Anses Opinion
Request No 2023-AUTO-0123
Related Request No 2020-SA-0083

The Director General

Maisons-Alfort, 19 juin 2025

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on « Preparation of the application dossier for the establishment of maximum residue limits (MRLs) of tea tree essential oil (EO) for inclusion in table 1 of regulation (EU) No 37/2010.»

Anses undertakes independent and pluralistic scientific expert assessments.
Anses primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 8 December 2021 shall prevail.

On June 7th, 2023, Anses issued an internal request to conduct the following expert appraisal: preparation of the application dossier for the establishment of maximum residue limits (MRLs) of tea tree essential oil (EO) for inclusion in table 1 of regulation (EU) No 37/2010.

1. BACKGROUNG AND PURPOSE OF THE REQUEST

Maximum residue limits (MRLs) correspond to regulatory thresholds, in food from treated animals, of substances residues contained in veterinary medicinal products. MRLs are defined for a given substance, species and tissue or food. They aim to ensure a level of exposure without risk to consumers. One of the main difficulties in veterinary phyto/aromatherapy is the lack of MRL status for the majority of plants, plant preparations and essential oils (EO) of interest. Without MRL status, their use in veterinary medicinal products for food-producing animals is not authorised. The concept of veterinary medicinal product is defined in the regulation (EU) No 2019/6.

Concerning the classification of pharmacologically active substances administered in food-producing animals with regard to maximum residue limits, regulation (EU) No 37/2010 contains two tables:

- Table 1 corresponding to the authorized substances (with the possibility of restrictions on use and/or species);
- Table 2 for prohibited substances (no MRLs can be set).

Some substances are considered, after evaluation by the European Medicines Agency (EMA), to fall outside the scope of the MRLs as defined in regulation (EC) No 470/2009 and are then included on a list named “Out of scope” of the Committee for Veterinary Medicinal Products (CVMP) of the EMA (EMA/CVMP/519714/2009). These include substances naturally present in the body or foodstuffs entering in human consumption, which do not pose a risk to the health of the consumer.

If the future veterinary medicinal product is intended for food-producing animals, each EO or plant containing it must be classified in Table 1 of regulation (EU) No 37/2010 or be included in the list “Out of scope”. Thus, the question of the MRL status of plants and EO is fundamental for the treatment in phyto/aromatherapy of food-producing animals both in the context of the assessment of the marketing authorization dossier and in the case of prescription of an extemporaneous preparation based on plants or EO. However, the vast majority of EO and plants frequently used have not been assessed within the meaning of regulation (EC) No 470/2009 and therefore cannot, at present, be included in veterinary medicinal products intended for food-producing animals or be prescribed in an extemporaneous preparation by a veterinarian. Plant-based products are often supplied to animals as supplementary (additive-based) feeds.

The assessment of MRLs under the current European regulations for veterinary medicinal products is difficult, if not impossible, due to the complex and variable chemical composition of a plant or plant preparation such as an EO.

This request is a continuation of previous Anses opinions and reports:

1) ‘Assessment of applications for marketing authorizations for herbal medicinal products’ (Request No 2014-SA-0081) One of the conclusions concerned the main difficulty with regard to the lack of adequate MRL status for a large majority of plants of interest in veterinary medicine.

2) ‘State of knowledge on EO and plants of interest for phytotherapy and aromatherapy in food-producing animals and proposed human health risk assessment methodology’ (Request No 2020-SA-0083). Proposed methodology is adapted to plants and their preparations. The data used are primarily those published by national organizations (French Health Products Safety Agency (ANSM), Anses...), European organizations (EMA, European Food Safety Authority (EFSA), European Chemicals Agency (ECHA)...) and even international organizations (World Health Organization (WHO), Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives (JECFA), Joint FAO/WHO Meeting on Pesticide Residues (JMPR)...) in the context of plant assessments and of their preparations. These data may be supplemented and/or updated by bibliographic searches. A two-steps decision tree can guide the assessor and classify the plants and EOs into one of the following categories:
    - No concern for the consumer of food from animals having received it,
    - Insufficient data to conclude that there is no concern for the consumer of food derived from animals that have received it,
    - Preparation of food from animals of concern to the consumer on the basis of available data.

In this report, a preliminary assessment of consumer risks for the plants and EOs of interest in veterinary medicine was carried out (10 plants, 5 EOs) as well as the evaluation of major substances present in essential oils (8 compounds).

The methodology proposed by Anses must now be officially evaluated at the European level. As part of the French Presidency of the Council of the European Union in 2022, this work was presented to the EMA and the European Commission. Anses was then encouraged to continue its work by submitting the first MRL dossiers to the EMA on the basis of this methodology.

This request is the application of the methodology adapted to plants and their preparations on a first example of drawing up a dossier for setting MRLs: tea tree EO, with a view to adding it to the list of authorized substances (table 1 of regulation (EU) No 37/2010). The choice was made for this EO due to the frequency of use in the field and the large number of data available. This EO has been the subject of a preliminary consumer risk assessment in request No 2020-SA-0083. The purpose of this request is to complete the assessment in accordance with the requirements of regulation (EU) No 2018/782. The format used in this report is therefore the one required by this regulation.

2. ORGANISATION OF THE EXPERT APPRAISAL

Anses entrusted the examination of this request to the Expert Committee for the Assessment of physico-chemical risks in food (CES ERCA). The Agency also mandated the Working Group (WG) on Plants for this expert appraisal.

CES ERCA was asked to give its opinion on the application of the methodology proposed in the request report No 2020-SA-0083 and on the logic and functionality of the decision tree. This initial application also highlights the limitations of this methodology and proposes recommendations for improvement. The purpose of the CES ERCA is not to give an opinion on management measures, as this is the responsibility of EMA and European Commission. Once the MRL dossier has been submitted by Anses-ANMV as an applicant, it will be evaluated by the EMA. Through this dossier, the proposed methodology will also have been evaluated by the EMA.

The methodological and scientific aspects of this group's work were regularly submitted to the CES. The report produced by the WG takes account of the observations and additional information provided by the CES experts. CES drew up and validated its conclusions between 12/12/2023 and 27/03/2024. The expert appraisal work was adopted by the CES ERCA on 27/03/2024, unanimously by the experts present.

This work was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise Activities".

Anses analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals. The experts' declarations of interests are made public on website: https://dpi.sante.gouv.fr

3. ANALYSIS AND CONCLUSIONS OF THE WG

The analysis and conclusions presented below summarise the expert reports of the rapporteurs of the Plants WG and the examination conducted by the ERCA CES.

3.1. Administrative information

See Annex 2: Application form

3.2. Data for scientific risk assessment

3.2.1. Introduction

The substance is the EO of *Melaleuca alternifolia* (Maiden & Betch) Cheel, commonly named "tea tree oil" (tea trea EO). It is a complex plant extract, with multiple uses.

The substance is intended for use as an **active ingredient** in **veterinary herbal products**. Tea tree EO is intended for use as an antiseptic / general antimicrobial in all food producing species, by oral route, oro-mucosal route, as well as in external use. It is mainly used in bovine, but also in small ruminants and horses.

Its uses are based on tradition of use, which might comply with the conditions depicted in directive 2004/24/EC for herbal medicinal products of human use (Anses 2016).

The main usual doses, as noted for field uses (Anses 2022), can be summarized as follows:

- Oral route: up to 1 mL of tea tree EO/day in bovine (approx. 885 - 906 mg/day), up to 0.2 mL in sheep and goat (approx. 177 - 181 mg/day) (diluted in fixed oil);
- Cutaneous use: 10 drops (approx. 250 - 400 µL, 220 - 360 mg), twice a day in bovine, 5 drops (approx. 125 - 200 µL, 110 - 330 mg), twice a day in sheep and goat.

Recommendations published by veterinarians in specialized literature (Labre 2012), based on empirical observations, are in accordance with these indications, routes and dosages. It can be noted that, according to the bibliography:

- Vaginal use (ovules) is mentioned in cows (1 mL/day) for metritis;
- Maximum level of use is for bovine, with 50 drops/day (1.25 - 2 mL/day, oral route, 5 % dilution);
- Tea tree EO is usually proposed in association with other EOs.

Tea tree EO is not listed in regulation (EU) No 37/2010 and doesn't have a MRL status yet. Several compounds of tea tree EO are present in EOs listed in table 1 of regulation (EU) No 37/2010 (Annex 3) and are natural ingredients of grass present in pasture that can be detected in milk. There is no applicant, and no dossier in compliance with the regulation (EU) No 2019/6 on veterinary medicinal products is available.

3.2.2. Safety file

3.2.2.1. Precise identification of the substance concerned by the application

3.2.2.1.1. *General description*

According to the European Pharmacopoeia (Eur. Pharm.) (monograph 01/2008:1837), tea tree EO is the EO obtained by stem distillation of the leaves and terminal stems of *Melaleuca alternifolia* (Maiden & Betch) Cheel (Myrtaceae) (syn. *Melaleuca linariifolia* var. *alternifolia* Maiden & Betch). It can also be obtained from *M. linariifolia* Smith, *M. dissitiflora* F. Mueller and/or from other *Melaleuca* species.

These origins and modes of production are in accordance with that given for tea tree EO for its commercial trade and non-pharmaceutical uses (fragrance and other cosmetic uses, food flavouring) by the International Organization for Standardization (ISO 4730:2004; addendum ISO 4730/A1:2018) (ISO 2018) and by the European Council for its use as a food flavouring substance.

