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Safety and efficacy of two solvent extracts of rosemary (*Rosmarinus officinalis* L.) when used as feed additive for cats and dogs (Kemin Nutrisurance Europe SRL)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of two rosemary extracts obtained from the [REDACTED] of *Rosmarinus officinalis* L., as a technological (antioxidant) feed additive for cats and dogs. The two rosemary extracts were obtained through two different solvent extraction methods, acetone and ethanol. The additives were specified to contain carnosic acid and carnosol as the reference antioxidative compounds at a minimum content of their sum as $\geq 10\%$ in the case of the acetone extract and $\geq 5\%$ for the ethanol extract. Based on the data available, the FEEDAP Panel concluded that the maximum safe concentrations of the additives in feed were 300 and 50 mg/kg feed, for dogs and cats, respectively. No data on potential effects on respiratory system were available; however, as the products are in liquid form, the FEEDAP Panel considered that the exposure through inhalation is unlikely. The additives were shown to be irritant to skin and consequently they were considered also eye irritants. Due to the lack of data, the FEEDAP Panel cannot conclude on the potential of the additives to be skin sensitisers. The food and feed matrices in which the additives are intended to be used are of comparable nature. Therefore, the antioxidant effect observed when the additives are used in food is expected to be observed also when the additives are used in feed at the recommended concentrations.

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Table of contents

Abstract.....	1
1 Introduction.....	4
1.1 Background and Terms of Reference.....	4
1.2 Additional information.....	4
2 Data and methodologies.....	5
2.1 Data.....	5
2.2 Methodologies.....	5
3 Assessment.....	5
3.1 Origin and extraction.....	5
3.2 Characterisation.....	6
3.2.1 Characterisation of the additives.....	6
3.2.2 Characterisation of the active substances.....	8
3.2.3 Stability and homogeneity.....	8
3.2.4 Conditions of use.....	8
3.3 Safety.....	8
3.3.1 Toxicological studies.....	9
3.3.1.1 Genotoxicity studies.....	9
3.3.1.2 Subchronic repeated dose toxicity study.....	10
3.3.2 Safety for the target species.....	11
3.3.2.1 Conclusions on safety for the target species.....	11
3.3.3 Safety for the user.....	11
3.4 Efficacy.....	11
4 Conclusions.....	12
5 Documentation provided to EFSA/Chronology.....	12
References.....	12
Abbreviations.....	13
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for rosemary extract.....	14

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Kemin Nutrisurance Europe SRL² for the authorisation of the additives consisting of rosemary extract (*Rosmarinus officinalis* L), when used as a feed additive for dogs and cats (category: technological additives; functional group: antioxidants).

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 11 March 2021.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals and user and on the efficacy of the rosemary extract, when used under the proposed conditions of use (see Section 3.2.4).

1.2. Additional information

In 2012, the FEEDAP Panel issued an opinion on the safety and efficacy of rosemary extract liquid of natural origin to be used as an antioxidant in feed for dogs and cats (EFSA FEEDAP Panel, 2012).

The EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) issued one opinion on the safety of rosemary extract when used as a food additive (E 392) (EFSA AFC Panel, 2008). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) issued another opinion on the extension of use of rosemary extract in fat-based spreads (EFSA ANS Panel, 2015) and a refined exposure assessment of extracts of rosemary (EFSA ANS Panel, 2018).

Rosemary leaf (*Rosmarini folium*) is described in a monograph of the European Pharmacopoeia 10.0 as the whole, dried leaf of *R. officinalis* L. with a minimum content of 12 mL/kg of essential oil (anhydrous drug) and a minimum content of 3% of total hydroxycinnamic derivatives, expressed as rosmarinic acid (anhydrous drug).

Rosemary oil (*Rosmarini aetheroleum*) is described in a monograph of the European Pharmacopoeia 10.0 (PhEu, 2020) as an essential oil obtained by steam distillation from the flowering aerial parts of *R. officinalis* L.

