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Safety and efficacy of a dried aqueous ethanol extract of *Melissa officinalis* L. leaves when used as a sensory additive for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of a dried aqueous ethanol extract of *Melissa officinalis* L. leaves when used as a sensory feed additive for all animal species. The aqueous ethanol extract is specified to contain ≥ 10% of hydroxycinnamic acid derivatives including ≥ 3% of rosmarinic acid. Considering the contradictory data from the Ames tests and uncertainty about the qualitative and quantitative presence of flavonoids and other compounds in the extract from *M. officinalis* L. leaves, the FEEDAP Panel could not conclude on the genotoxicity of the additive under assessment. Although the identified components of the extract do not raise concerns for the safety of target species, the analysis of the extract is incomplete. In the absence of adequate analytical and safety data, the FEEDAP Panel is unable to conclude on the safety of the additive for the target species. The use of *M. officinalis* L. leaf dried extract in animal feed at the proposed use level does not raise significantly the exposure levels of the consumer for compounds derived from this plant. However, in the absence of adequate data on genotoxicity, the Panel cannot conclude on the safety for the consumer. In the absence of specific studies, the FEEDAP Panel cannot conclude on the safety of the additive for the user. *M. officinalis* L. is a native species to Europe and its use in animal nutrition is not expected to pose a risk for the environment. Since *M. officinalis* L. and its extracts are recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary for the extract.

Keywords: sensory additives, flavouring compounds, dry extract, *Melissa officinalis L.*, Safety, efficacy

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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 and 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 the company NOR FEED SUD[2] for the authorisation and the re-evaluation of the product *Melissa officinalis* L. dry extract (Nor-Balm®), when used as a feed additive for all animal species (category: sensory additives; functional group: flavouring compounds).

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). The particulars and documents in support of the application were considered valid by EFSA as of 19 January 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product *M. officinalis* L. dry extract (Nor-Balm®), when used under the proposed conditions of use (see Section 3.2.4).

1.2. Additional information

The preparation under assessment, *M. officinalis* L. balm leaves extract, is currently authorised as 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 feed additive in the European Union (EU).

For *M. officinalis* L. folium, the European Medicines Agency (EMA) issued an assessment report and a monograph for human medicinal use (EMA, 2013a,b).

'Melissa Leaf Dry Extract' is described in a monograph of the European Pharmacopoeia 9.0 (PhEur, 2017). It is defined as a dry extract which is produced from the dried leaves of *M. officinalis* L. which has a minimum content of 2% of rosmarinic acid. The extract is produced by using either hot water (not less than 70°C) or a hydroalcoholic solvent usually equivalent in strength to ethanol (70%, v/v). As identity test, the monograph describes thin-layer chromatography using reference solutions of hyperoside, rutoside trihydrate and rosmarinic acid.

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[3] in support of the authorisation request for the use of a dry extract *M. officinalis* L. as a feed additive.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the *M. officinalis* dry extract in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] NOR FEED SUD, Rue Fleming 1, 49066 Angers CEDEX 1, France.
[3] FEED dossier reference: FAD-2010-0251.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0251.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *M. officinalis* L. dry extract is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA, 2009), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), Guiduance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2017b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a), Genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b).

3. Assessment

This opinion deals with the assessment of a dried aqueous ethanol extract from dried leaves of *M. officinalis* L. when used as sensory additive (functional group: flavouring compounds) in feed for all animal species.

3.1. Origin and extraction

M. officinalis L. belongs to the Lamiaceae, a family of flowering plants, and is commonly referred to as lemon balm or common balm. It is indigenous to the Western Asia and the Eastern Mediterranean region, and is widely cultivated in other temperate regions.[5]

The applicant provided a description and a flow chart of the manufacturing process of the additive.

[redacted]

3.2. Characterisation

3.2.1. Characterisation of the extract

The dried aqueous ethanol extract from *M. officinalis* L. leaves is listed by the Chemical Abstract Service (CAS) No 84082-61-1, the European Inventory of Existing Commercial Chemical Substances (EINECS) No 282-007-0, the Flavor Extract Manufacturers Association (FEMA) No 2112 and the Council of Europe (CoE) No 280.

M. officinalis L. leaf extract contains rosmarinic acid and other hydroxycinnamic acids (namely, caffeic acid, chlorogenic acid, ferulic acid, *m*-coumaric acid and *p*-coumaric acid) as active ingredients. No information has been provided on the qualitative and quantitative composition of the extract under assessment. The applicant has proposed rosmarinic acid and hydroxycinnamic acid derivatives as marker compounds for this product.