3.2.2.1.2. *International Nonproprietary Names (INN)*

There is no INN.

It is designated as "*Melaleuca aetheroleum*" in pharmaceutical systems (e.g. Eur. Pharm., EMA), or as "*Aetheroleum Melaleucae Alternifoliae*" by the WHO (WHO/FAO 2002).

Its Unique Ingredient Identifier (UNII) code as designated by the FDA is VIF565UC2G, which corresponds to the definition of this substance as described by the Eur. Pharm. (FDA 2023).

3.2.2.1.3. *International Union of Pure and Applied Chemistry (IUPAC) name*

Tea tree EO is a complex substance and thus doesn't belong to IUPAC nomenclature.

IUPAC designations of major components are indicated in Annex 4.

3.2.2.1.4. *Chemical Abstracts Service (CAS) number*

The CAS No of tea tree EO is 68647-73-4.

ECHA (2022) describes "*Melaleuca alternifolia*, ext.", corresponding to extracts and encompassing tea tree EO, with the EC / List No: 285-377-1 and the CAS No 85085-48-9. It can correspond to tea tree EO (encompassing several chemotypes), as well as other extracts such as absolutes or concretes, which don't comply with the mode of production of pharmaceutical essential oils and are not considered in this MRL dossier.

3.2.2.1.5. *Therapeutic, pharmacological and chemical classification*

Tea tree EO is mainly used as an antiseptic and antimicrobial agent (see 3.2.1. "Introduction") ,but also as an antifungal, antiparasitic and anti-inflammatory (see 3.2.2.2.1 "Pharmacodynamics").

3.2.2.1.6. *Synonyms and abbreviations*

The synonyms and abbreviations for this essential oil used in the bibliographical references cited in this report are as follows:

Tea tree essential oil

Tea Tree EO

Tea tree oil

Melaleuca aetheroleum

Aetheroleum Melaleucae Alternifoliae

TTO

3.2.2.1.7. *Chemical composition*

Composition of tea tree EO, according to the Eur. Pharm., is presented in Table 1:

Table 1: Composition of tea tree EO (Eur. Pharm. 01/2008:1837 corr. 7.0)

Component	Minimum (%)	Maximum (%)
α-Pinene (pin-2(3)-ene)	1.0	6.0
Sabinene (4(10)-thujene)	Maximum 3.5	
α-Terpinene	5.0	13.0
Limonene	0.5	4.0
1,8-Cineole	Maximum 15.0	
γ-Terpinene	10.0	28.0
p-Cymene	0.5	12.0
Terpinolene	1.5	5.0
Terpinen-4-ol (4-terpineol)	Minimum 30.0	
Aromadendrène	Maximum 7.0	
α-Terpineol	1.5	8.0

Several chemotypes are described for this EO (ISO 2018, Anses 2020, 2022, Gafner et Dowell 2018), with more than 35 individual compounds. Bibliographical data confirm the chemotype described in the Eur. Pharm. as being the most usual for tea tree EO (Tisserand et Young 2014, Anses 2020). It corresponds to CT 1 of ISO 4730:2017. The active substance considered here complies with Eur. Pharm.

3.2.2.1.8. *Structural formula*

Not applicable.

The chemical structures of major constituents of tea tree EO are presented in Annex 5.

3.2.2.1.9. *Molecular formula*

Not applicable.

For individuals components, see Annex 4.

3.2.2.1.10. *Molecular weight*

Not applicable.

For individuals components, see Annex 4.

3.2.2.1.11. *Degree of impurity*

The substance complies with the following Eur. Pharm. monographs: 01/2008:2098 (*Aetheroleum*) and 01/2008:1837 (*Melaleuca aetheroleum,* tea tree oil).

3.2.2.1.12. *Qualitative and quantitative composition of impurities*

The American Botanical Council (ABC), the American Herbal Pharmacopoeia (AHP), and the University of Mississippi's National Center for Natural Products Research (NCNPR) published the following documents: “Tea Tree Oil Laboratory Guidance Document” (Gafner et Dowell 2018) and “Tea Tree Oil Bulletin” (Bejar 2017), as part of the “Botanical Adulterants Program”. Adulterations mostly consist in the addition of synthetic (racemic) terpinen-4-ol. The controls imposed by the Eur. Pharm. and the GACP / GMP guidelines rule out the use of adulterated material.

Ascaridole, a monoterpenic peroxyde formed from α-terpinene, exceptionally occurs at low levels in batches which underwent long storage in poor conditions. This compound is not considered as a putative impurity in the Eur. Pharm. nor by the AHP/NCNPR consortium (Bejar 2017, Gafner et Dowell 2018). Tisserand and Young (2014) discussed the good stability of this EO under proper storage conditions and mention unusually high ***p*-cymene** content as the main oxidization marker (see Table 1 in 3.2.2.1.7). Ascaridole was therefore not considered here.

Methyleugenol is contained in tea tree EO in low proportions ranging from 0.01 to 0.4% (Anses 2020). A content of 0.4 % is exceptional, concentrations are generally below 0.01 % or no detection of this substance (Vázquez, Tabanca, et Kendra 2023, Raymond, Davies, et Larkman 2017, Gafner et Dowell 2018, Tisserand et Young 2014). Chemical structure is presented in Annex 6. Quantification of this substance is not required in the European Pharmacopoeia monograph.

Methyleugenol is registered under REACH (under the name 4-allylveratrole) and classified as mutagenic (class 2), carcinogenic (class 2) and toxic by single administration (class 4). In 2023, methyleugenol was classified by the IARC (International Agency for Research on Cancer) as probably carcinogenic to humans (group 2A) on the basis of sufficient evidence of carcinogenic activity in rodents and strong mechanistic evidence (IARC 2013).

In 2005, EMA published a public statement on the use of herbal medicinal products containing methyleugenol (EMA 2005). Methyleugenol is naturally present in food due to its natural occurrence in herbs and spices. The exposure resulting from consumption of herbal medicinal products (short time use in adults at recommended posology) does not pose a significant cancer risk.

The mutagenicity and genotoxicity of methyleugenol have been assessed in a multitude of *in vitro* and *in vivo* studies. All the results suggest that methyleugenol is a pro-mutagenic agent *in vitro* and *in vivo via* the formation of genotoxic metabolites. Methyleugenol is metabolised to 1'-hydroxymethyleugenol (1'-HME) by cytochromes P450. Subsequent sulphoconjugation of 1'-HME by sulphotransferases leads to the formation of highly reactive electrophilic metabolites that can form DNA adducts and induce mutations. A 2-year oral carcinogenesis study in rats and mice showed that methyleugenol mainly induced tumours of the liver, stomach, kidney, mammary gland and skin (NTP 2000).

In experimental animals, methyleugenol is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products at the levels present in tea tree EO (WHO/FAO 2009, EFSA 2023b).

3.2.2.1.13. *Description of physical properties*

Chemical and physical properties of tea tree EO are described in the Table 2 below.

Table 2: Chemical and physical properties of tea tree EO

Melting point:	-22°C (ECHA)
Boiling point:	97 - 220°C at 100.4 - 100.7 kPa (ECHA)
Vapor pressure:	21 hPa at 25°C (ECHA)
Solubility in water and in organic solvents (in g/L, in regard to temperature):	Water: 1.42 g/L at 20°C (ECHA) Soluble in two volumes of 85% ethanol at 20°C (WHO)
Density	0.885 - 0.906 g/mL (Eur. Pharm.)
Refraction index, optical rotation angle, etc.	Refraction index: 1.475 - 1.482 (Eur. Pharm.) Optical rotation angle: + 5° to + 15° (Eur. Pharm.)

3.2.2.2. Pharmacology

3.2.2.2.1. *Pharmacodynamics*

Tea tree EO exerts broad spectrum antimicrobial activity with little evidence for inducing tolerance and resistance (Sharifi-Rad *et al.* 2017, EMA 2015a). Moderate to strong antibacterial activities against Gram-positive and Gram-negative bacteria are well documented *in vitro* (*e.g*. *Cutibacterium acnes*, *Enterococcus faecium*, *Staphylococcus aureus*, *Streptomyces* spp., etc.; MIC 0.04 – 0.50 mg/mL) (Carson, Hammer, et Riley 2006, Casarin *et al.* 2018, EMA 2015a, WHO/FAO 2002, Winska *et al.* 2019).

Moderate to strong antifungal activities were also observed, in various dermatophytes and other fungal pathogens (*Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, *Malassezia furfur*, *Candida albicans*, *Cryptococcus neoformans*, *Pityrospermum ovale*, *Trichosporon cutaneum*; MIC 1.1 – 2.2 mg/mL) (EMA 2015a, Gupta, Nicol, et Batra 2004, Jain, Arora, et Nainwal 2022, WHO/FAO 2002)[1]. Antiparasitic potential of tea tree EO was also evidenced, mostly *in vitro* (helminths, protozoa) (Lam *et al.* 2020, WHO/FAO 2002).

Membrane disruption of microbial species is presented as the main mechanism of action.

Antiviral activity of tea tree EO was also addressed, against HSV-1, *in vitro* and in a small clinical trial (Winska *et al.* 2019).There is also some evidence of tea tree EO possessing anti-inflammatory activity (no mechanism of action was clearly evidenced) (He *et al.* 2023, EMA 2015a, Pazyar *et al.* 2013) and having an interest in wound-healing, mainly due to its antiseptic potential (Halcon et Milkus 2004, Pazyar *et al.* 2013).

