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Safety and efficacy of an essential oil from *Origanum vulgare* subsp. *hirtum* (Link) letsw. var. Vulkan when used as a sensory additive in feed 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 from *Origanum vulgare* subsp. hirtum var Vulkan (DOS 00001) when used as a sensory feed additive for all animal species. Analysis of the oil identified 34 components accounting for > 99% of the oil, with carvacrol being the most prevalent (> 60%). Five tolerance studies in three species (chickens for fattening, weaned piglets and dairy cows) were made to assess the safety for the target species. The recommended use level of 150 mg additive/kg feed was shown to be safe for chickens for fattening and weaned piglets and this conclusion is extended to all poultry and porcine species grown for meat production. A dose of 500 mg additive/head and day (equivalent to ~ 25 mg/kg complete feed) was also demonstrated safe for the dairy cow. The Panel concluded that since the recommended use level differs between the dairy cow and the non-ruminants tested the lower use level of 25 mg additive/kg feed could be applied to all target animals not included above. Residue studies (meat, liver, fat milk and eggs) showed that the exposure of consumers to products from animals given the additive at the recommended use level did not raise safety concerns. The additive should be considered as an irritant to skin and eyes, and to have a potential for sensitisation of susceptible individuals. Use in animal production of the essential oil extracted from *O. vulgare* is not expected to pose a risk for the environment. Since oregano and its extracts is recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

Keywords: *Origanum vulgare*, essential oil, carvacrol, consumer safety, target animal safety, environment, efficacy

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Question number: EFSA-Q-2016-00112

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Note: This scientific opinion has been amended according to the confidentiality claim made by the applicant. The modified sections are indicated in the text.

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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.

The European Commission received a request from the company Dostofarm GmbH[2] for authorisation of the product natural essential oil from *Origanum vulgare* L. subsp. *hirtum* var. Vulkan (DOS 00001), when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings).

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 14 March 2016.

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 natural essential oil from *O. vulgare* L. subsp. *hirtum* var. Vulkan (DOS 00001), when used under the proposed conditions of use (see Section 3.2.4).

1.2. Additional information

Oregano oil from *O. vulgare* L. 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).

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 an essential oil from *O. vulgare* L. subsp. *hirtum* var. Vulkan (DOS 00001) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[4] and the applicable EFSA guidance documents.

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 oregano essential oil 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 *Origanum vulgare* L. subsp. *hirtum* 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 Scientific Committee, 2009),

[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] Saqual GmbH on behalf of Dostofarm GmbH, Klosterstrasse 39, 5430 Wettingen, Switzerland.
[3] FEED dossier reference: FAD-2016-0004.
[4] 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.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2016-0004.pdf

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: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d), Technical guidance - Compatibility of zootechnical microbial additives with other additives showing antimicrobial activity (EFSA, 2008b).

3. Assessment

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). The related species *Origanum onites* (Pot marjoram or Turkish oregano) and *Origanum syriacum* (Syrian oregano) are similarly used as culinary herbs and a source of flavours. Cuban oregano also belongs to the mint family but from another genus (*Plectranthus ambonicus*), while Mexican oregano (*Lippia graveolens*) belongs to an entirely different plant family (Verbenaceae).

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 from the subspecies *hirtum* is considered of particular quality and oils from this subspecies from different locations and varieties have been extensively analysed. Analysis has shown that within this subspecies, two main chemotypes can be recognised (carvacrol-rich or thymol-rich oils) together with intermediate types containing both isomers and other types in which the precursors, *p*-cymene and γ-terpinene, also are present in significant amounts (D'Antuono et al., 2000).

The essential oil is extracted from the leaves and shoots of the plant by steam distillation in yields which typically vary between 0.4% to 1.2% dry matter (DM) but can be as high as 2.0%. Supercritical fluid extraction with CO_2 may be used to limited extent.

3.2. Characterisation

3.2.1. Characterisation of the essential oil

This application concerns only the essential oil derived by steam distillation from a single registered variety (Vulkan) of *O. vulgare* subsp*. hirtum* (Link) letsw. The product is a yellow-green to dark brown clear liquid with a density of 0.935–0.950 g/cm^3. The product specifications as proposed by the applicant are based on the main components of the essential oil, namely carvacrol, thymol, γ-terpinene, *p*-cymene (also known as 1-isopropyl-4-methylbenzene), linalool, β-caryophyllene, α-terpinene, terpinen-4-ol and *trans*-sabinene hydrate. Analysis of 11 batches of the additive[6] showed compliance with these specifications (Table 1). These nine compounds account for about 89.2% of the product (expressed as area %).

[6] Technical dossier/Supplementary information September 2016/SIn1_03.