Rosmarinic acid (IUPAC name: (2*R*)-3-(3,4-dihydroxyphenyl)-2-[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid) is identified with the CAS No 20283-92-5. The molecular formula of rosmarinic acid is $C_{18}H_{16}O_8$ and its molecular mass 360.31 g/mol. The structural formula of rosmarinic acid is given in Figure 1.

[5] Technical dossier/Section II/Annex_II_37.

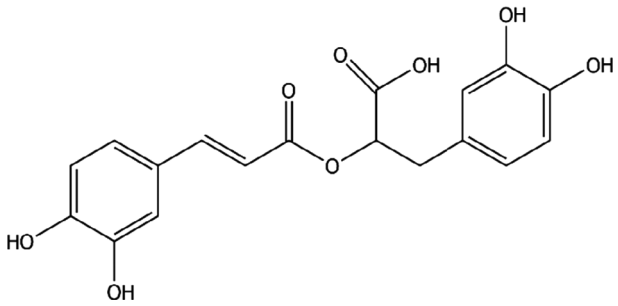

Figure 1: Molecular structure of rosmarinic acid

3.2.2. Characterisation of the formulated additive

Owing to the variable concentration of the marker compound rosmarinic acid in the native *M. officinalis* L. extract, the additive is formulated by adding maltodextrin and other materials (silicon dioxide as anti-caking agent) in order to ensure batch-to-batch consistency. The formulated additive is marketed with the trade name Nor-Balm®.

According to the specification provided by the applicant, the additive under assessment contains ≥ 10% of hydroxycinnamic acid derivatives (measured by UV spectrophotometry) including ≥ 3% of rosmarinic acid determined by high-performance liquid chromatography (HPLC).

The applicant provided data on five batches of the formulated additive. In these batches, the native dry extract accounted for 50–74% of the additive, maltodextrin for 24–8% and silicon dioxide for 2%.[6] The analysis of the same batches showed compliance with the specifications proposed by the applicant. Table 1 summarises the results of proximate analysis[7] and the characterisation of the phenolic fraction[8] of the five batches of the additive.

Table 1: Major constituents of the formulated product containing the dried aqueous ethanol extract from *Melissa officinalis* L. leaves based on the analysis of five batches (mean and range)

Constituent	Method	Percentage in the additive	
		Mean	Range
Proximate analysis			
Water	Gravimetry	4.0	2.7–5.9
Dry matter		96.0	94.1–97.3
Protein (as DM)	Kjeldahl	4.2	2.5–5.7
Fibre (as DM)	Gravimetry	< 0.4	< 0.4
Fat (as DM)	Gravimetry	0.75	0.4–1.6
Ash (as DM)	Gravimetry	13.2	9.4–16.3
Carbohydrates (as DM)	By difference	81.8	78.1–87.7
Total		100	–
Characterisation of the phenolic fraction			
Total phenolic compounds(a)	UV	14.0	12.7–15.0
Rosmarinic acid	HPLC-UV	5.4	5.0–5.7

DM: dry matter.
(a): Named in the dossier 'Total hydroxycinnamic acid derivatives'.

The carbohydrate fraction was further analysed using the Luff Schoorl method (according to Regulation (EC) No 152/2009[9]). The content of simple sugars (expressed as glucose, as dry matter (DM)) in three batches was found to range between 15.7 and 20.3 g/100 g.[10]

[6] Technical dossier/Supplementary information February 2019.
[7] Technical dossier/Supplementary information February 2019/Annexes 2–6.
[8] Technical dossier/Supplementary information February 2019/Annexes 16–20.
[9] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. OJ L 54, 26.2.2009, p. 1.
[10] Technical dossier/Supplementary information February 2019/Annexes 8–10.

A single batch of the formulated extract was analysed by HPLC with UV detection for the presence of hydroxycinnamic acids and their derivatives. Besides rosmarinic acid (which represented 93.37% of the area under the curve), another five components were identified: chlorogenic acid (0.35%), caffeic acid (2.35%), p-coumaric acid (2.19%), ferulic acid (0.89%) and m-coumaric acid (0.86%).[6]

Although rosmarinic acid is the major component of the hydroxycinnamic acids when determined by HPLC, it represents only about one-third of the fraction referred to by the applicant as 'total hydroxycinnamic acid derivatives.' The UV method used to determine this fraction is unspecific and will detect a range of compounds including, in particular, other phenolic compounds (e.g. flavonoids). Therefore, the so-called 'total hydroxycinnamic acid derivatives' fraction is given the more general title of 'phenolic compounds' in Table 1 and in the rest of the opinion. Gas chromatography-mass spectrometry (GC-MS) analysis confirmed that the organic fraction of the extract does not contain the volatile compounds found in the essential oil derived from *M. officinalis* L.