For veterinary medicinal uses, the European Medicines Agency (EMA) published summary report on *Rosmarini folium* (EMA, 1997).

For human traditional medicinal uses, EMA issued an assessment report and herbal monographs on *R. officinalis* L., *aetheroleum* and *R. officinalis* L., *folium* including its extracts (EMA, 2010a,b).

Rosemary extract is not authorised as a technological additive for use in feed in the European Union (EU) market.

Rosemary oil, oleoresin, extract and tincture are currently included as feed flavouring in the list of authorised additives in feedingstuffs according to Council Directive 70/524/EEC.³

Rosemary extract (E 392) is currently authorised as a food additive to be used in a wide variety of food categories.

¹ Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

² Kemin Nutrisurance Europe SRL, Via della Tecnica, 11. 37040, Veronella (Verona, Italy).

³ List of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ C 50, 25.2.2014, p. 1.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁴ in support of the authorisation request for the use of rosemary extracts (*Rosmarinus officinalis* L.) as feed additives.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance (carnosic acid) in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of rosemary extracts (*R. officinalis* L.) is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The additives under assessment are two extracts of *R. officinalis* L. intended for individual use as technological additives (functional group: antioxidants) in feed for cats and dogs.

The current application refers to two rosemary extracts obtained through two different solvent extraction methods, with acetone or ethanol.

3.1. Origin and extraction

R. officinalis L., commonly referred to as rosemary, is an evergreen shrub belonging to the Lamiaceae family. It is native to the Mediterranean region and to parts of Asia but is now widely distributed because of its decorative value, the use of its leaves as a culinary herb and its use in traditional systems of medicine. Rosemary extracts are used for flavouring purposes and as natural alternative to synthetic antioxidants in food.

Following DNA sequence studies in 2017, the taxonomic standing of the genus *Rosmarinus* has been brought into question as it appears more closely related to members of the genus *Salvia* than previously thought. The outcome of this analysis is at present unclear as it may lead to an overall revision of the genus *Salvia* or, as some have already accepted, the incorporation of *Rosmarinus* into *Salvia*. As a result, the binomial *Salvia rosmarinus* Schleid. can be found in the literature both as the currently accepted name for rosemary and as a synonym. The Panel has opted to use in this opinion the more commonly used name of *Rosmarinus officinalis* but recognises that this designation may not be retained.

The current application concerns two extracts of rosemary obtained through different solvent extraction methods, using either acetone or ethanol.

The final additive, hereinafter referred to as RE-A,

The method used to obtain the second additive differs from that used for the acetone extraction.

⁴ FEED dossier reference: FAD-2020-0084.

⁵ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2020-0084-rosemary-extract.pdf>

⁶ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[REDACTED]

The final additive, hereinafter referred to as RE-E, [REDACTED]

3.2. Characterisation

3.2.1. Characterisation of the additives

Both extracts are liquid preparations, dark amber in colour, with a density of 940–970 kg/L and are insoluble in water.

The specifications proposed for the two extracts are based on those set for comparable extracts when used as food additives under EU Regulation No 231/2012 which identifies the phenolic diterpenes carnosic acid and carnosol as the reference antioxidative compounds and establishes a minimum content of their sum as $\geq 10\%$ in the case of the acetone extract and $\geq 5\%$ for the ethanol extract. It also specifies that carnosic acid and carnosol should represent $\geq 90\%$ of the total phenolic diterpenes in the extracts and that the ratio of the concentrations of the two reference diterpenes to total volatiles, measured in % (w/w), should be ≥ 15 when measured by gas chromatography–mass spectrometry (GC–MS).

To demonstrate compliance with the existing specifications, the applicant submitted analytical results on the batch-to-batch variation measured on five batches of each additive.⁷ The average contents of carnosic acid was [REDACTED] and [REDACTED] for RE-A and [REDACTED] for RE-E (Table 1). The sum of the two reference antioxidative compounds [REDACTED]. The ratio of antioxidant and volatile compounds was [REDACTED] for RE-A and RE-E, respectively. The ratio [REDACTED]

[REDACTED] Table 1 shows the data for those components each contributing to one or both extracts. The remaining identified compounds (ranging between [REDACTED]) and accounting for [REDACTED] of extract RE-A and [REDACTED] of extract RE-E are listed in the footnote.