Table 1: Constituents of the essential oil from *Origanum vulgare L.* subsp. *hirtum:* specifications and batch to batch variation based on 11 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	Specification	Analysis of 11 batches (GC area %)	
			GC area %	Mean	Range
Carvacrol	499-75-20	04.031	60–65	61.06	60.07–61.85
Thymol	89-83-8	04.006	1.0–3.0	2.10	1.97–2.34
γ-Terpinene	99-85-6	01.020	4.0–9.0	7.95	7.72–8.45
p-Cymene	99-87-6	01.002	5.0–10.0	8.77	8.38–9.10
Linalool	78-70-6	02.013	< 5.0	3.96	4.89–3.52
β-Caryophyllene	87-44-5	01.007	2.0–5.0	3.39	3.21–3.79
α-Terpinene	99-86-5	01.019	< 1.5	0.72	0.62–0.90
Terpinen-4-ol	562-74-3	02.072	< 2.0	0.80	0.71–0.85
trans-Sabinene hydrate	546-79-2	02.085	0.3–1.0	0.44	0.36–0.56
Total				89.2	

CAS No: Chemical Abstract Service No.; FLAVIS number: EU Flavour Information System numbers; GC: gas chromatography.

The applicant provided the full characterisation of five batches obtained by gas chromatography (GC) coupled with a flame ionisation detector and mass spectrometry.[7] Besides the nine compounds indicated in the product specifications, 25 other compounds have been identified and quantified in the five batches and accounted on average for 99.06% (99.03–99.11%) of the product. Seven out of these 25 compounds were > 0.5% and are listed in Table 2; the remaining 18 ranged between 0.05% and 0.45% and are listed in the footnote.[8]

Table 2: An extended analysis of the essential oil from *Origanum vulgare L.* subsp. *hirtum* based on five batches, including the main components described in Table 1 and other seven constituents above 0.5%. The remaining 18 compounds are listed in footnote[8]

Constituents	CAS no	FLAVIS no	Analysis of 5 batches (GC area %)	
			Mean	Range
Carvacrol	499-75-20	04.031	60.8	60.4–61.4
Thymol	89-83-8	04.006	2.26	2.1–2.4
γ-Terpinene	99-85-6	01.020	7.62	7.1–7.8
p-Cymene	99-87-6	01.002	8.40	7.9–8.8
Linalool	78-70-6	02.013	3.82	3.7–4.0
β-Caryophyllene	87-44-5	01.007	3.56	3.4–3.7
α-Terpinene	99-86-5	01.019	0.60	0.60
terpinen-4-ol	562-74-3	02.072	0.80	0.80
trans-Sabinene hydrate	546-79-2	02.085	0.42	0.4–0.5
Pin-2(10)-ene (β-pinene)	127-91-3	01.003	1.02	1.02–1.02
d-Limonene	5989-27-5	01.045	0.82	0.79–0.85
1,8-Cineole (Eucalyptol)	470-82-6	03.001	1.57	1.52–1.59
Camphor	464-49-3	07.215	0.95	0.92–0.98
3,7,10-Humulatriene	6753-98-6	01.043	0.62	0.59–0.65

[7] Technical dossier/Section II/Annex_II_3 and Annex_II_4.

[8] Remaining compounds: sabinene, α-pinene, camphene, myrcene, α-phellandrene, octan-3-one, terpinolene, octan-3-ol, 1-octene-3-ol, α-copaene, bornyl acetate, carvacryl methyl ether, β-bisabolene, δ-cadinene, cumin aldehyde, α-calacorene, caryophyllene oxide and eugenol.

Constituents	CAS no	FLAVIS no	Analysis of 5 batches (GC area %)	
			Mean	Range
α-Terpineol	98-55-5	02.014	1.05	1.02–1.08
D,L-Borneol	507-70-0	02.016	1.59	1.55–1.64
Total			95.92	

CAS No: Chemical Abstract Service No.; FLAVIS number: EU Flavour Information System numbers; GC: gas chromatography.