[1] as well as against fungal plants pathogens (EFSA 2012)

Note that for tea tree EO used in herbal traditional medicinal products, the definition of pharmacodynamic properties is not required as per Article 16c(1)(a)(iii) of directive 2001/83/EC as amended.

3.2.2.2.2. *Pharmacokinetics in laboratory animals (ADME)*

No published data regarding the ADME of tea tree EO in laboratory animals are available.

3.2.2.2.3. *Conclusions on pharmacology, pharmacodynamics and pharmacokinetics*

Tea tree EO exerts antimicrobial, antifungal and antiviral activities. Its mechanism of action is not well known. But its seems to be due to a membrane disruption. An anti-inflammatory activity is also cited.

No kinetics data is available in laboratory animals following tea tree EO administration.

3.2.2.3. Toxicological studies

3.2.2.3.1. *Acute oral toxicity*

- **Rats**

1.Hammer *et al.* (2006). **A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil.**

After a single oral administration of tea tree EO, LD_{50} were reported to be 2 300 mg/kg bw in SPF rats and 1 682 mg/kg bw in non-SPF rats. Above 1 500 mg/kg bw, clinical signs were lethargy and ataxia. Animals showed depressed activity levels 72 h post dosing. By day 4, all but one rat had regained all locomotor functions.

2. EMA (2015a). **Assessment report on *Melaleuca alternifolia* (Maiden and Betch) Cheel, *M linariifolia* Smith, *M dissitiflora* F. Mueller and/or other species of Melaleuca, aetheroleum.**

An acute oral LD_{50} of 1 400 – 2 700 mg/kg bw was reported in rats.

3. ECHA (2022). **Registration dossier of *Melaleuca alternifolia*, ext.**

Tea tree EO is registered under the REACH regulation. According the registration dossier of ECHA, the acute oral (gavage) LD_{50} in rats Sprague-Dawley (female and male) was found to be 2.6 mL/kg bw (*i.e.* 2 300 mg/kg bw) in specific pathogen free (SPF) rats and 1.9 mL/kg bw (1 700 mg/kg bw) in non-SPF rats.

- **Mice**

1. Wei *et al.* (2021). **Characterization of tea tree oil nanoemulsion and its acute and subchronic toxicity**.

In this study, the acute oral toxicity (gavage) of tea tree EO and tea tree EO nanoemulsion was assessed for male ICR mice (6 – 8 weeks old, 10 in each group) according to OECD 423. The nano tea tree EO was composed of tea tree EO (4 % w/w – no information about quality), tween 80 (2 % w/w) and carboxymethylcellulose (0.2 % w/w) and water. The acute oral LD_{50}

values of tea tree EO and nano tea tree EO was estimated to be 854 mg/kg bw and 1 656 mg/kg bw respectively.

2. ECHA (2022). **Registration dossier of *Melaleuca alternifolia*, ext.**

The acute oral (gavage) toxicity was assessed in female mice (8 weeks old). The maximal tested dose was 2 000 mg/kg bw. The duration of observation period following administration was 14 days. No mortality was reported at 2 000 mg/kg bw. At this dosage, observed adverse effects were decreased activity, hunched back position, uncoordination, piloerection, decreased grip reflex. The acute oral LD_{50} of tea tree EO was higher than 2 000 mg/kg bw in female mice.

- **Conclusion on acute toxicity**

From provided data, the acute oral toxicity LD_{50} of tea tree in rats and mice is estimated to be 854 mg/kg bw for the tea tree EO nanoemulsion and 1 400 mg/kg bw for *Melaleuca alternifolia* extract). Main adverse effects observed were neurological (lethargy, ataxia, locomotor troubles ...).

3.2.2.3.2. *Repeat-dose toxicity*

3.2.2.3.2.1. Repeat-dose toxicity (28 days)

- **Mice**

1. Wei *et al.* (2021). **Characterization of tea tree oil nanoemulsion and its acute and subchronic toxicity.**

A repeated dose toxicity study (28 days) in mice used a formulation of nano tea tree EO (ultrasonic emulsification of tea tree EO (4 % w/w) with Tween 80 (2 % w/w) and carboxymethylcellulose (0.2 % w/w) and water). Mice were orally (gavage) dosed with of 0, 50, 100 and 200 mg/kg bw/day nano tea tree EO for 28 days.

No significant differences for hematological and biochemical parameters were noted between the treatment groups and the control group. No histopathological findings were noted.

The No Observable Adverse Effect Level (NOAEL) for nano tea tree was at least 200 mg/kg bw/day from this 28-day oral toxicity test in mice.

3.2.2.3.2.2. Repeat-dose toxicity (subchronic)

- **Rats**

1. EFSA (2018). **Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for extract from tea tree in light of confirmatory data.**

After a 90 days short term toxicity study in Wistar rats by oral route, the NOAEL of tea tree EO is 30 mg/kg bw/day (based on the observed effects on male reproductive organs and sperm parameters at 60 and 120 mg/kg bw/day).

3.2.2.3.2.3. Repeat-dose toxicity (chronic)

No data available.

3.2.2.3.2.4. Conclusion on repeat-dose toxicity

From an oral 28 days toxicity study in mice conducted with nano tea tree EO, a NOEL of 200 mg/kg bw/day was established.

From an oral 90 days toxicity study in rats, a No observed effect level (NOEL) for the tea tree EO of 30 mg/kg bw/day was retained by EFSA.

3.2.2.3.3. *Tolerance in target species of animal*

The constituents of tea tree EO are present in various plant species belonging to the normal diet of grazing animals. They are usual monoterpenes, monoterpenols and sesquiterpenes belonging to chemical groups 6, 16 and 31 of flavouring substances, as defined by EFSA.

To our knowledge, data on veterinary trials in target species using tea tree EO alone are scarce. Recent trials in broilers (Liu *et al.* 2023) and goats (Lv *et al.* 2022), as a feed additive, evaluated influence on general and immune parameters.

3.2.2.3.4. *Reproductive toxicity, including developmental toxicity*

3.2.2.3.4.1. Study of the effects on reproduction

No data

3.2.2.3.4.2. Study of developmental toxicity

- **Rats**

1. EFSA (2018). **Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for extract from tea tree in light of confirmatory data.**

Development toxicity and prenatal developmental toxicity study of extract from tea tree in Wistar rats by oral route was reported. Skeletal variations (delays in ossification) were reported at 60 and 120 mg/kg bw/day. The ossification delay in the high dose group was observed at maternal toxicity doses and did not have teratological significance because there was no interference with viability or function, and it was considered secondary to maternal toxicity.

EFSA has retained a NOAEL for maternal toxicity and developmental toxicity of 30 mg/kg bw/day based on this study.

No teratogenicity was identified.

2. ECHA (2022). **Registration dossier of *Melaleuca alternifolia*, ext.**

ECHA has reported results of a GLP prenatal developmental toxicity study with tea tree EO in 2011. In this study pregnant rats were orally administered with tea tree EO at doses of 0, 20, 100 or 250 mg/kg bw/day from D5 to D19 of gestation. The study was conducted in accordance with requirements of OECD GL 414. Embryotoxic effects were only reported at maternal

toxicity doses. A NOAEL of 20 mg/kg bw/day was proposed by ECHA for both maternal toxicity and prenatal developmental toxicity.

No teratogenicity was identified.

3.2.2.3.4.3. Conclusions on reproductive toxicity

ECHA has retained an oral NOAEL of 20 mg/kg bw/day was retained for both maternal toxicity and developmental toxicity from a GLP prenatal and developmental toxicity study in rats.

In both available developmental studies, tea tree EO was neither considered embryotoxic nor teratogen.

3.2.2.3.5. *Genotoxicity*

3.2.2.3.5.1. In vitro studies

1. ECHA (2022). **Registration dossier of *Melaleuca alternifolia*, ext.,** Ames test.

Negative result with and without an extrinsic metabolic activation system in *Salmonella typhimurium* TA 98 and TA 100 strains and *Escherichia coli* WP2 *uvr*A strain (Evandri *et al.* 2005), and in *Salmonella typhimurium* TA 98, TA 100 and TA 102 (Fletcher *et al.* 2005) were observed. However, the antimicrobial activity of tea tree EO certainly reduces the relevance of the results obtained with bacterial test systems.

2. ECHA (2022). **Registration dossier of *Melaleuca alternifolia*, ext.,** gene mutation in mammalian cells with and without S9 (OECD 476).

Not mutagen.

3. ECHA (2022). **Registration dossier of *Melaleuca alternifolia*, ext.,** *in vitro* mammalian chromosomal aberration test (Chinese Hasmter lung fibroblasts) (OECD 473).

Tea tree EO tested up to cytotoxic concentration (58.59 µg/mL), both with and without metabolic activation, did not induce structural chromosome aberrations in this test in V79 Chinese Hamster lung cells. Therefore, tea tree EO and its metabolites are not considered to be clastogenic in this test system.

A *in vitro* chromosome aberration test (human lymphocytes) (OECD 473) and *an in vitro* micronucleus test (human lymphocyte) were performed (Pereira *et al.* 2014). Authors concluded that tea tree EO in the tested concentrations, is not genotoxic in *in vitro* mammalian cells.

3.2.2.3.5.2. In vivo studies

EFSA (2018) has assessed results of a micronucleus assay performed in mouse in compliance with GLP, conducted in accordance with OECD GL 474. In this test, tea tree EO was orally administered (gavage) at 1 000, 1 350 and 1 750 mg/kg bw in Swiss mice.