The applicant performed a targeted analysis based on high-performance liquid chromatography (HPLC) to provide the concentration of caffeic acid and rosmarinic acid in five batches of both rosemary extracts. The average content of caffeic acid was [REDACTED] in RE-A. All the five batches of RE-E reported a level of caffeic acid of [REDACTED]. The content of rosmarinic acid was [REDACTED] in all the RE-A samples analysed and measured [REDACTED] in RE-E.⁹

⁷ Technical dossier/Section II/Annex II_1_3_2.

⁸

⁹ Technical dossier/Section II/Supplementary Information (July 2021).

Table 1: Composition of rosemary (*Rosmarinus officinalis* L.) extracts [REDACTED] and based on the analysis of five replicate batches

Components	Extract RE-A		Extract RE-E	
	Mean%	Range%	Mean%	Range%
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

The applicant proposed specifications for camphor as 150 mg/kg additive. Analyses of five batches¹⁰ of each additive demonstrated compliance with the proposed specifications [REDACTED]

Impurities

Three batches of each additive¹¹ were analysed for impurities and for evidence of microbial contamination. Heavy metals (cadmium, lead and mercury) levels were below the limit of quantification (LOQ)¹² and arsenic concentrations ranged from 0.037 to 0.062 mg/kg (RE-A) and from 0.101 to 0.125 mg/kg (RE-E). The levels of lead and arsenic were below the limits set in the specifications for the use of comparable extracts as food additives (Pb < 2 mg/kg extract and As < 3 mg/kg extract). Pyrethroids, organochlorine, organophosphorus and other pesticides¹³ were analysed and all were found below the respective LOQs. Tetrafluoroethane was measured in RE-A¹⁴ and RE-E¹⁵ batches and was found to be below the LOQ.¹⁶ The sum of dioxins plus dioxin like PCBs levels were 0.532 and 0.523 WHO-PCDD/F-PCB-TEQ/kg for RE-A and RE-E, respectively.¹³ Results from the analysis of the mycotoxins¹⁷ aflatoxins (B1, G1, B2, G2), deoxynivalenol, fumonisins, zearalenone and ochratoxin A were below the LOQ of the applied methods.¹⁸

The content of residual solvents (acetone or ethanol) was below 250 mg/kg extract in all the batches tested. This meets the specifications set in the Food Additives Regulation which stipulates that residual solvent should not exceed 500 mg/kg extract.

Microbiological contamination was assessed by the determination of counts for *Escherichia coli* (< 3 CFU/g), Enterobacteriaceae (< 10 CFU/g), total aerobes (< 10 CFU/g), total coliforms (< 3 CFU/g), *Salmonella* spp. (absent in 25 g), filamentous fungi (< 10 CFU/g) and yeasts (< 10 CFU/g).

Based on the results above, no safety concerns were identified.

¹⁰ Technical dossier/Section II/Annex_II_1.4.2.2.

¹¹ Technical dossier/Section II/Annex_II_1.4.2.2-1.4.2.4. and Annex_II_1.4.2.7-1.4.2.9.

¹² LOQ cadmium, lead and mercury: < 0.01 mg/kg.

¹³ Technical dossier/Section II/Annex_II_1.4.2.11 and 1.4.2.6.

¹⁴ Technical dossier/Section II/Annex_II_1.4.2.12.

¹⁵ Technical dossier/Section II/Annex_II_1.4.2.13.

¹⁶ LOQ tetrafluoroethane 5 mg/kg.

¹⁷ Technical dossier/Section II/Annex_II_1.4.2.10.