Some information on the possible nature of the approximately 60% of 'phenolic compounds' not identified as hydroxycinnamic acid derivatives is found in the literature. Several publications were cited by the applicant concerning the analysis of the phenolic fraction of *M. officinalis* L. leaf dried extracts.[11]

Fialová et al. (2008) analysed an aqueous ethanolic extract (50% ethanol) of a specimen from Slovakia and found that total cinnamic acid derivatives accounted for 13.1% of the extract including 3.8% rosmarinic acid. In this analysis, total flavonoids represented only 0.46% of the extract. Similar results were obtained by Carnat et al. (1998), who found 11.3% of total cinnamic acids with 4.0% of rosmarinic acid and 0.44% total flavonoids in an aqueous extract of *M. officinalis* L. leaves of commercial origin. Rosmarinic acid (9.6%) was also identified as the main phenolic compound in an aqueous ethanolic extract of *M. officinalis L.* by Dastmalchi et al. (2008), who also observed other cinnamic acids in smaller amounts and several flavonoids of which luteolin was most abundant.

A more extensive analysis of an aqueous extract of *M. officinalis* L. dry leaf powder by HPLC by Ieri et al. (2017) identified 3.2% of rosmarinic acid with 0.17% of a luteolin glycoside as the only flavonoid detected. Luteolin was also identified as the major flavonoid in *M. officinalis* L. by Heitz et al. (2000). Barros et al. (2013) analysed an aqueous extract of cultivated *M. officinalis* L. dry leaves with HPLC-MS and observed caffeic acid dimers, trimers and tetramers beside rosmarinic acid. Luteolin-7-O-glucoside was identified as the main flavonoid, which occurred in much smaller concentrations in the extract compared to the organic acid fraction.

A different composition of the phenolic fraction was reported by Kamden et al. (2013), who analysed an alcoholic (70%) extract of *M. officinalis* L. dry leaf powder. The authors identified 10 phenolic compounds, 20% of which were flavonoids (mainly quercetin and its glycosides), gallic and ellagic acid accounted for 9.3% and a further 8.7% was due to the presence of cinnamic acid group compounds, including caffeic acid (6.3%), chlorogenic acid (1.1%) and rosmarinic acid (1.4%).

The FEEDAP Panel notes that the qualitative and quantitative presence of flavonoids in the fraction 'phenolic compounds' of the additive under assessment is unknown.

3.2.2.1. Impurities

Data on impurities were provided for three batches of the formulated additive. Pesticide residues [redacted] and microbial contamination [redacted] The level of residual solvents [redacted]

The levels of mycotoxins [redacted] and heavy metals (cadmium, mercury and lead) and arsenic determined in three batches of *M. officinalis* L. leaves [redacted] The levels of dioxins were not determined, but the applicant considered that the risk for contamination was very low.

The applicant made a literature search on the composition of *M. officinalis* L. and its extracts.[6] The presence of estragole and methyleugenol was reported in lemon balm (SCF, 2001, EFSA ANS Panel 2010). Estragole and methyl eugenol were not detected in one batch of the extract (limit of

[11] Technical dossier/supplementary information April 2019.
[redacted]

detection, LOD 1 mg/kg).[15] The FEEDAP Panel notes that the analysis of more batches (at least three) would be needed to exclude the presence of these compounds in the extract.

3.2.2.2. Physicochemical properties

The formulated additive is a brown free-flowing powder. It has a bulk density [redacted] The solubility in water is [redacted] Particle size distribution determined by sieving in three batches of the extract resulted in [redacted] The dusting potential (according to Stauber–Heubach) determined in two batches of the extract was [redacted]

3.2.3. Stability

The stability of the additive [redacted] The additive was shown [redacted]

The stability of the additive in water for drinking [redacted]

3.2.4. Conditions of use

The additive is intended to be used in feed and water for drinking for all animal species. The recommended level of inclusion of the formulated additive in feed is 5–100 mg/kg of complete feedingstuffs. The maximum recommended level in feed corresponds to up to 15 mg/kg phenolic compounds (including up to 6 mg rosmarinic acid/kg complete feed). The recommended level of inclusion of the formulated additive in water for drinking is from 2.5 to 100 mg/kg.

3.3. Safety

The safety assessment is based on the highest use level proposed by the applicant (100 mg formulated additive/kg complete feed).