Twelve minor peaks detected in the chromatograms, accounting in total for 0.89–0.97% (0.94% on average) of the total area, could not be identified.[9]

3.2.2. Impurities

Analysis of three batches found that the heavy metals (mercury, cadmium and lead) and arsenic were not of concern. The sum of dioxins was 0.13 ng WHO PCDD/F-TEQ (World Health Organization polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) toxic equivalents)/kg oil and dioxin-like PCBs was 0.08 ng WHO-PCDD/F-PCB-TEQ (World Health Organization PCDD, PCDF and polychlorinated biphenyl (PCB) toxic equivalents)/kg oil. Organochloride, organophosphorous and organonitrogen pesticides, halogen containing fungicides and pesticides, pyrethroids and pyrethrins and nitrogen-containing herbicides could not be detected in any of the batches examined.[10]

3.2.3. Shelf-life

Three batches of the additive under application were stored in light-protected sealed containers at ambient temperatures not falling below 12°C for periods of 35–50 months. Each batch was analysed for the content of carvacrol, thymol, γ-terpinene, *p*-cymene and linalool at the start and end of the storage period. No changes in composition were observed supporting the declared shelf-life of 3 years.[11] The applicant advises that storage temperature should not exceed 25°C.[12]

3.2.4. Conditions of use

The additive is intended for use in feed for all animal species at a concentration of 15–150 mg/kg complete feed. No withdrawal period is foreseen.

These conditions of use were modified during the assessment for the dairy cow for which the additive is intended to be given with a highest recommended dose of 500 mg/head and day (equivalent to approximately 25 mg/kg complete feed).

3.3. Safety

3.3.1. Genotoxicity

No genotoxicity studies are available for the essential oil under application. As an alternative, the FEEDAP Panel considered individually each of the component compounds identified. With two exceptions, all of the identified compounds (34 compounds, listed in Table 2 and footnote 8) have been assessed for use in food and are all currently listed in the European Union (EU) database of flavouring substances.[13] For the two components not previously assessed for use in food, α-copaene, and α-calacorene, a quantitative structure–activity relationship (QSAR) analysis (Toxtree) was made for potential genotoxicity and mutagenicity. No alerts were identified.[14]

[9] Technical dossier/Section II/Annex_II_4.
[10] Technical dossier/Section II/Annex_II_18.
[11] Technical dossier/Section II/Annexes_II_11, 12, 13.
[12] Technical dossier/Section II/Annex_II_15.
[13] 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.
[14] Technical dossier/Section III/Annex_III_19.

3.3.2. Safety for the target species

A total of five tolerance studies in three species (chickens for fattening, weaned piglets and dairy cows) were made to assess the safety for the target species. The test preparation used in all studies was a commercial formulation in powder form (DOSTO 500 concentrate) containing 50% of essential oil under application, the remainder of the formulation being food-grade silicic acid (E551a).[15] The carvacrol content of the test preparation is approximately 31%.[16] The amount of DOSTO 500 concentrate incorporated into feed, adjusted based on the analysis of the specific batch, was used to deliver the stated amount of the essential oil (i.e. ~ 300 mg DOSTO 500 concentrate was used to deliver 150 mg essential oil).

3.3.2.1. Safety for weaned piglets

Two trials of similar design were made, both measuring performance. In addition, gross pathology was examined in the first trial with tissue samples retained for examination of residues and in the second trial haematology and blood chemistry were monitored.

In the first trial, 128 piglets of unspecified breed (around 28 days of age, ~ 8.4 kg) were distributed on the basis of body weight to one of four treatments. Each treatment had eight replicate pens with four piglets per pen (two males and two females).[17] Animals were given *ad libitum* a basal mash diet based on wheat, oats, whey powder and full-fat soya either unsupplemented (control), or supplemented with intended use level of 150 mg essential oil/kg feed (maximum use level), 450 mg essential oil/kg feed (x3 maximum use level) or 750 mg essential oil/kg feed (x5 maximum use level). However, analysis of feed based on the known carvacrol content of the test item gave recoveries between approximately 65% and 86% of the expected value.[18] The duration of the study was 42 days. Body weight and feed intake were measured daily, and from this data, total weight gain and feed to gain ratio were calculated. After 42 days, two animals from control and two from the 150 mg oil group were sacrificed and subjected to necropsy and gross pathology. At the same time, samples from liver, abdominal fat and skeletal muscle were taken and frozen for analysis of residues.[19] Data was subjected to an analysis of variance with standard error of the means tested for significance using the pen as the experimental unit. Significance was set at ($p \leq 0.05$).

The second trial mirrored the conditions of the first except the x3 treatment group was excluded, leaving three treatments groups (0, x1 and x5 maximum use level). Pigs were housed in pairs (one male and one female) and each treatment was replicated ten times, giving a total of 60 piglets.[20] The duration of the study also was 42 days and the same basal feed used. Body weight and feed intake were measured daily, and from this data, total weight gain and feed to gain ratio were calculated. After 42 days, blood samples were taken from all pigs. As previously, data was subjected to an analysis of variance with standard error of the means tested for significance. The pen was treated as the experimental unit for the analysis of performance characteristics.

Animals remained in good health throughout the studies and no deaths were recorded. The performance data for both trials are summarised in Table 3.