¹⁸ LOQ aflatoxin B1, B2, G1, G2: 1 µg/kg, deoxynivalenol: 0.1 mg/kg, fumonisin B1, B2 and B3: 0.1 mg/kg, ochratoxin A: 1 µg/kg, zearalenone: 12.5 µg/kg.

3.2.2. Characterisation of the active substances

Rosemary extracts derive from *Rosmarinus officinalis* L. and contain compounds belonging mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes which are known to exert antioxidative functions.

The phenolic diterpenes carnosic acid (chemical name: 4a(2*H*)-phenanthrenecarboxylic acid, 1,3,4,9,10,10a-hexahydro-5,6 dihydroxy-1,1-dimethyl-7-(1-methylethyl)-, (4*aR*,10*aS*)-, Chemical Abstracts Service (CAS) number 3650-09-7, 609-253-7, molecular weight: 332.4 g/mol, molecular formula: C₂₀H₂₈O₄ and melting point 185–190°C) and carnosol (chemical name: (1*R*,8*S*,10*S*)-3,4-dihydroxy-11,11-dimethyl-5-propan-2-yl-16-oxatetracyclohexadeca-2,4,6 trien-15-one, CAS No: 5957-80-2, molecular weight: 330.4 g/mol, molecular formula: C₂₀H₂₆O₄ and melting point: 210–220°C) are the principal antioxidative components present in the extracts. The chemical structures of carnosic acid and carnosol are shown in Figure 1.

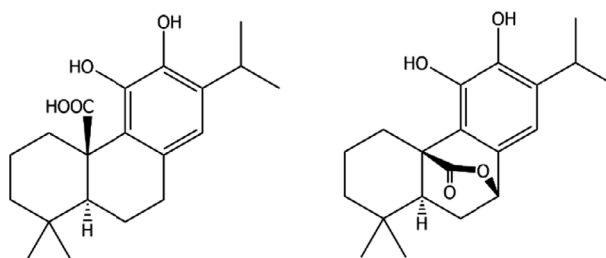


Figure 1: Structural formulas of carnosic acid and carnosol

3.2.3. Stability and homogeneity

The shelf life of the RE-A (three batches) was investigated when stored at 22°C in amber glass vials with screw top caps for 13 months. Losses (in terms of carnosic acid content) at the end of the storage period ranged from 0% to 1.8%.¹⁹

The shelf life of the RE-E (five batches) was investigated when stored at 21°C in high-density polyethylene containers for 15 months. Losses (in terms of carnosic acid content) at the end of the storage period ranged from 0% to 0.4%.²⁰

No data on stability and homogeneity in feed were provided.

3.2.4. Conditions of use

The additives are intended for use in feed for cats and dogs without minimum or maximum use levels proposed. Rosemary extracts are intended to be added to the complete feed via feed materials or premixtures (through fish/vegetable oils, other fats or protein meals). The applicant described typical inclusion levels of 200–10,000 mg/kg in fish and vegetable oils, 50–800 mg/kg in protein meals, 70–1,000 mg/kg in animal fat, 150–200 mg/kg in dry mix. These levels would provide 500 mg rosemary extracts/kg typical finished petfood (corresponding to 46.7 mg carnosic acid/kg feed and to 1.57 mg carnosol/kg feed).

3.3. Safety

In support of the safety of the additives, the applicant provided two Ames tests (one for each extract under assessment), two *in vitro* micronucleus tests (one for each extract under assessment), a 90-day toxicity study performed in rats administered with RE-A and two skin irritation studies (one for each extract under assessment). The FEEDAP Panel considered the two extracts (RE-A and RE-E) as equivalent considering the chemical similarity. Consequently, the read-across between the two extracts is considered acceptable.

The applicant specified the content of carnosol and carnosic acid in the test items used for the toxicological studies (genotoxicity battery and studies provided in support of the safety for the user):

- RE-A contains 9.42% carnosic acid and 1.79% carnosol,
- RE-E contains 9.47% carnosic acid and 1.29% carnosol.

¹⁹ Technical dossier/Section II/Annex_II.4.1.1.