There was a statistically significant depression of PCE and PCE + NCE ratio in the high dose test item treated groups in both sexes when compared with the vehicle control groups at 48 hours as an indication of the toxicity of the test item at this dose.

The test item tea tree EO was considered to be non-clastogenic and or aneugenic in the mouse micronucleus test under the conditions of the study.

3.2.2.3.5.3. Conclusions on genotoxicity

EFSA, EMA and ECHA have assessed *in vitro* and *in vivo* genotoxicity/mutagenicity data. Pereira et *al.* (2014) have also reported results of an *in vitro* micronucleus assay on human lymphocytes. Based on these publications tea tree EO is unlikely to be genotoxic.

3.2.2.3.6. *Carcinogenicity*

No carcinogenicity studies were available on tea tree EO.

Based on results of genotoxic/mutagenic tests assessed by EFSA, EMA and/or ECHA and because none of the substances listed in the Eur. Pharm. have carcinogenic concerns, the tea tree EO is very unlikely to have carcinogenic potential.

3.2.2.3.7. *Conclusions on toxicology*

A comprehensive review of the above literature allows the following to be concluded about tea tree EO:

- The lowest NOAEL of 20 mg/kg bw/day was retained from a GLP rats prenatal developmental toxicity study;
- Tea tree EO is neither embryotoxic nor teratogen in provided studies;
- Tea tree EO was reported to be not mutagenic / genotoxic;
- Tea tree EO is very unlikely to have carcinogenic potential.

3.2.2.4. Other requirements

3.2.2.4.1. *Special studies (e.g. immunotoxicity, neurotoxicity)*

See 3.2.2.4.3. Observations in humans for details about estrogenic effects.

3.2.2.4.2. *Microbiological properties of residues*

Tea tree EO has antibacterial activity. No data about intestinal bacteria or on bacteria used in the food industry are available but several Minimum Inhibitory Concentrations (MIC) are published (Table 3).

Table 3: Minimum inhibitory concentrations

Bacteria	MIC (mg/mL)	Reference
Staphylococcus aureus ATCC2523	0.25	(Liu *et al.* 2020)
Escherichia coli	3.1	(Puvaca *et al.* 2021)
Escherichia coli ATCC25922	2.7	(Puvaca *et al.* 2021)
Escherichia coli ATCC25922	0.25	(Liu *et al.* 2020)
Escherichia coli ATCC25922	0.3125	(Wei *et al.* 2021)*
Escherichia coli C83903	0.625	(Wei *et al.* 2021)*
Escherichia coli 0310E1-3	0.3125	(Wei *et al.* 2021)*
Salmonella typhimurium	6.2	(Puvaca *et al.* 2021)
Salmonella typhimurium J2L4	0.625	(Wei *et al.* 2021)*
Salmonella A2L2	0.625	(Wei *et al.* 2021)*
Citrobacter koseri	3.4	(Puvaca *et al.* 2021)

**: tea tree EO nanoemulsion*

According to the guideline VICH GL36, a microbiological ADI derived from MIC data has been calculated.

$$\text{ADI} = \frac{\text{MICcalc x Volume of Colon Content}}{\text{fraction of oral dose available to microorganisms x 60}}$$

The MIC available are derived from a very limited number of *in vitro* studies carried out with very few strains of bacteria (poor representatives of the human intestinal flora). To account for the limited database, the lowest MIC available (0.25 mg/mL) was used with an uncertainty factor (UF) of 100. There was no information on the percentage of the ingested residues entering in the colon and on the microbiological activity of the residues in the colon, it was assumed that 100 % of the ingested residues enter the colon and that all residues are microbiologically active.

$$\text{ADI} = \frac{0.25\,\frac{\text{mg}}{\text{mL}}\ \text{x}\,\frac{1}{100}\,\text{x}\ 500\,\frac{\text{mL}}{\text{day}}}{1\ \text{x}\ 60\ \text{kg}}$$

$\text{ADI}_{\text{microbiological}}$ = 0.021 mg/kg/day

3.2.2.4.3. *Observations in humans*

Tea tree EO is widely used as an antiseptic in humans. The most commonly reported adverse drug reactions (Bekhof *et al.* 2023) in cutaneous uses are contact dermatitis. After massive oral exposure (10 - 15 mL), resolutive ataxia and central nervous depression were described in children (EMA 2015a, Tisserand et Young 2014).

An *in vitro* study has shown estrogenic effects (Henley *et al.* 2007). Residues of pesticide may have been responsible of the estrogenic effects observed in this study. It has been suggested that tea tree EO causes prepubertal gynecomastia and premature thelarche in children. A systematic review of the literature (Hawkins *et al.* 2020) with a small number of cases (11) did not find evidence to support the claim that tea tree EO is related to endocrine disruption in children.

The safety of use of tea tree EO, as an ingredient or as pure EO and as a dietary supplements, was assessed by Anses. Conclusions were: the risk identified for tea tree EO is associated with the content in terpinen-4-ol and methyleugenol (as well as with ascaridole, a degradation product of γ-terpinene). Depending on the composition of the essential oil, no sanitary preoccupation exists below 80 to 240 mg/day for an adult (60kg) (Anses 2020).

3.2.2.5. Determination of ADI or alternative limit

Tea tree EO was included in Annex I to directive 91/414/EEC on 1 January 2009 by Commission directive 2008/127/EC, and has been deemed to be approved under regulation (EC) No 1107/2009, in accordance with Commission Implementing regulation (EU) No 540/2011, as amended by Commission Implementing regulation (EU) No 541/2011. In this context, based on a 90-day repeated dose toxicity study in Wistar rats and a developmental toxicity study in rat , derivation of an ADI of 0.03 mg/kg bw/day was deemed possible, using a conservative safety factor of 1 000 based on NOAEL of 30 mg/kg bw. The safety factor of 1 000 was chosen because of limited available data. In the technical document, it is stated that "*dietary intake calculations indicate that exposure from natural occurrence clearly exceeds exposure from intended uses*" (EFSA 2018).

However, ECHA reported results of a more recent (2022) GLP prenatal-developmental toxicity study in rats and retained a NOAEL of 20 mg/kg for the maternal toxicity and the developmental toxicity. The study was conducted in accordance with requirements of OECD GL 414. By applying a safety factor of 1 000 (to take into account the limited available toxicological data package for tea tree EO), an **ADI of 0.02 mg/kg bw/day** can be proposed.

The value of toxicological and microbiological ADI are similar.

3.2.3. Residue file

3.2.3.1. Metabolism and residue kinetics

The major part of the terpene components of tea tree EO are commonly occurring compounds found in many plants. Food producing species, as cattle, sheep and goat, are naturally exposed when grazing.

Several compounds of tea tree EO have been detected in milk or fatty tissue of ruminants but not quantified (only arbitrary units):

- α-Pinene (Valdivielso *et al.* 2017, Lejonklev *et al.* 2013, Serrano *et al.* 2011, Moran *et al.* 2019, Basdagianni *et al.* 2019, Tornambé *et al.* 2006, Poulopoulou *et al.* 2012);
- Limonene (Moran *et al.* 2019, Basdagianni *et al.* 2019, Tornambé *et al.* 2006, Valdivielso *et al.* 2017, Lejonklev *et al.* 2013, Poulopoulou *et al.* 2012, Borge *et al.* 2016);
- α-Terpinene (Moran *et al.* 2019, Tornambé *et al.* 2006);
- γ-Terpinene (Moran *et al.* 2019, Basdagianni *et al.* 2019, Tornambé *et al.* 2006, Borge *et al.* 2016, Valdivielso *et al.* 2017, Lejonklev *et al.* 2013, Serrano *et al.* 2011);
- α-Terpineol (Valdivielso *et al.* 2017, Serrano *et al.* 2011, Lejonklev *et al.* 2013);
- 1,8-Cineole (Moran *et al.* 2019);
- *p*-Cymene (Moran *et al.* 2019, Basdagianni *et al.* 2019, Tornambé *et al.* 2006);
- Terpinen-4-ol (syn. 4-terpineol) (Moran *et al.* 2019);
- α-Terpineol (Moran *et al.* 2019);
- Terpinolene (Basdagianni *et al.* 2019, Tornambé *et al.* 2006).

3.2.3.1.1. *Pharmacokinetics in food producing species (ADME)*

No kinetic data is available following administration of tea tree EO in food producing species. However, kinetic data are available for several constituents of tea tree EO (EFSA 2012, JECFA 1999).

Data available for **terpinen-4-ol** were reviewed by Anses (2020). Its absorption is rapid and complete by oral and cutaneous routes in animal species (salmon, calf, broiler, cow, pig). Hepatic metabolism appears to occur rapidly *via* several CYP (Human CYP1A2, 2A6, 3A4 *in vitro*) followed by glucuroconjugation. No toxic metabolites were identified.

3.2.3.1.2. *Depletion of residues*

No residue study following administration of tea tree EO in food producing species is available.

3.2.3.2. Monitoring and exposure data

Tea tree EO is not present in the normal diet of animals in Europe, where *Melaleuca* spp. were not introduced and are therefore not grazed.

However, tea tree EO is approved as a flavouring feed additive[2]. In addition, components of tea tree EO can be found in the normal animal diet. Most of its major constituents belong to the Register of feed additives, Annex I, 2020, with no restrictions (regulation (EC) No 1831/2003), without the exception of *p*-cymene, sabinène and aromadendrene.

