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Safety and efficacy of an essential oil from *Origanum vulgare* ssp. *hirtum* (Link) Ietsw. 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 an essential oil extracted from *Origanum vulgare* ssp. *hirtum* (Link) Ietsw. when used as a sensory feed additive for all animal species. Analysis of the oil identified 13 components accounting for > 99% of the oil, with carvacrol being the prevalent (78%). The FEEDAP Panel concludes that the proposed use level of 150 mg/kg complete feed is safe for ornamental fish. For the other species, the calculated maximum safe concentration of the essential oil in complete feed was 22 mg/kg for chickens for fattening, 33 mg/kg for laying hens, 30 mg/kg for turkeys for fattening, 40 mg/kg for piglets, 48 mg/kg for pigs for fattening, 63 mg/kg for sows, 57 mg/kg for dairy cows, 100 mg/kg for veal calves, 88 mg/kg for cattle for fattening, sheep, goats and horses, 35 mg/kg for rabbits, 101 mg/kg for salmonids, 106 mg/kg for dogs, and 18 mg/kg for cats. No concerns for consumer safety were identified following the use of the additive up to the maximum safe concentration in feed. The additive is considered as irritant to skin and eyes and a potential skin and respiratory sensitiser. Use in animal husbandry of the essential oil extracted from *O. vulgare* ssp. *hirtum* is not expected to pose a risk for the environment. Since oregano and its preparations are recognised to flavour food and their function in feed would be essentially the same, no further demonstration of efficacy is considered necessary for the essential oil.

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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.....	4
2.1. Data.....	4
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Origin and extraction.....	5
3.1.1. Characterisation of the essential oil.....	5
3.1.2. Impurities.....	6
3.1.3. Shelf-life.....	6
3.1.4. Conditions of use.....	6
3.2. Safety.....	7
3.2.1. Absorption, distribution, metabolism and excretion.....	7
3.2.2. Toxicological studies.....	7
3.2.2.1. Genotoxicity.....	7
3.2.2.2. Subchronic oral toxicity studies.....	7
3.2.3. Safety for the target species.....	8
3.2.3.1. Microbial studies.....	9
3.2.3.2. Conclusions on the safety for the target species.....	9
3.2.4. Safety for the consumer.....	9
3.2.5. Safety for the user.....	10
3.2.6. Safety for the environment.....	10
3.3. Efficacy.....	10
4. Conclusions.....	10
5. Recommendations.....	10
Documentation provided to EFSA/Chronology.....	10
References.....	11
Abbreviations.....	12
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for oregano essential oil.....	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 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Ropapharm International B.V.² for re-evaluation of the product oregano oil, 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 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 7 March 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 oregano oil, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

Oregano oil from *Origanum vulgare* is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined).

There is no specific European Union (EU) authorisation for any *O. vulgare* preparation when used to provide flavour in food. However, according to Regulation (EC) No 1334/2008³ flavouring preparations produced from food, may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer'.

The EFSA FEEDAP Panel issued two opinions on the safety and efficacy of an essential oil from *O. vulgare* ssp. *hirtum* (Link) Ietsw. var. Vulkan, when used as a feed additive for all animal species (EFSA FEEDAP Panel, 2017a, 2019a) and another opinion an essential oil of *O. vulgare* ssp. *hirtum* (Link) leetsw. for all poultry species (EFSA FEEDAP Panel, 2019b).

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 oregano oil as a feed additive.

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

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

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 Ropapharm International B.V., Kleine Tocht 13, 1507 CB, Zaandam, The Netherlands.

³ Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

⁴ FEED dossier reference: FAD-2010-0245.

⁵ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2010-0245_oregano_oil.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of an essential oil from *O. vulgare* ssp. *hirtum* (Link) Ietsw. is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Technical guidance – Compatibility of zootechnical microbial additives with other additives showing antimicrobial activity (EFSA, 2008b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a) and Genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b).

3. Assessment

This application covers an essential oil derived by steam distillation from *O. vulgare* ssp. *hirtum* (Link) Ietsw. when used as a sensory additive (functional group: flavouring compounds) in feed for all animal species.

3.1. Origin and extraction

Origanum is a genus of herbaceous plants belonging to the mint family (Lamiaceae), native to Europe, North Africa and temperate areas of Asia. The genus contains a number of species widely used for culinary purposes and as medicinal plants. The most commonly encountered are *O. vulgare*, known as oregano in most European countries, and *Origanum majorana* (sweet marjoram).

Five subspecies of *O. vulgare* are presently recognised of which the subspecies *viridulum*, *viride* and *hirtum* are the most commonly found in Europe.