²⁰ Technical dossier/Section II/Annex_II.4.1.2.

The applicant also clarified that the lot used in the subchronic toxicity study contains carnosic acid and carnosol at levels of 11.55% and 1.36%, respectively.

The FEEDAP Panel considered that the test items used in the safety studies are representative of the additives under assessment.

In support of the safety of rosemary extracts for the target species, the applicant has submitted the results of an extensive literature search. Three databases were used (CAB abstracts, Veterinary Science database and PubMed) without any time and language restriction. The applicant selected as relevant species dogs, cats and rodents. The keywords and strings used for the search were provided by the applicant and were considered appropriate. The inclusion/exclusion criteria used for the selection of relevant papers were also submitted. Overall, 1,444 hits were retrieved, 17 out of which were considered relevant by the applicant. The FEEDAP Panel noted that some of the studies reported effects of rosemary extracts (e.g. antimicrobial activity) which were not considered relevant for the present assessment. Other studies were not considered for the risk assessment, because of limitations such as the insufficient characterisation of the extract investigated or the lack of investigation of adverse effects under the chosen test conditions. Only one study on genotoxicity was considered and addressed in the specific section.

3.3.1. Toxicological studies

3.3.1.1. Genotoxicity studies

Bacterial reverse mutation test

RE-A was tested for the induction of reverse mutations in *Salmonella* Typhimurium tester strains (TA1535, TA1537, TA98 and TA100) and in *Escherichia coli* WP2 *uvrA*.²¹ The experimental protocol was in line with Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 471 and good laboratory practice (GLP). Precipitation was observed beginning at 333 µg per plate with all conditions. Toxicity was observed at 3,333 and 5,000 µg per plate with tester strain TA1535 in the presence or absence of S9 activation. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

RE-E was tested for the induction of reverse mutations in *Salmonella* Typhimurium tester strains (TA1535, TA1537, TA98 and TA100) and in *Escherichia coli* WP2 *uvrA*.²² The experimental protocol was in line with OECD TG 471 and GLP. Precipitation was observed beginning at 1,000 µg per plate with all conditions. No toxicity was observed. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

In vitro mammalian cell micronucleus test

RE-A was evaluated in an *in vitro* micronucleus assay performed in human peripheral blood lymphocytes for its ability to induce structural chromosomal damage and aneuploidy.²³ The study was in line with OECD TG 487 (2016) and GLP. The maximum tested concentrations were limited by cytotoxicity and precipitation. Three dose levels were selected for the analysis of micronuclei in binucleated cells applying a short treatment (4 + 20 h of recovery) in the presence and absence of metabolic activation (16.1, 32.3 and 76.8 µg/mL), and a continuous treatment in the absence of metabolic activation (24 + 0 h recovery) (32.3, 76.8 and 96 µg/mL). Appropriate positive and negative controls were used, and the results confirmed that the experimental system was sensitive and valid. No significant increase in the frequency of micronuclei was observed in any experimental condition.

RE-E was evaluated in an *in vitro* micronucleus assay performed in human peripheral blood lymphocytes for its ability to induce structural chromosomal damage and aneuploidy.²⁴ The study was in line with OECD TG 487 (2016) and GLP. The maximum tested concentration was limited by precipitation observed at the end of treatment at dose-levels ≥ 40 µg/mL. The concentrations selected for the analysis of micronuclei in binucleated cells were 10, 20, and 40 µg/mL applying a short treatment (4 + 20 h of recovery) in the absence and presence of metabolic activation (S9-mix) and a continuous treatment (24 + 0 h recovery) without S9-mix. Appropriate positive and negative controls were used, and the results confirmed that the experimental system was sensitive and valid. No

²¹ Technical dossier/Section III/Annex_III.2.2.2.3.

²² Technical dossier/Section III/Annex_III.2.2.2.4.

²³ Technical dossier/Section III/Annex_III.2.2.2.1.