Table 3: Effect of the essential oil on zootechnical performance in piglets

Performance parameter	Essential oil (mg/kg feed)			
	0	150	450	750
Trial 1				
Final body weight (kg)	35.1	34.7	34.5	36.6
Total weight gain (kg)	26.7	26.2	26.1	28.1
Total feed intake (kg)	51.8	50.9	50.6	53.9
Feed to gain	1.94[(a)]	1.94[(a)]	1.94[(a)]	1.92[(b)]

[15] Technical dossier/Section II/Annex_II_20.
[16] Technical dossier/Section III/Annex_III_4.
[17] Technical dossier/Section III/Annex_III_7.
[18] Technical dossier/Section III/Annexes_III_5 and 6.
[19] Technical dossier/Section III/Annex_III_20.
[20] Technical dossier/Section III/Annex_III_8.

Performance parameter	Essential oil (mg/kg feed)			
	0	150	450	750
Trial 2				
Final body weight (kg)	32.2	32.8	–	33.2
Total weight gain (kg)	22.0	22.7		23.2
Total feed intake (kg)	46.5	47.9		49.3
Feed to gain	2.12	2.11		2.13

(a), (b): Means within a column not sharing a common superscript are significantly different ($p < 0.05$).

No adverse effects on performance were seen in either trial. Gross pathological observation of the euthanised animals from the first trial did not reveal any abnormalities. However, it should be noted that this involved only two animals from the use level group and none from either of the overdose groups.

Except a decrease in the monocytes numbers in both test groups (0.7×10^9/L) compared to the control value (1.3×10^9/L), no significant changes in haematology or clinical chemistry parameters were observed in the second trial. The difference in monocyte numbers are considered to be without pathological consequences and might have derived from the higher numbers in the control group.

3.3.2.2. Safety for chickens for fattening

The two trials made with chickens for fattening followed the same pattern as the piglet studies. In both, performance parameters were measured. In addition, in the first trial, a gross pathology examination was made on a limited number of animals and tissue sample taken for residue determination, while in the second blood samples were taken for haematology and clinical chemistry.

In the first trial, a total of 1,680 one-day-old male Ross 308 broilers were distributed in groups of 35 birds to four dietary treatments (four treatments groups of 12 pens) for a period of 42 days.[21] The dietary treatments resulted from the supplementation of a pelleted wheat–soybean meal diet with the essential oil at 0, 150, 300 or 600 mg/kg complete feed (confirmed by the analysis of carvacrol).[22] Body weight and feed intake were measured on days 0, 14 and 42, and from this data, body weight gain and feed to gain ratio were calculated. Four birds from the control and four from the 150 mg oil group were taken. A gross pathological examination was made and duplicate samples from fat, skeletal muscle and liver retained for residue studies. Data was subjected to an analysis of variance with standard error of the means tested for significance using the pen as the experimental unit. Significance was set at ($p \leq 0.05$).

The second study used the same dietary treatments as the first, but involved only a total of 80 one- day-old male Ross 308 broilers. These were distributed in groups of two birds to four dietary treatments, giving 10 replicates per treatment. As previously, the diets were analysed for carvacrol content as confirmation of use levels. The duration of the study was 35 days.[23] Body weight and feed intake were measured on days 0, 14 and 35, and from this data, weight gain and feed to gain ratio were calculated. Blood samples were taken from all birds at the end of the trial. Data was analysed as in the previous study with the pen used as experimental unit for the performance data (Table 4).

Table 4: Effect of the essential oil on zootechnical performance in chickens for fattening

Performance parameter	Essential oil (mg/kg feed)			
	0	150	300	600
Trial 1				
Final body weight (kg)	2.91	2.90	2.89	2.83
Total weight gain (kg)	2.87	2.86	2.86	2.80
Total feed intake (kg)	4.99	4.94	4.93	5.00
Feed to gain	$1.74^{(b)}$	$1.72^{(c)}$	$1.73^{(b),(c)}$	$1.79^{(a)}$

[21] Technical dossier/Section III/Annexes_III_09.
[22] Technical dossier/Section III/Annexes_III_10-15.
[23] Technical dossier/Section III/Annex_III_10.

Performance parameter	Essential oil (mg/kg feed)			
	0	150	300	600
Trial 2				
Final body weight (kg)	2.14	2.19	2.18	2.17
Total weight gain (kg)	2.10	2.15	2.14	2.13
Total feed intake (kg)	3.49	3.51	3.51	3.52
Feed to gain	1.64	1.62	1.64	1.65

(a), (b), (c): Means within a column not sharing a common superscript are significantly different ($p < 0.5$).