The essential oil under assessment is extracted from the stem, leaves and flowers of the plant *O. vulgare* ssp. *hirtum* by steam distillation.

3.1.1. Characterisation of the essential oil

The essential oil under assessment is described as a colourless to pale yellow liquid with spicy odour. It has a viscosity of 7.10 mPa.s (25°C) and a surface tension 34 mN/m. It is insoluble in water.

The applicant provided the full characterisation of five batches of the essential oil under application by gas chromatography coupled with mass spectrometry but did not propose a specification.⁷ Besides carvacrol, 12 other compounds each representing > 0.2% of the oil were identified and quantified. These compounds accounted for 99.8% of the product and are listed in Table 1. Two minor peaks detected in the chromatograms, accounting each for about 0.1% of the total area could not be identified.

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.

⁷ Technical dossier/Supplementary information/October 2018/Annexes 1a-f.

Table 1: Constituents of the essential oil from *Origanum vulgare* ssp. *hirtum* (Link) Ietsw. based on five batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituents	CAS no	FLAVIS no	Analysis of five batches (% GC area)	
			Average	Range
Carvacrol	499-75-2	04.031	78.1	77.9–78.3
Thymol	89-83-8	04.006	2.63	2.61–2.64
p-Cymene	99-87-6	01.002	6.11	6.07–6.15
γ -Terpinene	99-85-6	01.020	3.73	3.66–3.79
Linalool	78-70-6	02.013	3.41	3.37–3.53
β -Caryophyllene	87-44-5	01.007	1.67	1.59–1.72
Myrcene	123-35-3	01.008	0.93	0.92–0.94
Pin-2(3)-ene (α -pinene)	80-56-8	01.004	0.80	0.79–0.82
α -Terpineol	98-55-5	02.014	0.71	0.71–0.72
4-Terpineol	562-74-3	02.072	0.68	0.66–0.70
Limonene	5989-27-5	01.045	0.37	0.37–0.38
Sabinene hydrate	546-79-2	02.085	0.33	0.33–0.34
Camphene	79-92-5	01.009	0.29	0.28–0.30
Unknown 1			0.113	0.109–0.120
Unknown 2			0.083	0.078–0.087
Total (without unknown)			99.79	99.71–99.81
Total (with unknown)			99.98	99.92–100

CAS: Chemical Abstracts Service; FLAVIS: The EU Flavour Information System; GC: gas chromatography.

3.1.2. Impurities

Analysis of three batches⁸ of the essential oil from *O. vulgare* ssp. *hirtum* showed that heavy metals (mercury, cadmium and lead) and arsenic were below the limit of detection (LOD < 0.01 mg/kg) and not of concern. The upper limit of the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) was in the range of 0.271–0.274 ng WHO PCDD/F-PCB-TEQ (World Health Organization polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) and PCB toxic equivalents)/kg oil and thus below the level of concern. Mycotoxins (deoxynivalenol, ochratoxin A, zearalenone, aflatoxin B1, B2, G1 and G2) were below the corresponding LODs.⁹ Pesticides were not detected in a multiresidue analysis.

3.1.3. Shelf-life

One batch of the essential oil was stored in a sealed container in the absence of light and air at a temperature of 25°C for 12 months. The batch was analysed every 2 months for the content of carvacrol and thymol during the storage period. At the end of the storage period, carvacrol loss was < 7% and there was no measurable loss in the content of thymol, supporting the declared shelf-life of 1 year under the storage conditions described.¹⁰

3.1.4. Conditions of use

The additive is intended for use in feed for all animal species at a concentration of 150 mg essential oil/kg complete feed without withdrawal.

⁸ Technical dossier/Supplementary information October 2018/Annexes 3a-3c.

⁹ Limit of detection (LOD, μ g/kg): deoxynivalenol < 1,000, ochratoxin A < 4-6, zearalenone < 44-48, aflatoxin B1, B2, G1 and G2 < 1.

¹⁰ Technical dossier/Section II/Annex 2.4.1a.

3.2. Safety

The assessment of safety is based on the use level proposed by the applicant (150 mg essential oil/kg complete feed).

3.2.1. Absorption, distribution, metabolism and excretion

As no specific studies on absorption, distribution, metabolism and excretion (ADME) with the oil under assessment were provided, the ADME of the individual constituents is therefore considered.