²⁴ Technical dossier/Section III/Annex_III.2.2.2.2.

significant cytotoxicity was induced by the test item. The frequencies of micronuclei were comparable between treated and vehicle control cultures both in the presence and absence of metabolic activation.

The FEEDAP Panel concluded that both rosemary extracts did not induce gene mutations in bacteria nor induce chromosome damage *in vitro* in mammalian cells under the experimental conditions employed in these studies.

In addition, the applicant has submitted results from an extensive literature search. The study by Maistro et al. (2010), reported positive results in a battery of genotoxicity and mutagenicity studies. However, the test item used in the studies was an essential oil which is not representative of the additives under assessment.

3.3.1.2. Subchronic repeated dose toxicity study

Groups of 10 Sprague–Dawley rats of each sex were given a suspension of RE-A (11.55% carnosic acid and 1.36% carnosol) by gavage twice daily at doses of 0, 563, 1,126 or 1,558 mg/kg body weight (bw) per day for 90 days.²⁵ An additional group of 10 rats of each sex received the 1,126 mg RE-A/kg bw per day dose as a single daily gavage administration. The study was conducted in compliance with GLP and followed OECD TG 408 (1998).

There was one death early in the study associated with intubation error. Otherwise, there were no consistent effects on animal condition although some unusual in-cage behaviours (circling, excessive sniffing, head bobbing, and head tilt) were noted in all treated groups, mainly at the higher doses. There was a lower body weight gain in males given the highest two doses but not in females. Food intake was increased compared with controls for both sexes receiving 1,126 mg carnosic acid/kg bw per day and above. The functional observational battery and ophthalmoscopy did not reveal any test-article related effects.

At day 40 of the experiment, haematology measurements showed higher lymphocyte counts in males at mid (single daily dose only) and high dose and females at the mid dose. The same effects were seen at 90 days in males and females and additionally in the low-dose females. All of the differences were small and only occasionally statistically significant. There were no other treatment-related differences in the haematology results including the prothrombin and thromboplastin times.

Clinical chemistry results showed small differences between treated and control groups in urea nitrogen, globulin, and total protein levels at both 40 and 90 days. There was a small decrease in the triglyceride levels of both sexes at mid and high doses at both measurement times.

Urinalysis showed a dose-related increase in urine volume in both sexes at 40 and 90 days associated, in some instances, with slightly reduced specific gravity.

At necropsy, no treatment-related differences were observed in the macroscopic appearance of organs and tissues. Liver weight (both absolute and relative) was increased, compared with controls, in all treated groups of both sexes. Relative kidney weight was increased compared with controls in both sexes at mid and high dose and in males at the lowest dose.

Microscopic examination revealed hepatocellular hypertrophy at all doses which was dose-related in terms of incidence and/or severity. The distribution of the hypertrophy was primarily centrilobular, but often appeared more diffuse with involvement of the entire hepatic lobule in animals with moderate severity and was characterised by large hepatocytes that compressed the sinusoidal spaces.

In the kidney, there was an increased incidence and/or severity of basophilic tubules and mononuclear cell infiltrates in animals given the mid and high dose. An increased incidence and/or severity of proteinaceous casts were present in males at the mid and high dose and in low- and mid-dose females. Increased hyaline droplets were present in tubule cells in most males at the mid and high dose, and in one female given 1,558 RE-A mg/kg per day.

Minimal to slight degeneration/vacuolation of the non-glandular stomach mucosa was present in several animals given the mid and high dose. This finding was characterised primarily by swelling of the superficial stratified squamous epithelium with variably sized vacuoles containing clear space or proteinaceous material.

In the colon and cecum, minimal to slight mucosal epithelial hypertrophy/hyperplasia was present in animals given the mid and high dose. This finding was characterised by an increased thickness of the mucosa which often formed villous-like projections along the luminal surface. Colonic and caecal glands often contained closely arranged epithelial cells with fewer mucous (goblet) cells as compared with controls.

²⁵ Technical dossier/Section III/Annex_III.2.2.3.