The applicant provided a literature search on the absorption, distribution, metabolism and excretion (ADME) and the toxicology of *M. officinalis* L. extracts and some of its individual components (rosmarinic acids and other hydroxycinnamic acids).[11]

3.3.1. Absorption, distribution, metabolism and excretion

The pharmacokinetics of rosmarinic acid has been studied in rats and humans and showed a similar profile in both species (Nakazawa and Ohsawa, 1998; Baba et al., 2004; Konishi and Kobayashi, 2005; Wang et al., 2017; Noguchi-Shinohara et al., 2015). The data obtained from the rat studies show that rosmarinic acid after oral administration has a rapid, but limited absorption, and is then extensively metabolised and excreted via urine and bile. Free rosmarinic acid and its metabolites appear in plasma, bile and urine shortly after application. Maximum plasma levels appear 30–60 min after administration. Several metabolites have been identified. Absorbed rosmarinic acid is metabolised in the liver and plasma by methylation and conjugation with glucuronic acid and sulfate. After hydrolysis of the ester bond, the resulting caffeic acid is partly methylated to ferulic acid and iso-ferulic acid. The cinnamic acid derivatives are conjugated to glucuronide and sulfate esters and excreted via bile and urine. *m*-Coumaric acid and *m*-hydroxyphenylpropionic acid together with their conjugated forms appear in the plasma with a delay. Both compounds are mainly excreted via the kidneys. The delay suggests that the *p*-dehydroxylation and reduction of the double bond of caffeic acid is performed by intestinal bacteria in the distal parts of the gut.

Caffeic acid, ferulic acid and *m*- and *p*-coumaric acid were also detected in the *M. officinalis* L. leaf extract under consideration. The pharmacokinetic behaviour of these cinnamic acid derivatives follows the same scheme as that of the compounds, formed via metabolism of rosmarinic acid in the body. Chlorogenic acid also was detected in the *M. officinalis* L. leaf extract in small amounts. The compound

[15] Technical dossier/Supplementary information February 2019/Annex_25.

[redacted]

consists of caffeic acid conjugated with quinic acid at different sites of its hydroxyl groups. The bioavailability of chlorogenic acid derivatives is limited. Although some metabolism occurs in the stomach and the small intestine, most takes place in the gut by the intestinal microflora. Caffeic acid resulting from the hydrolysis of chlorogenic acid is absorbed and metabolised by methylation, glucuronidation, sulfation and reduction as described above (Azuma et al., 2000; Farell et al., 2011).

With exception to rosmarinic acid and some other hydroxycinnamic acid derivatives, the phenolic fraction of the *M. officinalis* L. leaf extract proposed as feed additive has not been characterised. Literature cited by the applicant indicates the presence of hydroxybenzoic acid derivatives and flavonoids, such as luteolin and quercetin glycosides. A comprehensive review of the ADME of flavonoids has been published by D'Archivio et al. (2007). Only the aglycones and some glycosylated derivatives (e.g. quercetin) are directly absorbed. The majority of glycosides are hydrolysed by glycosidases, occurring in the intestinal mucosal cells or by the gut microflora. The liberated aglycones are absorbed and conjugated with glucuronic acid or sulfate, either by cells of the intestinal wall or in the liver or kidney. Methylation of hydroxyl groups or aromatic ring oxidation by CYP-450 is also possible. Hydrophilic conjugates are excreted via bile or urine and can be routinely found in the excreta of animals and humans.

ADME studies in target species were not available. The FEEDAP Panel notes that the enzymes involved in the biotransformation pathways of rosmarinic acid, hydroxycinnamic acids and flavonoids are present in all the target (food-producing and non-food producing) species, with the exception of feline species and some other pure carnivorous animals, which have a reduced capacity for glucuronidation (Court and Greenblatt, 1997).

3.3.2. Genotoxicity

For mixtures containing a substantial fraction of substances that have not been chemically identified, the EFSA Scientific Committee recommends that first the chemically defined substances be assessed individually for their potential genotoxicity and, if these prove negative, only then should the potential genotoxicity of the mixture as a whole be considered (EFSA Scientific Committee, 2019b). Therefore, the potential genotoxicity of rosmarinic acid is first considered.

3.3.2.1. Genotoxicity studies with rosmarinic acid

In vitro *study*

Furtado et al. (2009) investigated the DNA-damaging effects of rosmarinic acid (100–400 μg/mL) in Chinese hamster lung fibroblasts (V79-cells) in culture. The compound did not induce micronuclei or DNA-strand breaks investigated by the comet assay technique. However, the study did not extend to the top dose indicated in the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 487, and no information was given on the rationale for the selection of the highest concentration tested. Consequently, the FEEDAP Panel concluded that genotoxic effects cannot be excluded at higher concentrations.