In mammals, carvacrol and thymol are rapidly absorbed from the gastrointestinal tract, mainly conjugated with sulfate and glucuronic acid and excreted in the urine (WHO, 2001; CIR, 2006; EFSA, 2006). Besides carvacrol and thymol, the compounds identified in the essential oil are monoterpenes (see Table 1). The most abundant of these terpenoids is *p*-cymene, a precursor of carvacrol and thymol.

In general, monoterpenes are expected to be absorbed from the gastro-intestinal tract and oxidised to polar oxygenated metabolites (by the cytochrome P450 enzymes, alcohol dehydrogenase and aldehyde dehydrogenases). The resulting hydroxylated metabolites and carboxylic acids may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites that are also excreted in conjugated form in the urine. Oxidation of the double bond leads to epoxide intermediates which are rapidly transformed either by hydrolysis to yield diols, or by conjugation with glutathione (WHO, 2005). The enzymes involved in the biotransformation pathways of these compounds are present in all the target species.

3.2.2. Toxicological studies

3.2.2.1. Genotoxicity

No genotoxicity studies are available for the essential oil under application. As an alternative, the FEEDAP Panel applied a component-based approach considering individually each of the components identified in the mixture (EFSA Scientific Committee, 2019b). All of the 13 identified compounds (listed in Table 1), accounting for 99.8% of the composition of the additive, have been assessed for use in food and are all currently listed in the EU List of authorised flavouring substances.¹¹ In the assessment for food use, it was established that these compounds were not genotoxic.

3.2.2.2. Subchronic oral toxicity study

A subchronic 90-day oral toxicity rat study with an essential oil of *O. vulgare* has been published (Llana-Ruiz-Cabello et al., 2017). Although the test item was derived from another subspecies (*O. vulgare* ssp. *virens* (Hoffmanns & Link) Jetsw.), the analysis of constituents shows that it is similar in composition and content to the essential oil under application (Table 2). Among the major components, the main differences are due to the different percentage of *p*-cymene and thymol (decreased by 37% and 51%, respectively) and carvacrol (increased by 40%). However, these three compounds accounting together for about 80% of the composition of the oils are structurally related (as *p*-cymene is the precursor of the hydroxylated derivatives carvacrol and thymol) and have similar toxicological profile.

Table 2: Comparison of the test item used in the subchronic oral toxicity study (A, *O. vulgare* ssp. *virens*) and the essential oil under application (B, *O. vulgare* ssp. *hirtum*)

Compound	Essential oil A (%)	Essential oil B (%)
Carvacrol	55.82	78.1
Thymol	5.14	2.63
γ -Terpinene	4.71	3.73
<i>p</i> -Cymene	16.31	6.11
Linalool	nr	3.41
β -Caryophyllene	2.40	1.67

¹¹ Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

Compound	Essential oil A (%)	Essential oil B (%)
α -Terpinene	1.62	0.60
β -Myrcene	1.52	0.93
Terpinen-4-ol	1.33	0.67
α -Pinene	1.10	0.80
α -Thujene	1.69	–
Total	91.6	98.7

A total of 80 male and female Wistar rats were given 0, 50, 100 or 200 mg essential oil/kg body weight (bw) per day via the diet for 90 days following the Organisation for Economic Co-operation and Development (OECD) Guideline 408. Doses were selected based on the results of an acute oral toxicity study showing no mortality at 2,000 mg/kg bw, and a palatability study in which doses above 200 mg/kg bw per day resulted in feed refusal. In the 90-day study, no mortality was reported and no significant differences in growth were observed between treated and control groups. The results of haematology, blood chemistry, gross pathology and histology showed no evidence of any treatment related adverse effects. Consequently, the authors of the study identified a no observed adverse effect level (NOAEL) of 200 mg/kg bw per day, the top dose tested. The FEEDAP Panel agrees with the conclusions of the authors of the study.

3.2.3. Safety for the target species

Tolerance studies and/or toxicological studies made with the essential oil under application were not submitted. In the absence of such studies, the approach to the safety assessment of the whole mixture can be based on read-across from a sufficiently similar mixture (EFSA Scientific Committee, 2019a). The FEEDAP Panel considers the composition of the oregano oil tested in the 90-day study (Llana-Ruiz-Cabello et al., 2017) sufficiently similar to that of the oil under assessment (Section 3.2.2.2). In addition, the oregano oil under assessment is well characterised (up to 99.8%) and all the identified components have been assessed by EFSA for use in feed and/or food. Finally, the oil under assessment does not contain substances of concern in the characterised fraction. Therefore, the FEEDAP Panel identified the NOAEL of 200 mg/kg bw per day from the 90-day study as a suitable reference point to assess the safety of the oregano oil under assessment.