Tea tree EO is not present in the normal human diet. However, **tea tree EO is listed as a natural flavouring in food and drinks by the European Council under the name *Melaleuca linariifolia* Smith. No restriction is mentioned** (Council 2007) and **tea tree EO is approved in dietary supplements in almost all European countries** with at least the exemption of Belgium. This decision to prohibit the oral consumption of tea tree EO in Belgium is based on cases of neurological damage after ingesting a very high dose of these EO[3]. In addition, components of tea tree EO can be found in the normal diet and are most of its are approved as flavouring substances for use in foods, with no use restrictions (regulation (EU) No 872/2012), except for aromadendrene (Annex 4).

Regarding medicinal uses, monographs were published by WHO (2002) and EMA for its use in herbal medicinal products for cutaneous and oro-mucosal use (EMA 2023, 2015b, a).

3.2.3.3. Residue analytical method

Not relevant as no MRL value has been proposed.

3.3. Risk management considerations

The approach developed by French authorities (Anses 2022), was applied to establish a MRL status of the tea tree EO. This approach has been organised in the form of a decision tree (Figure 1).

First of all, it is necessary to ensure that the herbal preparation considered is indeed a traditional-use preparation as defined by directive 2004/24/EC of the European Parliament and of the Council of 31 March 2004 amending, as regards traditional herbal medicinal products, directive 2001/83/EC on the Community code relating to medicinal products for human use (Article 16c 1(c)).

Then it is necessary to verify if there is a pharmacopoeia standard in priority, followed by AFNOR standards, when available.

[2] https://ec.europa.eu/food/food-feed-portal/screen/feed-additives/search/details/POL-FEED-IMPORT-1116, consulted on 18/01/2024
[3] https://vigilanses.anses.fr/sites/default/files/VigilAnsesN14_Juin2021_Melaleuca_0.pdf, consulted on 07/02/2024

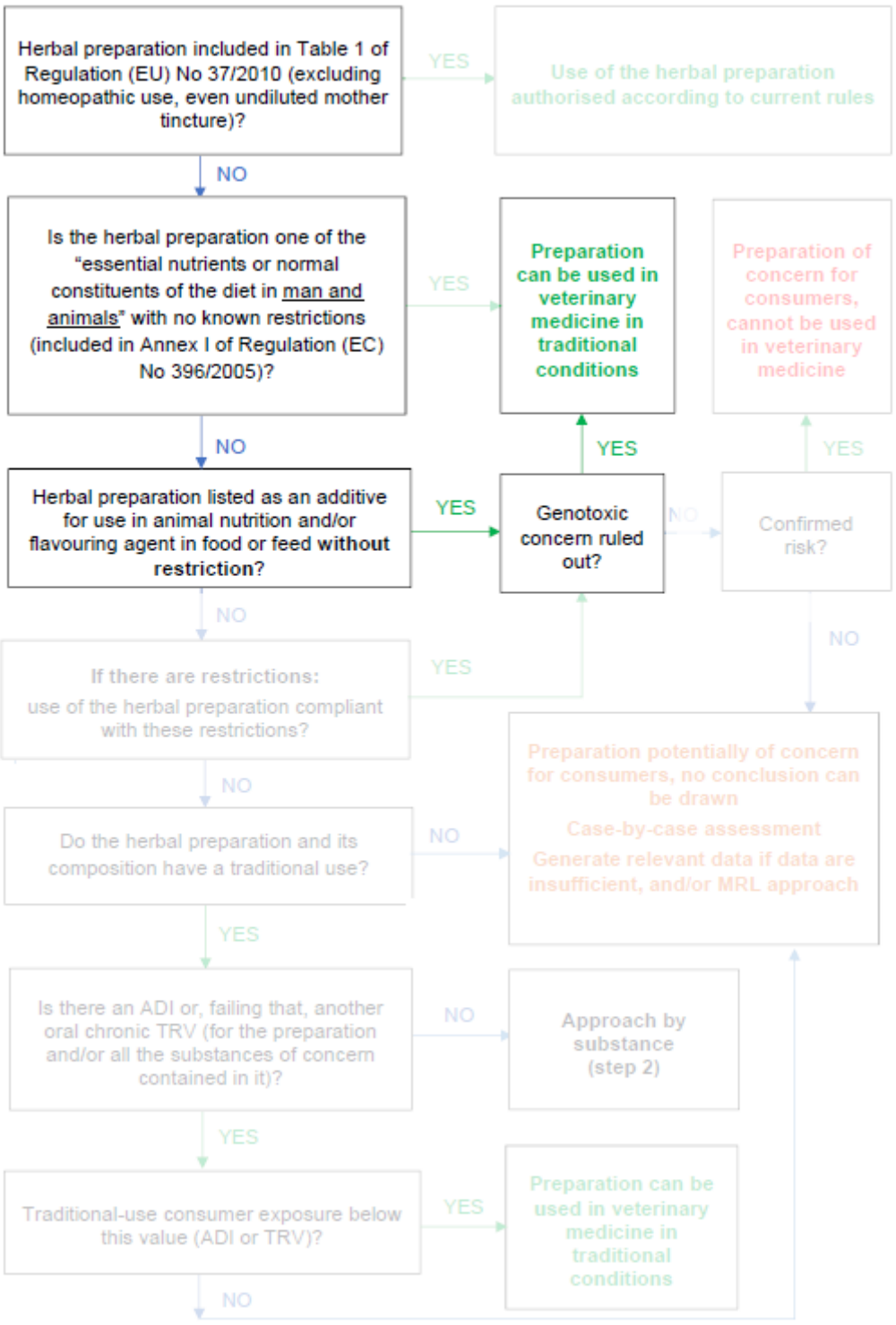

Figure 1: decision tree for tea tree EO

Considering that, for tea tree EO:

- Tea tree EO is traditional-use preparation as defined by directive 2004/24/EC of the European Parliament and of the Council 2001/83/EC on the Community code relating to medicinal products for human use (Article 16c 1(c));
- Tea tree EO has a composition described in the Eur. Pharm.;

- Is the herbal preparation included in Table 1 of regulation (EU) No 37/2010 (excluding homeopathic use, even undiluted mother tincture)?

  No, this EO is currently not listed in Table 1 and in Table 2 of regulation (EU) 37/2010.

  One of its constituents, 1,8-cineole (0-15%) is listed in Table 1 of regulation (EU) 37/2010;

- Is the herbal preparation one of the "essential nutrients or normal constituents of the diet in man and animals" with no known restrictions (included in Annex I of regulation (EC) No 396/2005)?

  No, this EO is not one of the "essential nutrients or normal constituents of the diet in man and animals".

  Components of tea tree EO can be found in the normal animal diet;

- Is the herbal preparation listed as an additive for use in animal nutrition and/or flavouring agent in food or feed **without restriction**?

  Yes, this EO is approved as a flavouring compound without restriction for use in animal nutrition and as a flavouring agent in human at European level.

  All *Melaleuca* essential oils, regardless of their composition, are not authorized only in Belgium in dietary supplements;

- Genotoxic concern ruled out?

  Yes, tea tree EO was reported to be not mutagenic / genotoxic.

  One of the constituents in trace amounts not mentioned in the composition of the European Pharmacopoeia, methyleugenol, is a pro-mutagenic agent. It is absorbed, metabolised and rapidly excreted and is not expected to accumulate in animal tissues and products;

According to the decision tree, the preparation can be used in veterinary medicine in food producing species in traditional conditions.

However, an ADI has been proposed for tea tree EO. Therefore, it seems necessary to check consumer exposure:

- An ADI of 0.02 mg/kg bw/day is proposed, *i.e* 1.2 mg/person
- Data on exposure are available in the rapporteur member state's assessment report on the tea tree EO for its renewal as active substance in plant protection products (Commission 2022). The daily average natural uptake of tea tree EO components with food is 12.5 mg/kg bw/day and the daily peak uptake is 154 mg/kg bw/day. Natural

exposure is a high multifold of the ADI value. As a consequence a comparison of exposure to the proposed ADI does not seem relevant.

Exposure through food consumption from animals treated with tea tree EO can be calculated as follows:

- The maximum doses are
  - 2 mL/cattle, *i.e*. approximately 1 800 mg/cattle. With a body weight of 700 kg, dose is 2.6 mg/kg bw.
  - 0.2 mL/goat*, i.e*. approximately 181 mg/goat. With a body weight of 60 kg, dose is 3 mg/kg bw;

- This is by the oral route. As there are no depletion or ADME data, exposures are the worst-case:

- If we consider the consumption of 500 g of meat[4] at a concentration of 3 mg/kg (worst case according to the maximum dose administered), human will be exposed to 1.5 mg of tea tree EO.

  This value is lower than the natural exposure of 12,5 mg/kg, which is a high multifold of the ADI (1.2 mg/person).

In conclusion, exposure through food consumption of animal treated with tea tree EO is lower than natural exposure and does not significantly increase consumers' exposure.

- An alternative to safety evaluation is proposed, based on literature data. A thorough study of the existing literature and of reports from European sanitary agencies was performed.

[4] muscle, fat, liver and kidney

4. CES CONCLUSION

As clearly stated in point 2 on the organization of expertise, in this opinion, CES ERCA was mandated to give an opinion on the application of the methodology proposed in the opinion report 2020-SA-0083 and on the logic and functionality of the decision tree. On the other hand, CES ERCA did not have the mandate to give an opinion on the management measures, which falls within the competence of the EMA and the European Commission.