Overall mortality was 1.6% in the first trial and not treatment related. There were no mortalities in the second trial. The results showed no difference in the zootechnical parameters except a significant ($p < 0.001$) increase in the feed to gain ratio for the highest use level group compared to the control in trial 1. Gross pathological observation of the euthanised animals from the first trial did not reveal any abnormalities. However, as in the pig studies, it should be noted that this involved only animals from the use level group and none from either of the overdose groups. There were no significant differences detected in the haematology and clinical chemistry data.

3.3.2.3. Safety for dairy cows

A total of 72 Holstein cows were distributed to four treatment groups each of 18 animals. Animals were assigned on the basis of a randomised complete block design with blocking by parity, calving date and start of lactation. All animals were fed a total mixed ration (total mixed ration (TMR): 62.7% DM, net energy of lactation (NEl) 1.53 Mcal/kg DM, crude protein (CP) 15.4% DM, neutral detergent fiber (NDF) 33.8% DM). The essential oil in the form of DOSTO 500 concentrate was diluted with a concentrate at 1% and added to the TMR to give 0, 500, 1,500 or 2,500 mg/head and day. Based on the data available, the highest dose tested (2,500 mg/head and day) would equate to around 115 mg/kg complete feed (88% DM). The content of carvacrol was analysed in the concentrate supplied to the control animals and that given to the treatment groups.[24] The lactating dairy cows (electronically tagged) were fed the TMR for 57 days and feed offered and refused was recorded daily. Animals were weighed daily and individual milk production and milk fat, protein and lactose were recorded after each milking. Somatic cell counts were counted every 18 days in four consecutive milking. Duplicate samples of milk were taken from the control and the recommended dose group at days 1, 28 and 56 for the analysis of residues and metabolites derived from the essential oil. One sample from each treatment group was taken at the beginning and at the end of the experiment to analyse the fatty acid profile. Blood samples were taken from five animals per treatment on days 1, 28 and 56.[25]

Data was analysed by a mixed-effects model for repeated measures. The model accounted for the fixed effects of treatment, week of study, parity (either primiparous or multiparous) plus the random effects of animal within treatment. Week of study entered the model as a repeated measure using the variance–covariance matrix that yielded the least Bayesian information criterion. Days in milk at the beginning of the study entered the model as a covariate.

As expected, primiparous cows produced significantly less ($p < 0.01$) milk (27.2 $\pm$ 0.99 kg/day) than multiparous cows (31.7 $\pm$ 1.17 kg/day) but there were no differences between treatment groups. Similarly, primiparous cows had a lesser milk fat content (3.58 $\pm$ 0.06%) than multiparous cows (3.82 $\pm$ 0.07%), but there were no further differences due to treatment. Milk protein or lactose concentration was unaffected by treatment or parity. There were also significant differences ($p < 0.001$) between the body weight of primiparous (643 $\pm$ 9.8 kg) and multiparous (736 $\pm$ 11.7 kg) cows. But body weight/weight gain was unaffected by treatment. Similarly daily feed intake (22.5–22.9 kg) and milk to feed ratio (1.27–1.30) was not significantly affected by treatment.

No significant treatment related effects were seen in clinical chemistry or haematological data.

3.3.2.4. Microbiological studies

The minimum inhibitory concentration (MIC) of a single batch of the additive under application[26] was tested against a number of indicator strains of bacteria as recommended in the guidance on

[24] Technical dossier/Section III/Annex 11.
[25] Technical dossier/Section III/Annex 16.
[26] Technical dossier/Section III/Annex 22.

microbial studies (EFSA, 2008b). A serial dilution of the additive in nutrient broth was prepared and inoculated with the individual indicator strains. After 24 h, incubation the broth was plated onto solid medium and any growth observed. Results for the indicator strains were *Escherichia coli* (American type culture collection (ATCC) 25922) 0.3 mg/mL, *Pseudomonas aeruginosa* (ATCC 27853) 1 mg/mL, *Enterococcus faecalis* (ATCC 29212) 0.5 mg/mL, *Staphylococcus aureus* (ATCC 25923) 0.5 mg/mL, *Bacillus subtilis* (ATCC 6633) 50 mg/mL and an unspecified strain of *E. coli* 0157:H7 5 mg/mL. The Guidance on compatibility indicates (EFSA, 2008b) that if a MIC value against a target organism is four times greater than the use level in feed then compatibility can be confirmed. As the maximum recommended use level of 150 mg/kg feed is substantially higher than the MIC values determined for some strains tested, effects on the gastrointestinal microflora cannot be excluded. However, there were no indications in the tolerance studies of gastrointestinal disturbance.