Applying an uncertainty factor 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 was calculated (Table 3).

Since glucuronidation of the hydroxylated or oxygenated metabolites of the individual constituents of oregano oil is an important metabolic reaction facilitating the excretion of these compounds (see Section 3.2.2.1), their use as additives in cat feed needs an additional uncertainty factor of 5. This factor was due to the unusually low capacity of glucuronidation reported in cats (Court and Greenblatt, 1997).

Table 3: Maximum safe concentration in feed for different target animals for the essential oil from *Origanum vulgare* ssp. *hirtum*

	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	22
Laying hens	2	106	53	33
Turkeys for fattening	3	176	59	30
Piglets	20	880	44	40
Pigs for fattening	60	2,200	37	48
Sow lactating	175	5,280	30	63
Veal calves (milk replacer)	100	1,890	19	100
Cattle for fattening	400	8,000	20	88
Dairy cows	650	20,000	31	57

	Body weight (kg)	Feed intake (g DM/day)	Daily feed intake (g DM/kg bw)	Maximum safe concentration (mg/kg feed) ⁽¹⁾
Sheep/goat	60	1,200	20	88
Horse	400	8,000	20	88
Rabbit	2	100	50	35
Salmon	0.12	2.1	18	101
Dogs	15	250	17	106
Cats	3	60	20	18 ⁽²⁾
Ornamental fish	0.012	0.54	5	391

DM: dry matter; bw: body weight.

(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.

At the concentrations considered safe for the target species (Table 3), the two unidentified compounds representing about 0.1% of the composition of the essential oil would lead to concentration in feed ranging from 0.02 mg/kg for poultry to 0.11 in mg/kg for dogs. These values are below the maximum acceptable concentration in feed for Cramer Class I and II compounds but they are close to the threshold value for Cramer Class III (ranging from 0.02 mg/kg feed for poultry to 0.08 mg/kg feed for salmonids and dogs). However, taking into account the origin and the manufacturing process, the Panel considers it very likely that these unidentified compounds are terpenoids and belong to Cramer Class I.

The applicant provided additional publications on the use of essential oils in the target species, particularly in poultry.¹²

Poultry have smaller olfactory epithelium and fewer olfactory receptor genes compared to pigs and ruminants (Roura et al., 2008). Three studies were retrieved which investigated the effects of supplementation of poultry feed with essential oils at high concentrations. For two studies, the test item was not considered relevant (Çabuk et al., 2006; Hassan and Awad, 2017). The third study with carvacrol essential oil (Liu et al., 2018) showed major limitations (the duration of the third study was limited to 2 weeks, the first and most sensitive period of growth was not covered and haematology and blood chemistry were not assessed).

3.2.3.1. Microbial studies

The minimum inhibitory concentration (MIC) for a wide range of Gram-positive and Gram-negative bacteria (43), including both animal pathogens and human enteropathogens was provided and presented in tabular form.¹³ However, no detailed report was provided and considering the small amount of poorly reported information, this study cannot be considered. Consequently, effects of the additive on the gastrointestinal microflora cannot be excluded.

3.2.3.2. Conclusions on the safety for the target species

The proposed use level of 150 mg/kg complete feed is safe for ornamental fish. For the other species, the calculated maximum safe concentration of oregano oil in complete feed was 22 mg/kg for chickens for fattening, 33 mg/kg for laying hens, 30 mg/kg for turkeys for fattening, 40 mg/kg for piglets, 48 mg/kg for pigs for fattening, 63 mg/kg for sows, 57 mg/kg for dairy cows, 100 mg/kg for veal calves, 88 mg/kg for cattle for fattening, sheep, goats and horses, 35 mg/kg for rabbits, 101 mg/kg for salmonids, 106 mg/kg for dogs and 18 mg/kg for cats.

3.2.4. Safety for the consumer

No data on residues in products of animal origin were made available for any of the constituents of the essential oil under assessment.

However, the Panel recognises that the constituents of essential oil are expected to be extensively metabolised and excreted in the target species (see Section 3.2.1). Therefore, it is expected that the concentration of any residues of the individual constituents in products of animal origin would be considerably less than the concentrations given by feed to the target species.

¹² Technical dossier/Supplementary information October 2018.

¹³ Technical dossier/Section III.

All the individual identified constituents of the oregano oil, accounting together for 99.8% of the composition of the additive, are currently authorised as food flavours without limitations.

Consequently, no safety concern would be expected for the consumer from the use of the essential oil from *O. vulgare* ssp. *hirtum* up to the highest safe level for the target species in feed.