Conclusion on the proposed methodology and functionality of the decision tree

Based on available toxicological and microbiological data, an ADI was calculated for tea tree EO. The study used to establish the ADI was a developmental and prenatal toxicity study conducted in rats (ECHA 2022). In its current version, the decision tree does not take into account the ADI when the plant or plant extract is listed as a food additive and flavouring agent in food and feed, and in the absence of genotoxic concern, as is the case for the tea tree EO. CES ERCA proposes that the decision tree and methodology be reviewed to include a question on the availability of an ADI from the early stages of the assessment and, where appropriate, to be able to compare it with the consumer exposure.

No available studies show that the tea tree EO is genotoxic. The composition of the tea tree EO is defined by the European Pharmacopoeia. No available studies show that the tea tree EO constituents defined by the European Pharmacopoeia are genotoxic. However, although absent from the list of constituents of tea tree EO according to the European pharmacopoeia, methyleugenol, may be present naturally. Methyleugenol is also naturally present in certain foods of plant origin. Dietary exposure to this substance indicates a health concern related to the consumption of these methyleugenol-containing foods in a worst-case scenario Current available data indicate that methyleugenol is metabolised into several genotoxic metabolites. EFSA and WHO/FAO (WHO/FAO 2009, EFSA 2023b) state that in food-producing species, methyleugenol would be absorbed, metabolised and rapidly excreted and would not be accumulated in food based on current knowledge. However, no residue data in foodstuffs of animal origin are available. CES ERCA proposes to clarify that any genotoxic substance present in an EO or a plant, even if it is not mentioned in the composition of the European pharmacopoeia, must be subject to a risk assessment to ensure that there is no health concern.

Limitations and questioning on the methodology

The methodology initially proposed is based on the status of plants and EOs in different regulations and therefore on the opinions of European Agencies without reassessing them. However, the need to consider the new published data appeared necessary. The need for certain verification points was highlighted through this first implementation.

5. AGENCY CONCLUSION AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety is in favour of submitting the MRL dossier for tea tree EO to the EMA by ANSES-ANMV for evaluation. According to the decision tree and the consumer risk assessment methodology adapted to plants and EO proposed in request No 2020-SA-0083, tea tree EO does not present a risk to the consumer of food from animals that have received it. If necessary, and after consulting the

CVMP, this EO may be included in table 1 of Regulation (EU) No 37/2010 by the European Commission with the statement ‘no MRL required’ and for all food-producing species.

Through the evaluation of this tea tree EO MRL dossier, the proposed methodology will be examined by the EMA. At the end, the EMA may suggest the creation of a working group to draft an European guideline to adapt the consumer risk assessment of food from animals treated with plants and EO from the methodology developed by ANSES. ESC ERCA proposals, in particular on the amendments and limitations of this methodology, will be shared with the EMA and may be included in this guideline.

Anses proposes to the EMA a different approach from that of Regulation (EU) No 2018/782 to adapt to the particularities of plants and EO. This with the aim of continuing to compile MRL dossiers for plants and H.E. already used in the field by veterinarians in food-producing animals. These MRL dossiers evaluated by the EMA will lead the European Commission to add these plants and H.E. as appropriate to Table 1 of Regulation (EU) No 37/2010 in the absence of an identified risk and thus allow for the use of these products within a defined regulatory framework.

KEYWORDS

Tea tree EO, TTO, essential oil, MRL, consumer risk

SUGGESTED CITATION

Anses. (2024). Preparation of the application dossier for the establishment of maximum residue limits (MRLs) of tea tree essential oil (EO) for inclusion in table 1 of regulation (EU) No 37/2010. (Request No 2023-AUTO-0123). Maisons-Alfort : Anses, 45 p.

LIST OF ABBREVIATION

ABC	:	American Botanical Council
ADI	:	Acceptable daily intake
AHP	:	American Herbal Pharmacopoeia
ADME	:	Absorption, distribution, metabolism and excretion
Anses	:	French Agency for Food, Environmental and Occupational Health & Safety
ANSM	:	French Health Products Safety Agency
BMDL		Benchmark Dose Lower Boud
BW	:	Body weight
CES ERCA	:	Expert Committee on Assessment of physico-chemical risks in food
CLP	:	Classification, labelling and packaging
CMR	:	Carcinogenic, mutagenic, reprotoxic
CT	:	Chemotype
CYP	:	Cytochrome
CVMP	:	Committee for Medicinal Products for Veterinary Use
ECHA	:	European Chemicals Agency
EFSA	:	European Food Safety Authority
EMA	:	European Medicines Agency
EO	:	Essential oil
Eur. Pharm.	:	European Pharmacopoeia
FDA	:	US Food and Drug Administration
GACP	:	Good Agricultural and Collection Practices

GLP	:	Good Laboratory Practices
GMP	:	Good Manufacturing Practices
HMPC	:	EMA Committee on Herbal Medicinal Products
IARC	:	International Agency for Research on Cancer
ICR	:	Institute of Cancer Research
INN	:	International Nonproprietary Names
ISO	:	International Organization for Standardization
IUPAC	:	International Union of Pure and Applied Chemistry
JECFA	:	Joint FAO/WHO Expert Committee on Food Additives
JMPR	:	Joint FAO/WHO Meeting on Pesticide Residues
LD	:	Lethal dose
MIC	:	Minimum inhibitory concentration
MRL	:	Maximum residue limit
NCE	:	Normo-chromatic erythrocytes
NCNPR		National Center for Natural Products Research
NOAEL	:	No observed adverse effect level
NOEL	:	No observed effect level
OECD	:	Organisation for Economic Co-operation and Development
PCE	:	Polychromatic erythrocytes
REACH	:	Registration, Evaluation, Authorisation and Restriction of Chemicals
SPF		Specific-pathogen free
UF	:	Uncertainty factor
VICH	:	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WG	:	Working Group
WHO	:	World Health Organization

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ANNEX 1

Presentation of the participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organisation.

WORKING GROUP

Chair

Mr Pierre CHAMPY – Professor (Paris-Saclay University) – Speciality: pharmacognosy

Vice-chair

Mr. Hervé POULIQUEN – Professor (ONIRIS, Nantes Veterinary School) – Speciality: toxicology

Members

Ms Martine AVENEL – Retired doctor – Speciality : dermatology

Ms Sabrina BOUTEFNOUCHET – Lecturer (Paris-Descartes University) – Speciality: pharmacognosy

Ms Céline DELERME – Preclinical assessor (ANSM) – Speciality: toxicology

Mr Thierry HENNEBELLE – Professor (Lille University) – Speciality: pharmacognosy

Mr Jean-Philippe JAEG – Lecturer (National Veterinary School of Toulouse) – Speciality: toxicology

Ms Marie-Aleth LACAILLE-DUBOIS – Emeritus professor (Bourgogne Franche-Comté University) – Speciality: pharmacognosy

Ms An LE – Scientific Delegate for Medicinal Plants and Homeopathy (ANSM)

Mr Ludovic LE HEGARAT – Deputy Head of Unit (Anses/Fougères Laboratory) – Speciality: toxicology

Ms Isabelle LUSSOT-KERVEN – Veterinary surgeon – Speciality: phytotherapy and aromatherapy

Mr Serge MICHALET – Lecturer (Claude Bernard University, Lyon I) – Speciality: pharmacognosy

Ms Céline RIVIERE – Lecturer (Lille University) – Speciality: pharmacognosy

Ms Sophie SIMAR MENTIEES – Head of toxicology laboratory (Institut Pasteur de Lille) - Speciality: toxicology

Ms Florence SOUARD – Lecturer (Grenoble Alpes university) – Speciality: pharmacognosy

WG « PLANTS » REVIEWERS

Mr Pierre CHAMPY – Professor (Paris-Saclay University) – Speciality: pharmacognosy

Mr Jean-Philippe JAEG – Lecturer (National Veterinary School of Toulouse) – Speciality: toxicology

EXPERT COMMITTEE

The work described in this report was monitored and adopted by the Expert Committee on Assessment of physico-chemical risks in food (CES ERCA):

2022-2026 mandate, dates: 13 January 2023, 19 June 2024, 22 January 2024 and 20 February 2024

Chair

Mr Bruno LE BIZEC – University Professor – Expertise in analytical chemistry and health risk assessment

Vice-chair

Ms Marie-Louise SCIPPO – University Professor – Expertise in analytical chemistry and health risk assessmen

Members

Mr Claude ATGIE – University Professor – Expertise in toxicology

Mr Pierre-Marie BADOT – University Professor – Expertise in contaminant transfer and ecotoxicology

Ms Marie-Yasmine BOTTEIN – Researcher in environmental toxicology – Expertise in marine biotoxins

Ms Rachide CHEKRI - Laboratory manager – Expertise in analytical chemistry

Mr Nicolas DELCOURT – University Lecturer – Hospital Practitioner – Expertise in biochemistry and clinical toxicology

Ms Christine DEMEILLIERS – University Professor – Expertise in toxicology

Ms Virginie DESVIGNES - Research Engineer – Expertise in exposure and risk assessment