3.3.2.5. Conclusions on safety for the target species

Chickens for fattening and weaned piglets showed no adverse effects when given feed containing the additive under application up to four or five times the maximum recommended use level. Consequently, the maximum recommended use level of 150 mg additive/kg feed is considered safe for these species and categories. This conclusion is extended to all poultry and all porcine species/categories grown for meat production at the same maximum use level, but excludes those used for reproduction.

The dairy cow is intended to be given the additive on a head and day basis with a highest recommended dose of 500 mg additive/head and day. This is equivalent to ~ 25 mg/kg complete feed, a value at the lower end of the range proposed for other species/categories. The results of the tolerance study with dairy cows would support the safety of this dose.

Safety has been demonstrated in three major species with a comparable margin of safety between the maximum proposed use level and the highest tolerated use level tested, allowing a conclusion on safety to be extrapolated to all species. However, since the recommended use level differs between the dairy cow and the non-ruminants tested, the lower use level of 25 mg additive/kg feed or, if appropriate, the equivalent dose on a head and day basis is applied to all target species or categories not included above.

3.3.3. Safety for the consumer

3.3.3.1. Toxicology

A subchronic 90-day oral toxicity rat study with the essential oil of *O. vulgare* has been published (Llana-Ruiz-Cabello et al., 2017). Although the test item derived from another subspecies *O. vulgare* subsp. *virens* (Hoffmanns & Link) letsw., analysis shows that it is similar in composition and content to the essential oil under application (Table 5).

Table 5: Comparison of the test item used in the subchronic oral toxicity study (A) and the essential oil under application (B)

Compound	Essential oil A (%)	Essential oil B (%)
Carvacrol	55.82	60.80
Thymol	5.14	2.26
γ-Terpinene	4.71	7.62
p-Cymene	16.31	8.40
Linalool	nr	3.82
β-Caryophyllene	2.40	3.56
α-Terpinene	1.62	0.60
Terpinen-4-ol	1.33	0.80
trans-Sabinene hydrate	nr	0.42
Total	87.3	88.3

Nr: not reported.

A total of 80 male and female Wistar rats were given 0, 50, 100 or 200 mg/kg body weight (bw) per day via the diet for 90 days following the Organisation for Economic Co-operation and Development (OECD) Guideline 408. Each treatment group consisted of 10 male and 10 female animals. Doses were selected after an acute oral toxicity study in which rats were given 2,000 mg/kg bw by gavage which did

not result in the death of any animal and a palatability study which indicated that a dose greater than 200 mg/kg bw per day was likely to result in feed refusal. There were no deaths in the study and no significant differences in growth between groups. The results of haematology, blood chemistry, gross pathology and histology showed no evidence of any treatment related adverse effects. Consequently, a no observed adverse effect level (NOAEL) of 200 mg/kg bw per day, the top dose tested, was identified.

3.3.3.2. Residue studies

Residue studies were made with chickens for fattening, laying hens, piglets and dairy cows. Appropriate tissue/products from animals fed a basal diet or one supplemented with the additive under application at the maximum recommended level were analysed for carvacrol and its isomer thymol, which collectively represent approximately 65% of the essential oil. Analysis was carried out using gas chromatography/mass spectrometry (GC/MS) with a limit of quantitation (LOQ) of 3 μg/kg tissue (Table 6).

Table 6: Carvacrol and thymol content (μg/kg) of tissues from chickens for fattening and piglets given a basal diet free from the essential oil or a diet supplemented with 150 mg additive/kg feed

Tissue	Control feed		*Origanum* supplemented diet	
	Thymol	Carvacrol	Thymol	Carvacrol
Chickens for fattening (n = 4)				
Fat	nd	nd	6.8	63.1
Liver	nd	nd	6.5	97.4
Skeletal muscle	nd	nd	8.0	42.0
Piglets (n = 2)				
Fat	nd	nd	5.6	120.4
Liver	nd	nd	32.3	316.1
Skeletal muscle	nd	nd	4.7	39.6

nd: not detected (LOQ 3 μg/kg, LOD not given).

To assess the concentration of possible residues in eggs, laying hens (Lohmann Brown classic) were fed with standard basal diet or standard basal diet supplemented with the additive 150 mg/kg feed for 28 days. After reaching the metabolic steady state (day 28), 10 eggs were taken from 10 birds from each group on three consecutive days. Eggs were cracked and immediately frozen. Two eggs per day and per group (n = 6) were analysed for carvacrol and thymol (LOQ 3 μg/kg). No monoterpene was detected in eggs from the control group. Thymol was not detected in eggs from the supplemented group, but a trace amount of carvacrol (mean 4.7 μg/kg) was found.[27]

From the tolerance study made with dairy cows, duplicate milk samples were taken on days 1, 28 and 56 from five animals from the control group and five from the group given the additive at the maximum recommended dose. Trace amounts of both carvacrol and thymol below the LOQ (8.1 μg/L) could be detected in all samples.[28]