The FEEDAP Panel considered the effects seen in liver as adaptive and non-adverse, and those in the kidney as background changes.

The effects observed in non-glandular stomach, colon and cecum in animals in the mid and highest dose groups are considered to be related to the treatment and adverse.

Based on the above considerations, the FEEDAP identified a no observed adverse effect level (NOAEL) of 563 mg RE-A/kg bw per day (which corresponds to the lowest dose tested of 65 mg carnosic acid/kg bw per day and 7.65 mg carnosol/kg bw per day).

Considering the chemical similarity among the two rosemary extracts (RE-A and RE-E), the FEEDAP Panel concluded that the results obtained with RE-A can be extended to RE-E.

3.3.2. Safety for the target species

No tolerance studies in dogs or cats have been provided by the applicant. A valid 90-day study performed in rats has been submitted (see Section 3.3.1.2) and from this study a NOAEL 563 mg RE-A/kg bw per day has been identified.

Applying an uncertainty factor (UF) of 100 to the NOAEL, the safe daily dose for the target species was derived following the EFSA Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), and thus the maximum safe feed concentration of the extracts was calculated. Since glucuronidation of the hydroxylated or oxygenated metabolites of the individual constituents of rosemary extracts is an important metabolic pathway facilitating the excretion of these compounds, the calculation of safe concentrations in cat feed needs an additional UF of 5. This factor is due to the unusually low capacity for glucuronidation in cats (Court and Greenblatt, 1997; Lautz et al., 2021).

The calculated maximum safe concentrations of the additives RE-A and RE-E in complete feed (88% dry matter, DM) are 300 and 50 mg/kg for dogs and cats, respectively.

Camphor

The additives are specified to contain < 150 mg camphor/kg additive [REDACTED] a compound belonging to Cramer class II (EFSA FEEDAP Panel, 2016a). At the safe use level in feed (50 and 300 mg/kg for cats and dogs, respectively), using the highest values reported [REDACTED] this would result in 0.0105–0.063 mg camphor/kg complete feed. These concentrations in feed would be 40-fold and 7-fold lower (for cats and dogs, respectively) than the maximum acceptable concentrations in feed for Cramer class II compounds (0.4–0.48 mg/kg complete feed for cats and dogs, respectively) and are considered not of concern for the target species.

3.3.2.1. Conclusions on safety for the target species

The additives RE-A and RE-E are safe for dogs at the use level of 300 mg/kg complete feed (88% DM) and at 50 mg/kg complete feed (88% DM) for cats.

3.3.3. Safety for the user

No data on potential effects on respiratory system have been provided; however, as the product is in liquid form, the FEEDAP Panel considered that the exposure through inhalation is unlikely.

Two acute skin irritation GLP studies (one for each extract under assessment) performed according to the OECD TG 439 were submitted.²⁷ The results of the studies showed that both extracts are irritants to skin, therefore, the extracts are also considered irritants to the eyes.

Due to the lack of data, the FEEDAP Panel cannot conclude on the potential of the additives to be skin sensitiser.

3.4. Efficacy

Rosemary extract is approved as food additive (E 392) under Commission Regulation (EC) 1333/2008 to be used in food matrices on a fat basis with the function of antioxidant (EFSA ACF Panel, 2008).

The food and feed matrices in which the additives are intended to be used are of comparable nature. Therefore, the antioxidant effect observed when the additives are used in food is expected to be observed also when the additives are used in feed at the recommended concentrations.

²⁶ Technical dossier/Section II/Annex_II_1.4.2.2.

²⁷ Technical dossier/Section III/Annex_III_3.1.2.1-3.1.2.2.

4. Conclusions

Rosemary extracts (RE-A and RE-E), from dried leaves of *Rosmarinus officinalis* L., are safe up to the maximum use levels of 300 and 50 mg/kg complete feed (88% DM) for dogs and cats, respectively.

The additives under assessment should be considered as irritants to skin and eyes. Due to lack of data the FEEDAP Panel cannot conclude on their potential to be skin sensitisers. The FEEDAP Panel considers that exposure through inhalation is unlikely.

