications on feed flavourings would be re-organised by giving priority to the assessment of the chemically defined feed flavourings, as agreed with the European Commission
20/01/2014	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
27/02/2019	Partial withdrawal by applicant (EC was informed) for the following additives: broom teatree oil, geranium oil, bay oil and vetiveria oil
17/12/2019	EFSA informed the applicant that the evaluation process restarted
18/12/2019	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*
18/11/2022	Partial withdrawal by applicant (EC was informed) for the following additive: bambusa tincture and allspice oil
06/06/2023	Reception of an amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to geranium rose oil, eucalyptus oil, lemongrass oil and clove oil
19/10/2023	Reception of supplementary information from the applicant (partial dataset: tea tree oil)—Scientific assessment remains suspended
28/02/2024	The application was split and a new EFSA-Q-2024-00119 was assigned to the additive included in the present assessment. Scientific assessment re-started for the additive included in the present assessment
01/03/2024	Reception of an amendment of the Evaluation report of the European Union Reference Laboratory for Feed Additives related to citronella oil, melaleuca oil, niaouli oil, tea tree oil, eucalyptus tincture, clove tincture.
18/09/2024	Opinion adopted by the FEEDAP Panel on tea tree oil (EFSA-Q-2024-00119). End of the Scientific assessment for the additive included in the present assessment. The assessment of other additives in BGD 07 is still ongoing

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food
BDG	Botanically defined group
BMD	benchmark dose
$BMDL_{10}$	BMD lower confidence limit for a benchmark response of 10%
bw	body weight
CAS	Chemical Abstracts Service
CDG	Chemically defined group
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CG	chemical group
CLH	Harmonised Classification and Labelling
CLP	Classification, Labelling and Packaging
CoE	Council of Europe
ECHA	European Chemicals Agency
EEIG	European economic interest grouping
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAO	Food Agriculture Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavor Extract Manufacturers Association
FFAC	Feed Flavourings authorisation Consortium of (FEFANA) the EU Association of Specialty Feed Ingredients and their Mixtures
FGE	Flavouring Group Evaluation
FLAVIS	the EU Flavour Information System
FLAVIS-No	FLAVIS number
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC–MS	gas chromatography–mass spectrometry
IARC	International Agency for Research on Cancer
ISO	International standard organisation

LOD	Limit of detection
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organization for Economic Co-operation and Development
PCBs	polychlorinated biphenyls
PhEur	European Pharmacopoeia
QSAR	Quantitative Structure–Activity Relationship
RAC	Committee for Risk Assessment
TG	Test Guideline
UF	uncertainty factor
WHO	World Health Organization

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CONFLICT OF INTEREST

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

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APPENDIX A

Methyleugenol in tea tree oil: Maximum daily intake and margin of exposure for the different target species

The maximum daily intake of methyleugenol for the different target species and categories was calculated based on the default values for body weight and feed intake (EFSA FEEDAP Panel, 2017b); the maximum proposed/safe use level (see Table 7) of the additive in feed for the different target animal categories (ranging from 1.1 to 15.0 mg/kg complete feed); and assuming that methyleugenol is present at a concentration corresponding to the analysed value in one batch of the additive (0.01%).

The margin of exposure (MOE) for each animal category is calculated as the ratio of the reference point to the intake: the $BMDL_{10}$ of 22.2 mg methyleugenol/kg bw per day for long-living and reproductive animals; the NOAEL of 10 mg methyleugenol/kg bw per day for target species for fattening (EFSA FEEDAP Panel, 2023b).

According to the general approach to assess the safety for the target species of botanical preparations which contain compounds that are genotoxic and/or carcinogenic (EFSA FEEDAP Panel, 2021),[46] 'for substances for which carcinogenicity studies in rodents are available, from which a $BMDL_{10}$ can be derived, the MOE approach (EFSA, 2005; EFSA Scientific Committee, 2012) can be applied. Similarly to human risk assessment, a margin of exposure (MOE) with a magnitude of ≥ 10,000, when comparing estimated exposure to genotoxic and/or carcinogenic substances with a $BMDL_{10}$ from a rodent carcinogenicity study, would be indicative of a low concern for the target species (EFSA Scientific Committee, 2019a).' For short-living animals, genotoxicity and carcinogenicity endpoints are not considered relevant, therefore a lower magnitude of the MOE (> 100) when comparing estimated exposure with a reference point based on non-neoplastic endpoints is considered adequate (EFSA FEEDAP Panel, 2021a).

The maximum daily intake of methyleugenol for the different target animal categories and the corresponding MOE are reported in Table A.1.

TABLE A.1 Target animal intake of methyleugenol (as µg/kg bw per day) and margin of exposure (MOE) at the maximum proposed use level of tea tree oil in feed for target animal category.

Animal category	Daily feed intake g DM/day	Body weight kg	Max safe use levels mg/kg	Intake[a] µg/kg bw/day	Lowest MOE[b]
Long-living and reproductive animals					
Laying hens	0.106	2	1.7	0.010	2,168,257
Sows lactating	5.28	175	3.1	0.011	2,088,710
Dairy cows	20	650	2.9	0.010	2,189,379
Sheep/goats	1.2	60	4.4	0.010	2,220,000
Horses	8	400	4.4	0.010	2,220,000
Rabbits	0.1	2	1.8	0.010	2,170,667
Dogs	0.25	15	5.3	0.010	2,211,623
Cats	0.06	3	1.8	0.004	5,426,667
Ornamental fish	0.00054	0.012	15.0	0.008	2,894,222
Target species for fattening					
Chickens for fattening	0.158	2	1.1	0.010	1,012,658
Turkeys for fattening	0.176	3	1.5	0.010	1000,000
Piglets	0.88	20	2.0	0.010	1000,000
Pigs for fattening	2.2	60	2.4	0.010	1000,000
Veal calves (milk replacer)	1.89	100	5.0	0.010	1000,000
Cattle for fattening	8	400	4.4	0.010	1000,000
Sheep/goats	1.2	60	4.4	0.010	1000,000
Horses	8	400	4.4	0.010	1000,000
Rabbits	0.1	2	1.8	0.010	977,778
Salmon	0.0021	0.12	5.0	0.010	1,005,714
Shrimps	0.0002	0.015	6.6	0.011	933,333

[a]The values of methyleugenol in feed are calculated considering the value (0.01%) analysed in one batch of the additive.

[b]The MOE for methyleugenol is calculated as the ratio of the reference point to the intake: for long-living and reproductive animals is based on $BMDL_{10}$ of 22.2 mg/kg bw per day derived from rodent carcinogenicity studies with methyleugenol; for target species for fattening based on a NOAEL of 10 mg/kg bw per day derived from a 90-day study with methyleugenol (NTP, 2000).

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