In vivo *studies*

The mutagenicity of rosmarinic acid was tested in Swiss albino mice investigated for the occurrence of micronucleated polychromatic peripheral erythrocytes (MNPCEs). Groups of six animals each received doses of 50, 100 or 200 mg/kg body weight (bw) of rosmarinic acid dissolved in water by gavage. No exposure of the target tissue was demonstrated, since comparable frequencies of polychromatic erythrocytes (PCE) were reported between negative controls and treated animals; in addition, no information was given on clinical signs of toxicity to be used as alternative to PCE frequency to evaluate systemic exposure. However, the Panel noted that the highest concentration tested is close to the lethal dose 50 (LD_{50} 561 mg/kg bw) reported by Petersen and Simmonds, 2003, and considered adequate the concentrations selected in the present study to assure a systemic exposure. The occurrence of MNPCEs was investigated 48 and 72 h after treatment. Intraperitoneal injections of 15 mg/kg bw of doxorubicin were used as positive control. Rosmarinic acid did not induce MNPCEs at any dose tested (Furtado et al., 2008).

De Oliveira et al. (2012) investigated the possible effect of rosmarinic acid by an *in vivo* Comet assay in peripheral blood and brain in association with a micronucleus test in bone marrow cells. The analysis was conducted in the frame of a study aimed to evaluate the protective activity of rosmarinic acid against the genotoxic effects of ethanol. No genotoxic effects of rosmarinic acid were observed in this experiment. However, only one concentration of rosmarinic acid was tested (100 mg/kg bw) and no evidence of bone marrow exposure was reported. The Panel noted that an indication of systemic

exposure could be deduced by the protective effects of rosmarinic acid against ethanol genotoxicity observed in brain tissue with the Comet assay. The FEEDAP Panel concluded that the study gave negative results, although a dose–response relationship could not be evaluated.

The FEEDAP Panel considers that the presence of rosmarinic acid in the extract does not raise a concern for genotoxicity.

3.3.2.2. Genotoxicity studies with *Melissa officinalis* L. extracts

Genotoxicity studies with the mixture under assessment were not submitted. Therefore, the FEEDAP Panel reviewed the information on the genotoxicity of *M. officinalis* L. extracts from literature made available by the applicant. Some of the test materials in these studies are aqueous extracts instead of aqueous ethanolic extracts. However, the FEEDAP Panel considers these studies as relevant since the two solvent systems are expected to extract a similar spectrum of compounds giving rise to comparable qualitative composition, although differences might occur in the quantitative composition.

In vitro *studies*

Negative results in the Ames test (Salmonella Typhimurium TA98 and TA100 with or without metabolic activation) are reported for a 70% aqueous ethanolic tincture of *M. officinalis* L., folium. (secondary reference in ESCOP, 2003). Since the test item (described as a tincture) is different from the additive under assessment and only a limited number of strains were tested, the FEEDAP Panel considers the results of this study of limited relevance.

The mutagenicity of an aqueous extract of *M. officinalis* L. (plant part and rosmarinic acid content not specified) was tested in S. Typhimurium using the Ames MPFTM 98/100 assay kit, a modified colorimetric liquid microplate version of the traditional Ames assay (Abudayyak et al., 2015). Mutagenicity was observed in strains TA98 without S9 and TA100 with and without S9. For extracts in methanol and chloroform, no mutagenicity was observed. The authors of the study suggested that the presence of phenolic compounds in *M. officinalis* L., particularly caffeic acid derivatives, could be correlated to its apparent genotoxicity. The FEEDAP Panel does not follow this hypothesis, because caffeic acid was not proven to be mutagenic in S. Typhimurium, in contrast to quercetin, and some related flavonoids, which induce frameshift mutations in the strain TA98.

Alves et al. (2009) investigated an aqueous lyophilised extract of *M. officinalis* L. leaves in a set of bacterial genotoxicity tests (Lysogenic induction assay in *E. coli*, SOS-chromotest), and mutagenesis assays (tryptophan mutagenesis assay in *E. coli*, Lactose mutagenesis assay and *Salmonella* mutagenesis assay). The extract represented 11% of the original leaf material and a qualitative analysis by HPLC indicated the presence of caffeic acid and flavonol derivatives. The extract was genotoxic and mutagenic in all the test systems. The mutagenic and genotoxic effects could be reduced in the presence of antioxidants, suggesting, that reactive oxygen species are involved in the DNA damaging effects.