3.3.3.3. Consumer exposure

Carvacrol and thymol are taken as marker compounds. The highest residue values were found for carvacrol in pig tissues. However not all tissues were examined and, with only two animals, it is not possible to derive a standard deviation. Nonetheless applying the available mean data to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) food basket,[29] as described in the guidance on consumer safety (EFSA FEEDAP Panel, 2012c), gives an estimated daily exposure from pig tissues of approximately 50 μg carvacrol. Additional exposure from milk/eggs would make a negligible contribution. This estimated exposure can be related to a daily safe exposure of 1,800 μg carvacrol/person and day, the value derived from an assessment based on the threshold of toxicological concern (TTC) for this Cramer class I compound. The estimate for thymol would result in a 10-fold lower value (5 μg thymol).

[27] Technical dossier/Supplementary information_September 2016/Annex Sin1_02.
[28] Technical dossier/Section III/Annex_III_21.
[29] Default daily adult human consumption figures according to Regulation (EC) No 429/2008: 300 g meat, 100 g liver, 50 g kidney and 50 g fat.

The other compounds identified in the essential oil are terpenoids (see Table 2 and footnote 8) with metabolisms similar to that shown by carvacrol. Since they occur in substantially lower concentrations than carvacrol, they are not expected to be individually detected in anything other than trace amounts in tissues and products and not to modify the conclusion on consumer safety derived from the study of carvacrol and thymol.

Support for this conclusion is provided by the oral toxicity study made with an essential oil of similar composition to that under application from which a NOAEL of 200 mg/kg bw per day (the upper dose tested) was derived (Llana-Ruiz-Cabello et al., 2017). Compared to the 50 μg carvacrol exposure, which represents over 60% of the extract, the margin of exposure would be 4,000.

3.3.3.4. Conclusions on safety for the consumer

No concerns for consumer safety were identified following the application of the additive at the proposed use level in animal nutrition.

3.3.4. Safety for the user[30]

A test of acute dermal toxicity of the additive was made following OECD 402 guideline,[31] which indicates that the LD_{50} for the essential oil under test is > 2,000 mg/kg bw. Although not the purpose of the study, the observations of the study point to a potential for skin (and eye) irritation. This was recognised by the applicant who indicated the hazard statements H315 and H319 in the safety data sheet.

No studies relating to sensitisation were provided. However, there are occasional reports of exposure to oregano and its extracts within the workplace and outside resulting in respiratory and skin disorders included allergic reactions (Campiglio et al., 1983; Futrell and Rietschel, 1993). It has also been noted that plants belonging to the Labiatae can induce cross-sensitivity in humans (Benito et al., 1996).

3.3.4.1. Conclusions on safety for the user

The additive should be considered as irritant to skin and eyes and a potential skin and respiratory sensitiser in susceptible individuals.

3.3.5. Safety for the environment

Origanum vulgare is a native species to Europe where it is widely grown both for commercial and decorative purposes. The oil content of oregano varies from 4,000 to 20,000 mg oil/kg oregano. The maximum concentration of the oil is 150 mg/kg feed. This amount is not likely to change the concentration of compounds from oregano oil in the environment. Use of the essential oil extracted from the plant in animal production is not expected to pose a risk for the terrestrial or fresh water environment.

3.4. Efficacy

Under the terms Regulation (EC) No 1334/2008 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'. Consequently, there is no specific EU authorisation for any *Origanum vulgare* extract when used to provide flavour in food. However, oregano and its extracts are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009a) and by the Flavour and Extract Manufactures Association (FEMA) with the reference number 2828.

Since oregano and its extracts is universally 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.

The applicant makes reference to two studies considering possible changes in the sensory properties of animal products following the use of the additive. However, the first of these on egg quality was not further considered as the use level of the essential oil used (12.5 mg additive/kg feed) was below the minimum use level described under conditions of use.

In the second study, milk samples generated in the tolerance study with dairy cows (see Section 3.3.2.3) were taken on day 0 and day 56 from four cows of each of the four treatments and

[30] This section has been amended according to the confidentiality claim made by the applicant.
[31] Technical dossier/Section III/Annex 24.

the fatty acid composition of each sample determined. The results showed that supplementation with the additive under application up to 2,500 mg/head and day did not alter the pattern of fatty acids present in the milk.

4. Conclusions

The recommended use level of 150 mg additive/kg feed is considered safe for chickens for fattening and weaned piglets. This conclusion is extended to all poultry and all porcine species/categories grown for meat production at the same maximum use level, but excludes those used for reproduction. The recommended dose of 500 mg additive/head and day (equivalent to ~ 25 mg/kg complete feed) is also demonstrated safe for the dairy cow. Since the recommended use level differs between the dairy cow and the non-ruminants tested, the lower use level of 25 mg additive/kg feed or, if appropriate, the equivalent dose on a head and day basis, is applied to all target species or categories not included above.