Mr Erwan ENGEL – Research Director – Expertise in analytical chemistry

Me Gauthier EPPE – University Professor – Expertise in analytical chemistry

Ms Anne-Sophie FICHEUX – Research Engineer – Expertise in toxicology

Mr Eric HOUDEAU – Researcher director – Expertise in toxicology

Mr Jean-Philippe JAEG - Lecturer (National Veterinary School of Toulouse) – Speciality: toxicology

Ms Emilie LANCE – University Lecturer – Expertise in ecotoxicology and cyanotoxins Mr Olivier LAPREVOTE – University Professor and Hospital Practitioner – Expertise in toxicology

M. Michel LAURENTIE - Directeur de recherche – Compétences en pharmacocinétique

Mr Ludovic LE HEGARAT – Deputy Head of Unit – Expertise in toxicology

Mr Jean-Charles LEBLANC - Head of Unit - Expertise in exposure and risk assessment

Mr Nicolas LOISEAU – Researcher director– Expertise in biochemistry

Mr David MAKOWSKI – Research Director – Expertise in statistics and modelling Ms Francesca MANCINI - Research associate - Expertise in epidemiology

Mr Eric MARCHIONI – University Professor – Expertise in analytical chemistry

Mr Jean-François MASFARAUD – University Lecturer – Expertise in contaminant transfer and ecotoxicology

Ms Mathilde MUNIER - Hospital researcher – Expertise in toxicology

Ms Isabelle OSWALD – Research Director – Expertise in toxicology

Ms Anne PLATEL - University Lecturer - Expertise in toxicology

Mr Yann SIVRY – University Lecturer – Expertise in analytical chemistry

Ms Paule VASSEUR – Emeritus University Professor – Expertise in toxicology

CES ERCA REVIEWERS

Appui à la coordination du CES ERCA

Ms Emilie LANCE – University Lecturer – Expertise in ecotoxicology and cyanotoxins

Mr Olivier LAPREVOTE – University Professor and Hospital Practitioner – Expertise in toxicology

Mr Nicolas LOISEAU – Researcher director– Expertise in biochemistry

Ms Marie-Louise SCIPPO – University Professor – Expertise in analytical chemistry and health risk assessmen

ANSES PARTICIPATION

CES ERCA coordination support

Ms Géraldine CARNE – Scientific Coordinator – Anses/DER

Mr Julien JEAN – Scientific Coordinator – Anses/DER

Scientific coordination

Ms Céline RENOUARD – Scientific Coordinator – Anses/ANMV

Scientific contribution

Ms Sophie BARRETEAU – Deputy Director of the Scientific Assessment Department – Anses/ANMV

Ms Anne-Marie JACQUES – Pharmacology and MRL expert – Anses/ANMV

Ms Lise LABORIEUX – Pharmaceutical quality expert – Anses/ANMV

Ms Anne SAGNIER – Expert toxicologist – Anses/ANMV

ANNEX 2 *:* APPLICATION FORM

APPLICATION FOR THE ESTABLISHMENT OF MRL(s) FOR A PHARMACOLOGICALLY ACTIVE SUBSTANCE TO BE USED IN VETERINARY MEDICINAL PRODUCTS IN ACCORDANCE WITH REGULATION (EC) No 470/2009

APPLICATION FORM

APPLICATION FOR THE ESTABLISHMENT OF MRL(s) FOR A PHARMACOLOGICALLY ACTIVE SUBSTANCE TO BE USED IN VETERINARY MEDICINAL PRODUCTS IN ACCORDANCE WITH REGULATION (EC) No. 470/2009

PART I: Administrative Data

Name of substance for review, using INN (where attributed):	Melaleuca aetheroleum					
Name and address of applicant:	ANSES 14 rue Claude Bourgelat PA de la Grande Marche Javené - CS 70611 35306 FOUGERES Cedex FRANCE					
Name, address, telephone number and fax number of company contact point for all correspondence arising in connection with the application:						
Type of application (please tick):	Full	☑	Extension	☐	Modification	☐
Legal basis (please tick):	Article 3	☐	Article 15	☐	Article 9a[1]	☐
	Article 9b	☑	Article 11	☐	Article 27[2]	☐
Marketing authorisation of veterinary medicinal products in the EU (please tick):	Does the applicant hold a marketing authorisation in the EU for a veterinary medicinal product containing the substance? Yes ☐ No ☑, or Has the applicant submitted a marketing authorisation application in the EU for a veterinary medicinal product containing the substance? Yes ☐ No ☑ If the response to both questions above is "No": Has the applicant the intention to submit an application for a marketing authorisation containing the substance and concerned species in the EU Yes ☐ No ☑					
Rapporteur:						
Co-rapporteur:						

PART II: SUMMARY OF THE EVALUATION PROPOSED BY THE APPLICANT

Name of Substance for review, using INN (where attributed):	Melaleuca aetheroleum			
Is the substance used in veterinary medicinal products as (please tick):	Active ingredient?	☑	Excipient, preservative, etc?	☐

Please summarise the anticipated pattern of veterinary use:

Target Species	Major indications	Dose regimen
All food producing species	antiseptic / general antimicrobial	

Overall NOEL used for the determination of ADI (mg/kg bw/day):	
Reference to relevant study (including location in the dossier):	
Uncertainty factor proposed:	/
ADI proposed (µg/kg bw):	20
ADI proposed (µg/60 kg person):	1200
MRL required? (Please tick)	Yes ☐ No ☑
If yes, what is the marker residue proposed:	/

Food commodity	Proposed MRLs (µg/kg)	
Muscle	/	/
Fat/Skin+Fat	/	/
Liver	/	/
Kidney	/	/
Milk	/	/
Eggs	/	/
Honey	/	/

Description of the proposed analytical method:	/
Limit of quantification (LOQ)	/
Reference (including location in the dossier):	/
Evaluations performed by other EU or international bodies:	Has the substance been evaluated by other EU or international bodies? Yes ☑ No ☐ If the response to the above question is "yes", please indicate the name of the EU body(ies), the date(s) of evaluation(s) and the outcome(s)

I hereby certify that all information relating to the establishment of MRLs for the above-mentioned substance, whether favourable or unfavourable, has been submitted with this application.

Date:		Signature:	

ANNEX 3

Melaleuca alternifolia essential oil constituents: Presence in EOs registered in table 1 of regulation (EU) No 37/2010

Component	Amount (%, tea tree EO) [a]	EO registered in table 1 of regulation (EU) No 37/2010 [b]	Amount (%, EO in table 1) (Eur. Pharm.)	Amount (%, EO in table 1) (Tisserand and Young, 2014)
α-Pinene (pin-2(3)-ene)	1.0 – 6.0	*Angelicae radix aetheroleum*		4.4 – 24.0
		Coriandri aetheroleum	3.0 – 7.0	
		Cupressi aetheroleum (all species, topical use only)		20.4 – 52.7 (EO from leaves and terminal branches)
		Eucalypti aetheroleum	0.05 – 10.0	
		Foeniculi aetheroleum	1.0 – 10.0	
		Lauri folii aetheroleum (Laurel leaf)		7.1 – 15.9
		Myristicae (fragrantis) aetheroleum (all species, use in newborn only)[5]	15.0 – 28.0	
		Rosmarini aetheroleum	18.0 – 26.0 (Spain CT) 9.0 – 14.0 (Morocco / Tunisia CT)	
		Terebinthinae aetheroleum rectificatum (all species, topical use only)	70.0 – 85.0	

[5] Eur. Pharm. : "*Myristicae fragrantis aetheroleum"*

Component	**Amount (%, tea tree EO)[a]**	**EO registered in table 1 of regulation (EU) No 37/2010 [b]**	**Amount (%, EO in table 1) (Eur. Pharm.)**	**Amount (%, EO in table 1) (Tisserand and Young, 2014)**
Sabinene (4(10)-thujene)	≤ 3.5	*Angelicae radix aetheroleum*		0.4 – 1.2
		Citri aetheroleum	1.0 – 3.0	
		Cupressi aetheroleum (all species, topical use only)		0.7 – 2.8 (EO from leaves and terminal branches)
		Eucalypti aetheroleum	≤ 0.3	
		Lauri folii aetheroleum (Laurel leaf)		4.5 – 6.5
		Myristicae (fragrantis) aetheroleum (all species, use in newborn only)[6]	14.0 – 29.0	
α-Terpinene	5.0 – 13.0	*Thymi typo thymolo aetheroleum*	0.9 – 2.6	

[6] Eur. Pharm. : "*Myristicae fragrantis aetheroleum"*

Component	Amount (%, tea tree EO) [a]	EO registered in table 1 of regulation (EU) No 37/2010 [b]	Amount (%, EO in table 1) (Eur. Pharm.)	Amount (%, EO in table 1) (Tisserand and Young, 2014)
Limonene	0.5 – 4.0	*Angelicae radix aetheroleum*		6.0 – 13.2
		Carvi aetheroleum	30.0 – 45.0	
		Citri aetheroleum [7]	56.0 – 78.0	
		Citronellae aetheroleum	1.0 – 5.0	
		Coriandri aetheroleum	1.5 – 5.0	
		Cupressi aetheroleum (all species, topical use only)		2.3 – 6.0 ((+)-limonene) (EO from leaves and terminal branches)
		Eucalypti aetheroleum	0.05 – 15.0	
		Foeniculi aetheroleum	0.9 – 5.0	
		Lavandulae aetheroleum	≤ 1.0	
		Menthae arvensis aetheroleum	1.5 – 7.0	
		Menthae piperitae aetheroleum	1.0 – 3.5	
		Myristicae (fragrantis) aetheroleum (all species, use in newborn only) [7]	2.0 – 7.0	
		Rosmarini aetheroleum	2.5 – 5.0 (Spain CT) 1.5 – 4.0 (Morocco / Tunisia CT)	
		Terebinthinae aetheroleum rectificatum (all species, topical use only)	1.0 – 7.0	