Kamdem et al. (2013) investigated the antioxidant activity, genotoxicity and cytotoxicity of an extract of *M. officinalis* L. leaf powder in 70% ethanol. The authors presented an analysis of the phenolic composition of the extract, which showed the presence of cinnamic and benzoic acid derivatives and several flavonoids, of which quercetin and its glycosides were most abundant. The *in vitro* Comet assay was applied in human peripheral leucocytes incubated for 3 h in the absence or presence of a *M. officinalis* ethanolic extract, at different concentrations (10, 50, 100 and 150 μg/mL). DNA damage was not observed at any concentration tested. No cytotoxicity was observed at the top concentration (almost 100% viability). The FEEDAP Panel concluded that genotoxic effects cannot be excluded at higher concentrations.

In vivo *study*

In a study by de Carvalho et al. (2011), ethanolic (500 mg/kg bw) and aqueous (100 mg/kg bw) extracts of *M. officinalis* leaves were administered daily to mice by gavage over a period of 15 days. To evaluate the genotoxic effects of the extracts, a micronucleus test was performed in bone marrow cells according to a protocol by the U.S. Environmental Protection Agency Gene-Tox Program, in combination with the Comet assay in peripheral blood performed according to the standard protocol by Singh et al. (1988). No cytotoxicity was reported with the Comet assay as well as no evidence of exposure of the bone marrow. In addition, no information was given on clinical signs of toxicity to be used as alternative to evaluate systemic exposure. No increase of DNA damage was observed in treated animals compared to negative controls in both assays. The FEEDAP Panel considered these results inconclusive, since no evidence of exposure of the target tissues was provided. In addition, the FEEDAP Panel noted that the study presented some limitations, such as: i) low number of cells

analysed in the bone marrow (1,000 instead of 2,000 per animal, as requested by the OECD TG 474 (1997) in force when the study was conducted; ii) no information reported on the number of cells analysed in the Comet assay; iii) only one concentration each of the ethanol and of the aqueous *M. officinalis* L. leaf extracts were tested.

Overall, the FEEDAP Panel notes that positive effects were obtained with aqueous and aqueous/ethanolic extracts of *M. officinalis* L. leaves in bacterial, but not in mammalian systems. Such effects were frequently observed with plant extracts and mostly associated with the presence of flavonoids, especially quercetin. The presence of these compounds in *M. officinalis* L. leaf extract has been consistently reported in the literature (Section 3.2.2), but was not analytically confirmed in the additive under assessment and in the extracts used as test items in the genotoxicity testing (with the exception of the study by Kamdem et al., 2013).

3.3.2.3. Conclusions on genotoxicity

Contradictory results were obtained in bacteria when the capacity of the extracts to induce gene mutations was evaluated. The data submitted did not fulfil the requirements for *in vitro* genotoxicity testing in mammalian cells.

Consequently, the FEEDAP Panel cannot conclude on the genotoxicity of the additive under assessment.

3.3.3. Other toxicological studies

No repeated-dose toxicity studies with the additive under evaluation were submitted.

The test materials used in the toxicity studies cited by the applicant are aqueous extracts or aqueous ethanolic extracts with different percentages of ethanol in the solvent compared to that used for the extract under application. However, the Panel considers these studies as relevant since the two solvent systems are expected to extract a similar spectrum of compounds giving rise to comparable qualitative composition, although differences might occur in the quantitative composition.

In a 28-day study in rat, no adverse effects on biochemical parameters[21] and liver morphology were observed when a dried aqueous extract of *M. officinalis* L. leaves was administered in water by gavage at 2,000 mg/kg bw per day (Bolkent et al., 2005).

An aqueous ethanolic extract (20% ethanol) of *M. officinalis* L. leaves concentrated under vacuum was given to male and female Sprague-Dawley srats at a dose of 600 or 1,200 mg/kg bw per day for 30 days (Hashemnia et al., 2017). No compositional data of the extract were given. Rats of both treatment groups showed a statistically significant reduction in red blood cell count and haemoglobin concentration in the serum. Liver damage was indicated by an increase of the ALT-concentration in serum. LDH concentrations were also increased, however, the difference to the control was not significant. Albumin and total protein concentrations in serum were statistically significantly reduced with respect to the control. Liver damage was also indicated by histopathological alterations which included hepatocyte degeneration, congestion and dilation of sinusoids, proliferation of bile ducts and infiltration of mononuclear cells around the portal area. Kidney damage was indicated by an increased serum concentration of creatinine in the high-dose group and a dose-dependent increase in tubular degeneration and necrosis, tubular and glomerular atrophy and congestion. The FEEDAP Panel noted that the study clearly shows an adverse effect of an aqueous ethanolic *M. officinalis* L. leaf extract on the liver and kidney of rats. Because both treatment groups were affected, a no observed adverse effect level (NOAEL) cannot be identified from this study.