No concerns for consumer safety were identified following the application of the additive at the maximum proposed use level in animal nutrition.

The additive should be considered as an irritant to skin and eyes and a potential skin and respiratory sensitiser in susceptible individuals.

Use in animal production of the essential oil extracted from *O. vulgare* is not expected to pose a risk for the terrestrial or fresh water environment.

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

Documentation provided to EFSA

1) Natural essential oil from *Origanum vulgare* L. subsp. *hirtum* var. Vulkan (DOS 00001) for all animal species. February 2016. Submitted by Dostofarm GmbH.
2) Natural essential oil from *Origanum vulgare* L. subsp. *hirtum* var. Vulkan (DOS 00001) for all animal species. Supplementary information. September 2016. Submitted by Dostofarm GmbH.
3) Natural essential oil from *Origanum vulgare* L. subsp. *hirtum* var. Vulkan (DOS 00001) for all animal species. Supplementary information. January 2017. Submitted by Dostofarm GmbH.
4) Natural essential oil from *Origanum vulgare* L. subsp. *hirtum* var. Vulkan (DOS 00001) for all animal species. Supplementary information. September 2017. Submitted by Dostofarm GmbH.
5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Oregano essential oil.
6) Comments from Member States.

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Abbreviations

ATCC	American type culture collection
bw	body weight
CAS	Chemical Abstracts Service
CP	crude protein
DM	dry matter
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavour and Extract Manufactures Association
FID	flame ionisation detector
FLAVIS	the EU Flavour Information System
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantitation
MIC	minimum inhibitory concentration
NDF	neutral detergent fiber
NEl	net energy of lactation
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDD/F	polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran
PCDF	polychlorinated dibenzofuran
QSAR	quantitative structure-activity relationship
TEQ	toxic equivalents
TMR	total mixed ration
TTC	threshold of toxicological concern
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 4(1) for natural essential oil from *Origanum vulgare L.,* subsp*. hirtum* var. Vulkan (DOS 00001) 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, authorisation is sought for the use of the *feed additive* for all animal species and categories.

The Applicant defined the product as 'natural essential oil from *Origanum vulgare L.* subsp*. hirtum* var. Vulkan (DOS 00001)', containing the following main constituents: Carvacrol (60–65%); *p*-Cymene (5–10%); γ-Terpinene (4–9%); β-Caryophyllene (2.0–5.0%); Thymol (1.0–3.5%); Linalool (less than 5.0%); α-Terpinene and Terpinen-4-ol (less than 2.0% each). The Applicant suggested using Carvacrol as the phytochemical marker. The *feed additive* is to be used in *feedingstuffs* with no proposed minimum or maximum concentration levels. However, recommended inclusion levels of the *feed additive* are ranging from 15 to 150 mg/kg complete *feedingstuffs*.

For the characterisation of the *feed additive*, the Applicant submitted a gas chromatography coupled with flame ionisation and mass spectrometric detection (GC-FID/MS) method – derived from the ISO 11024 and the European Pharmacopoeia monograph 8.0, 2.2.28 – to identify and quantify the main constituents. The Applicant reported a relative standard deviation for *intermediate precision* (RSDip), ranging from 0.8% to 6.1% when quantifying Carvacrol and Thymol in the pure essential oil. Based on the experimental evidence provided the EURL recommends the GC-FID/MS method for official control to identify the major constituents and quantify the phytochemical marker (Carvacrol), in the *feed additive.*

For the quantification of Oregano essential oil in *premixtures* the Applicant submitted a method based on volumetric analysis after water-steam distillation – derived from the ISO 6571 standard and European Pharmacopeia monograph 8.0, 2.8.12. The Applicant applied this method for the analysis of the essential oil in three different types of *premixtures* containing the essential oil on silica and fatty plant oil carriers and reported satisfactory recovery rates (Rrec) ranging from 89.7% to 90.9% for the volatile oil content. As for the *feed additive*, the EURL suggests applying the GC-FID/MS for the identification of the obtained essential oil. Based on the experimental evidence available, the EURL considers these methods suitable for determination of oregano essential oil in the *premixtures* investigated.

The accurate quantification of added essential oil from *Origanum vulgare L.* subsp*. hirtum* var. Vulkan (DOS 00001) in *feedingstuffs* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Origanum vulgare L.* subsp*. hirtum* var. Vulkan (DOS 00001) in *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) is not considered necessary.