[7] Eur. Pharm. : "*Myristicae fragrantis aetheroleum"*

Component	Amount (%, tea tree EO) [a]	EO registered in table 1 of regulation (EU) No 37/2010 [b]	Amount (%, EO in table 1) (Eur. Pharm.)	Amount (%, EO in table 1) (Tisserand and Young, 2014)
1,8-Cineole (eucalyptol)	≤ 15.0	*Cinnamomi ceylanici aetheroleum*	≤ 3.0	
		Eucalypti aetheroleum	> 70.0	
		Lauri folii aetheroleum (Laurel leaf)		38.1 – 43.5 %
		Lavandulae aetheroleum (all species, topical use only)	≤ 2.5	
		Menthae arvensis aetheroleum	≤ 1.5	
		Menthae piperitae aetheroleum	3.5 – 8.0	
		Rosmarini aetheroleum	16.0 – 25.0 (Spain CT) 38.0 – 55.0 (Morocco / Tunisia CT)	
		Angelicae radix aetheroleum		0.7 – 2.2
Terpinolene	1.5 – 5.0	*Cupressi aetheroleum* (all species, topical use only)		2.4 – 6.3 (EO from leaves and terminal branches)
		Lauri folii aetheroleum (Laurel leef)		0.1 – 1.1 %
		Myristicae aetheroleum (all species, use in newborn only)[8] (Nutmeg)		0.6 – 2.6 (Nutmeg, East Indian) 1.4 – 1.7 (Nutmeg, West Indian)
		Cupressi aetheroleum (all species, topical use only)		0.3 – 1.0 (EO from leaves and terminal branches)

[8] Eur. Pharm. : "*Myristicae fragrantis aetheroleum"*

Component	Amount (%, tea tree EO) [a]	EO registered in table 1 of regulation (EU) No 37/2010 [b]	Amount (%, EO in table 1) (Eur. Pharm.)	Amount (%, EO in table 1) (Tisserand and Young, 2014)
Terpinen-4-ol (4-terpineol)	≥ 30.0	*Lauri folli aetheroleum (Laurel leaf)*		2.1 – 2.2
		Lavandulae aetheroleum (all species, topical use only)	0.1 – 8.0	
		Myristicae (fragrantis) aetheroleum (all species, use in newborn only)[9]	2.0 – 6.0	1.0 – 10.9 (Nutmeg, East Indian) 3.0 – 6.4 (Nutmeg, West Indian)
		Rosmarini aetheroleum		1.8 – 6.8 (Rosemary, β-myrcene CT)
		Thymi typo thymolo aetheroleum	0.1 – 2.5	
		Eucalypti aetheroleum (Eucalyptus globulus)		0.1 – 2.2 ((+)-aromadendrene)
Aromandendrene	≤ 7.0	*Anisi aetheroleum*	≤ 1.2	
α-Terpineol	1.5 – 8.0	*Citri aetheroleum* [10]	≤ 0.6	
		Coriandri aetheroleum	0.1 – 1.5	
		Cupressi aetheroleum (all species, topical use only)		1.2 – 1.4 (EO from leaves and terminal branches)
		Lauri folii aetheroleum (Laurel leaf)		0.9 – 1.9
		Lavandulae aetheroleum (all species, topical use only)	≤ 2.0	
		Rosmarini aetheroleum	1.0 – 3.5 (Spain CT) 1.0 – 2.6 (Morocco / Tunisia CT)	

[a] European Pharmacopoeia (Monograph 01/2008:1837).

[b] Unless specified: All animal species; Other provisions: none.

[9] Eur. Pharm. : "*Myristicae fragrantis aetheroleum"*
[10] ibid *Limonis aetheroleum* in the Eur. Pharm.

ANNEX 4

Melaleuca alternifolia essential oil constituents: Use as food and feed additives

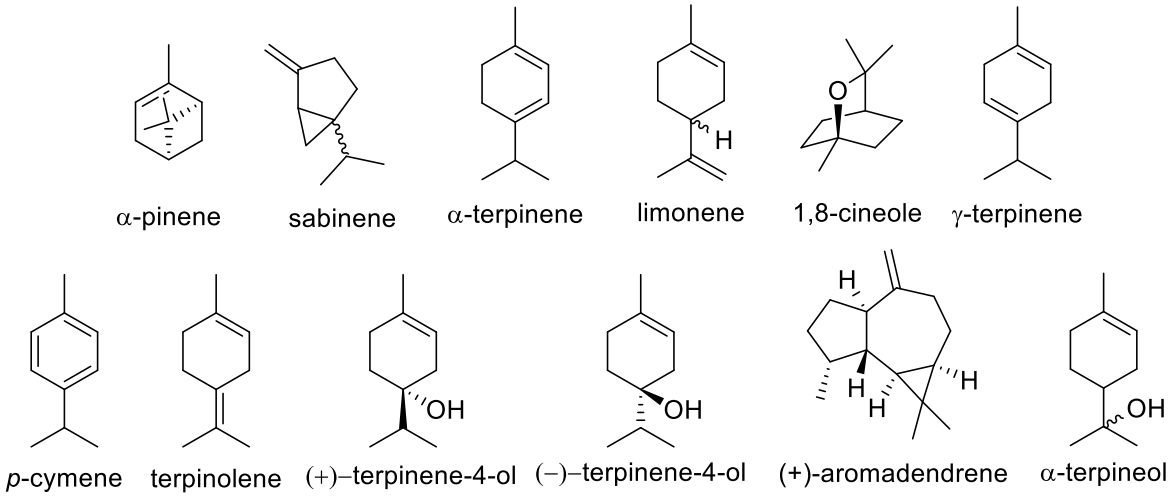
Component	CAS N°	IUPAC	Chemical formula	Molecular weight	Flavouring substance[11], Food: Flavis n°	Register of feed additives, Annex I[12]	NOAEL (mg/kg b.w. per day)* (EFSA 2023a, 2019)
α-Pinene (pin-2(3)-ene)	80-56-8 (RS)	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	136.2380	01.004	X	*222*
Sabinene	3387-41-5	1-(1-Methylethyl)-4-methylenebicyclo[3.1.0]hexane	$C_{10}H_{16}$	136.2380	01.059	not registered	*222*
α-Terpinene	99-86-5 (RS)	1-methyl-4-propan-2-ylcyclohexa-1,3-diene	$C_{10}H_{16}$	136.2380	01.019	X	*250*
Limonene	138-86-3 (RS)	1-methyl-4-prop-1-en-2-ylcyclohexene	$C_{10}H_{16}$	136.2380	01.045	X	**250**
1,8-Cineole (eucalyptol)	470-82-6	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane	$C_{10}H_{18}O$	154.2530	03.001	X	**100**
γ-Terpinene	99-85-4 (RS)	1-methyl-4-propan-2-ylcyclohexa-1,4-diene	$C_{10}H_{16}$	136.2380	01.020	X	*250*
p-Cymene	99-87-6	1-methyl-4-propan-2-ylbenzene	$C_{10}H_{14}$	134.2220	01.002	not registered	**154**
Terpinolene	586-62-9 (RS)	1-methyl-4-propan-2-ylidenecyclohexene	$C_{10}H_{16}$	136.2380	01.005	X	*250*
Terpinen-4-ol (4-terpineol)	562-74-3 (RS)	4-methyl-1-propan-2-ylcyclohex-3-en-1-ol	$C_{10}H_{18}O$	154.2530	02.072	X	*250*
Aromadendrene	489-39-4	[1a*R*-(1aα,7α,7aβ,7bα)]-1a,2,3,5,6,7,7a,7b-Octahydro-1,1,4,7-tetramethyl-1*H*-cycloprop[e]azulene	$C_{15}H_{24}$	204.35	-	not registered	*222*
α-Terpineol	98-55-5 (RS)	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	$C_{10}H_{18}O$	154.2530	02.014	X	*250*

*bold: NOAEL value available, *italics: NOAELs extrapolated by using read-across

[11] 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
[12] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition

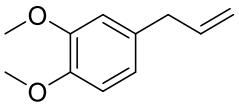
ANNEX 5

Structures of *Melaleuca alternifolia* essential oil constituents



ANNEX 6

Methyleugenol



Component	CAS N°	IUPAC	Chemical formula	Molecular weight	Flavouring substance, Food: Flavis n°	Register of feed additives, Annex I
Methyleugenol	93-15-2	4-Allyl-1,2-dimethoxybenzene	$C_{11}H_{14}O_2$	178.2310	Not registered Regulation (EC) No 1334/2008: - Annex III – part A[13] - Annex III – part B[14] : Maximum level mg/kg Dairy products 20 Meat preparations and meat products, including poultry and game 15 Fish preparations and fish products 10 Soups and sauces 60 Ready-to-eat savouries 20 Non-alcoholic beverages 1	Not registered *

* see: (EFSA 2023b, d, c, 2019, 2022)

[13] Annex III, Part A: “Substances which shall not be added as such to food”
[14] Annex III, Part B: “Maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in certain compound food as consumed to which flavourings and/or food ingredients with flavouring properties have been added”: Maximum level 1-20 mg/kg.