In a repeated dose 90-day oral study compliant with OECD guideline 408, spearmint extract (containing 15.4% rosmarinic acid) was administered to Sprague-Dawley rats (males and females) by gavage at concentrations of 0, 422, 844 and 1,948 mg/kg bw per day (corresponding to 0, 65, 130 and 300 mg rosmarinic acid/kg bw per day) (Lasrado et al., 2015). No adverse effects were observed up to the highest dose tested (1,948 mg/kg bw per day). From this study, an NOAEL of 300 mg/kg bw per day for rosmarinic acid was identified (the top dose tested).

3.3.4. Safety for the target species

In the absence of adequate tolerance studies or repeated dose studies from which an NOAEL for the additive could be identified, the safety of the additive for the target species is assessed based on its individual components.

[21] Serum cholesterol, total lipid, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP).

The additive is formulated with maltodextrin (12–48%) and silicon dioxide (2%). Since maltodextrin is a feed material and silica an authorised feed additive, they are considered of no concern. Similarly, the protein, fat and simple sugars identified by the proximate analysis are not of concern and are not further considered.

The additive contains a variety of phenolic compounds (up to 15%), including rosmarinic acid (up to 6%) and other hydroxycinnamic acids in minor percentages (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and *m*-coumaric acid).

At the maximum proposed use level of 100 mg formulated extract/kg feed, the concentration of rosmarinic acid would be up to 6 mg/kg feed. Toxicological data derived from a subchronic study are available for rosmarinic acid. Applying an uncertainty factor (UF) of 100 to the NOAEL of 300 mg/kg bw, the maximum safe intake for the target species was derived following the EFSA Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), and thus, the maximum safe feed concentration was calculated (see Table 2).

Table 2: Maximum safe concentration in feed for rosmarinic acid

	Body weight (kg)	Feed intake (g DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe concentration (mg/kg feed)(1)
Chickens for fattening	2	158	79	33
Laying hens	2	106	53	50
Turkeys for fattening	3	176	59	45
Piglets	20	880	44	60
Pigs for fattening	60	2,200	37	72
Sow lactating	175	5,280	30	94
Veal calves (milk replacer)	100	1,890	19	150
Cattle for fattening	400	8,000	20	132
Dairy cows	650	20,000	31	86
Sheep/goat	60	1,200	20	132
Horse	400	8,000	20	132
Rabbit	2	100	50	53
Salmon	0.12	2.1	18	151
Dogs	15	250	17	158
Cats(2)	3	60	20	26
Ornamental fish	0.012	0.54	5	587

(1): Complete feed containing 88% DM, milk replacer 94.5% DM.
(2): The uncertainty factor for cats is increased by an additional factor of 5 because of the reduced capacity of glucuronidation.

Because glucuronidation of the hydrolysis or oxidation products of rosmarinic acid is an important metabolic pathway to facilitate the excretion of this compound (see Section 3.3.1), an additional UF of 5 is applied when the additive is used in cat feed, because cats have an unusually low capacity for glucuronidation (Court and Greenblatt, 1997).

The concentration of 6 mg rosmarinic acid/kg complete feed resulting from the use of the additive at 100 mg/kg complete feed is safe for all animal species with a margin of safety of at least 4. When the additive is administered via water for drinking, only, and at the same use level (100 mg/L) as proposed for feed, the intake of the additive via water would be 2–3 times higher than the intake via feed for poultry, pigs and rabbits, and other monogastric animals like cats and dogs (EFSA FEEDAP Panel, 2010). In these species, the magnitude of the margin of safety would be proportionally reduced (range: 1.4–8.8).

The other identified hydroxycinnamic acids, namely caffeic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric acid and chlorogenic acid, are assigned to Cramer Class I and the data indicate that no one individual compound would exceed the threshold value for Cramer Class I (ranging from 0.3 mg/kg feed for poultry to 1.5 mg/kg feed for salmonids and dogs, EFSA FEEDAP Panel, 2017a). Therefore, no concern for the target species arises from these hydroxycinnamic acids.

Although the identified components do not raise concern for target species, the analysis of the extract is incomplete. The Panel notes that there is a significant unknown fraction which probably contains flavonoids, and which prevents the Panel to reach a final conclusion on the safety for target species.

3.3.4.1. Conclusions on safety for the target species

In the absence of adequate data on composition and in view of incomplete genotoxicity testing, the FEEDAP Panel is unable to conclude on the safety of the additive for the target species.