3.2.5. Safety for the user

No data on the safety for user was provided. According to the safety data sheet, hazards for skin and eyes and for respiratory exposure are recognised.¹⁴ Allergenic and irritation reactions of oil from *Origanum* spp. are known after skin or eye contact (CIR, 2006).¹⁵

Based on this, the additive is considered as irritant to skin and eyes and a potential skin and respiratory sensitiser.

3.2.6. Safety for the environment

Origanum vulgare is a native species to Europe where it is widely grown both for commercial and decorative purposes. Use of the essential oil under the proposed conditions of use in animal production is not expected to increase the environmental load of the constituents of the essential oil and to pose a risk for the environment.

3.3. Efficacy

Oregano and its preparations are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by the Flavour and Extract Manufacturers Association (FEMA) with the reference number 2828.

Since oregano and its preparations are universally recognised to flavour food and their function in feed would be essentially the same, no further demonstration of efficacy is considered necessary.

4. Conclusions

The proposed use level of 150 mg/kg complete feed is safe for ornamental fish. For the other species, the calculated maximum safe concentration of oregano oil in complete feed was 22 mg/kg for chickens for fattening, 33 mg/kg for laying hens, 30 mg/kg for turkeys for fattening, 40 mg/kg for piglets, 48 mg/kg for pigs for fattening, 63 mg/kg for sows, 57 mg/kg for dairy cows, 100 mg/kg for veal calves, 88 mg/kg for cattle for fattening, sheep, goats and horses, 35 mg/kg for rabbits, 101 mg/kg for salmonids, 106 mg/kg for dogs, and 18 mg/kg for cats.

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

The additive is considered as irritant to skin and eyes and a potential skin and respiratory sensitiser.

Use in animal production of the essential oil extracted from *O. vulgare* ssp. *hirtum* is not expected to pose a risk for the environment.

Since oregano and its preparations are recognised to flavour food and their function in feed would be essentially the same, no further demonstration of efficacy is considered necessary for the essential oil.

5. Recommendations

A specification should be set to ensure a minimum content of 75% carvacrol.

Documentation provided to EFSA/Chronology

Date	Event
03/11/2010	Dossier received by EFSA. Oregano oil for all animal species. November 2010. Submitted by Ropapharm International B.V.
13/02/2018	Reception mandate from the European Commission
07/03/2018	Application validated by EFSA – Start of the scientific assessment

¹⁴ Technical dossier/Supplementary information October 2018/Annex 6_SDS.

¹⁵ Technical dossier/Supplementary information October 2018/Literature/Anonymous 2006.

Date	Event
03/04/2018	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, conditions of use, safety for target species, safety for the consumer, and safety for the user</i>
04/05/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003. <i>Issues: method of analysis</i>
08/06/2018	Comments received from Member States
08/10/2018	Reception of supplementary information from the applicant - Scientific assessment remains suspended
20/06/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives – Scientific assessment restarted
12/11/2019	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
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufacturers Association
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
GC-FID	gas chromatography coupled to flame ionisation detection

GC–MS	gas chromatography-mass spectrometry
LOD	limit of detection
MIC	minimum inhibitory concentration
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo- <i>p</i> -dioxin
PCDF	polychlorinated dibenzofuran
TEQ	toxic equivalents
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for oregano essential oil

In the current application, authorisation is sought under Article 10(2) for the botanically defined *Oregano oil* (*Origanum vulgare ssp.*) 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. Specifically, the *feed additive* is sought to be used for all animal species and categories.

According to the Applicant, the phytochemical marker of the *feed additive* is *carvacrol*. The Applicant stated that the levels of *carvacrol* in *Oregano oil* may vary from 50% to 76% (w/w). The *feed additive* is intended to be used in feed with a proposed dose up to 150 mg/kg of complete *feedingstuffs*.

For the determination of the phytochemical marker in the *feed additive* the Applicant proposed a gas chromatography coupled to flame ionisation detection (GC-FID) method based on the international standard 'ISO 13171 – Essential oil of oregano' where a specific chromatographic profile derived from a GC-FID method is presented. Upon request of the EURL, the Applicant provided experimental data for the analysis of *carvacrol* in two different batches of the *feed additive* applying the method described in ISO 13171.

Based on the experimental evidence, the EURL recommends for official control for the determination of the phytochemical marker (*carvacrol*) in the *feed additive* the GC-FID method as indicated in the international standard 'ISO 13171 – Essential oil of oregano'.

The Applicant did not provide experimental data or an analytical method for the determination of *Oregano oil* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate or recommend any method for official control for the determination of *Oregano oil* 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.