Since rosemary extract is used as food additive, and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. Documentation provided to EFSA/Chronology

Date	Event
26/10/2020	Dossier received by EFSA. Rosemary extract for dogs and cats. Submitted by Kemin Nutrisurance Europe SRL
29/10/2020	Reception mandate from the European Commission
11/03/2021	Application validated by EFSA – Start of the scientific assessment
21/05/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
25/05/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No. 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation and safety</i>
14/06/2021	Comments received from Member States
23/07/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
17/09/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No. 1831/2003 – Scientific assessment suspended. <i>Issues: safety for the target species</i>
08/10/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
10/11/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
BSTFA	<i>N,O</i> -bis-(trimethylsilyl)trifluoroacetamide
BW	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FOB	functional observational battery
GC-FID	gas chromatography with flame ionisation detection
GC-MS	gas chromatography–mass spectrometry
GLP	good laboratory practice
HPLC	high-performance liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
MW	molecular weight
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PhEu	European Pharmacopeia
RRec	recovery rate
RSDip	relative standard deviation for intermediate precision
RSDr	relative standard deviation for repeatability
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 the Analysis for rosemary extract

In the current application an authorisation is sought under Article 4(1) for rosemary extract under the category/functional group 1(b) "technological additives/antioxidants", according to the classification system of Annex I of Regulation (EC) No. 1831/2003. Specifically, the authorisation is sought for cats and dogs. The feed additive (rosemary extract) is a liquid or solid preparation of natural origin, based on an extract from dried leaves of *Rosmarinus officinalis* L. According to the Applicant, the feed additive contains carnosic acid as an active substance with a minimum content of 9% (w/w). Additionally, the feed additive contains 1–2% (w/w) of carnosol as a minor component with antioxidative properties. The feed additive is intended to be used directly into feedingstuffs or through premixtures with no proposed minimum or maximum levels in feedingstuffs. However, the Applicant suggested inclusion levels of 500 mg rosemary extract/kg feedingstuffs. For the quantification of the active substance carnosic acid in the feed additive the Applicant submitted a single-laboratory validated and further verified method based on high performance liquid chromatography (HPLC) with spectrophotometric (UV) detection at 280 nm. The following performance characteristics of the HPLC-UV method have been obtained in the frame of the validation and verification studies for the quantification of carnosic acid in the feed additive: a relative standard deviation for repeatability (RSDr) ranging from 0.8% to 2.1%; a relative standard deviation for intermediate precision (RSDip) ranging from 1.3% to 2.5%; and a recovery rate (RRec) ranging from 96% to 112%. Based on the available performance characteristics the EURL recommends the singlelaboratory validated and further verified HPLC-UV method for official control for the quantification of carnosic acid in the feed additive. In addition, the feed additive was further characterised by the Applicant applying the above mentioned HPLC-UV method for the analysis of another minor component having antioxidative properties, namely carnosol. Based on the acceptable performance profile the EURL considers the HPLC-UV method as suitable for an additional characterisation of the feed additive. For the quantification of carnosic acid in premixtures and feedingstuffs the Applicant referred to a single-laboratory validated method based on gas chromatography with flame ionisation detection (GC-FID) after derivatisation with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA), which was submitted by the same Applicant and evaluated in the frame of the previous dossier FAD-2004-0003. Acceptable recovery and precision values were obtained in the frame of the validation studies for the quantification of carnosic acid in premixtures and feedingstuffs, but the Applicant did not present verification studies of the above mentioned GC-FID method for the quantification of carnosic acid in premixtures and feedingstuffs. Furthermore, the Applicant did not submit any method or data for the quantification of rosemary extract in premixtures and feedingstuffs as the accurate quantification of rosemary extract added to premixtures and feedingstuffs is not achievable experimentally. Based on the available information, the EURL is not able to recommend any method for official control for the quantification of carnosic acid or rosemary extract in premixtures and feedingstuffs. 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.