3.3.5. Safety for the consumer

The FEEDAP Panel recognises that aqueous and aqueous ethanolic extracts of the leaves of *M. officinalis* L., including herbal tea infusions, are widely used as food, food flavour and traditional herbal medicinal products (Burdock, 2010; EMA, 2013a,b; PhEur, 2017).

The ADME studies show that rosmarinic acid and other hydroxycinnamic acids present in the organic fraction of the additive are readily metabolised and excreted and are not expected to accumulate in animal tissues and products. Any flavonoids present would also be expected to be readily metabolised and not to accumulate.

Therefore, the FEEDAP Panel concludes that the use of the additive in animal feed would not appreciably increase the existing human exposure to the constituents of the additive. However, in the absence of adequate data on the genotoxicity, the FEEDAP Panel cannot conclude on the safety of the use of the additive in animal feed for the consumer.

3.3.6. Safety for the user

The data on the dusting potential indicate that there is a potential for user exposure by inhalation.

No specific studies were provided by the applicant regarding the safety of the additive for users. In the absence of such data, the FEEDAP Panel cannot conclude on the safety for the users when handling the additive.

3.3.7. Safety for the environment

M. officinalis L. is a species native to Europe where it is widely grown both for commercial and decorative purposes. Use of the extract from the plant in animal production is not expected to pose a risk for the environment.

3.4. Efficacy

M. officinalis L. (lemon balm) and its extracts are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2010) and by the FEMA with the reference number 2111 (lemon balm), 2112 (balm leaves extract) and 2113 (balm oil).

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

4. Conclusions

Toxicity and genotoxicity data of the identified components of the extract do not raise concerns. However, the analysis of the extract is incomplete. In the absence of adequate data on composition and in view of incomplete genotoxicity testing, the FEEDAP Panel is unable to conclude on the safety of the additive for the target species and the consumer.

The data on the dusting potential indicate that there is a potential for user exposure by inhalation. In the absence of specific studies, the FEEDAP Panel cannot conclude on the safety of the additive for the user.

M. officinalis L. is a species native to Europe and its use in animal feed is not expected to pose a risk for the environment.

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

Documentation provided to EFSA/Chronology

Date	Event
03/11/2010	Dossier received by EFSA. *Melissa officinalis dry extract* (Nor-Balm®) for all animal species and categories Submitted by NOR FEED SUD

Date	Event
17/11/2010	Reception mandate from the European Commission
06/05/2011	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
21/12/2011	EFSA informed the applicant that, in agreement with the European Commission and 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
19/01/2018	Application validated by EFSA – Start of the scientific assessment
20/02/2018	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 and safety for the user*
24/04/2018	Comments received from Member States
01/04/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
28/01/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
bw	body weight
CAS	Chemical Abstracts Service
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufactures Association
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
HPLC	high-performance liquid chromatography
LOD	limit of detection
MNPCEs	micronucleated polychromatic peripheral erythrocytes
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocytes
UF	uncertainty factor
UV	ultraviolet

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Melissa officinalis dry extract*

In the current application, authorisation is sought under articles 4(1) and 10(2) for *Melissa officinalis dry extract* under the category/functional group 2(b) 'sensory additives'/'flavouring compounds', according to the classification system of Annex I of Regulation (EC) No 1831/2003. *Melissa officinalis dry extract*, also known as lemon balm extract, is an extract from leaves of *Melissa officinalis L*. It contains 3–6% rosmarinic acid, used as a phytochemical marker to trace the active substance. Specifically, authorisation is sought for the use of *Melissa officinalis dry extract* for all animal species and categories. It is intended to be incorporated in complete *feedingstuffs* or in drinking *water* at a dose ranging from 2.5 to 100 mg/kg.

For the identification and determination of *Melissa officinalis dry extract* in the *feed additive*, the Applicant proposed the internationally recognised European Pharmacopoeia method, based on High Performance Liquid Chromatography (HPLC) coupled to ultraviolet detection. The method is based on the determination of rosmarinic acid (phytochemical marker of *Melissa officinalis dry extract)*, using reference solution of rosmarinic acid. Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method – Ph. Eur. 6.0, method 01/2010:2524, for the qualitative identification of *Melissa officinalis dry extract* in the *feed additive*.

The Applicant did not provide any experimental method or data for the determination of *Melissa officinalis dry extract* in *premixtures*, *feedingstuffs* and *water*. Furthermore, the unambiguous determination of the content of *Melissa officinalis dry extract* added to *premixtures* and *feedingstuffs* is not achievable by analysis. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Melissa officinalis dry extract* in *premixtures*, *feedingstuffs* and *water*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.