SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 17 March 2020

doi: 10.2903/j.efsa.2020.6070

Safety and efficacy of Nimicoat® (carvacrol) as a zootechnical additive for weaned piglets

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy Nimicoat® (carvacrol), as a zootechnical additive for weaned piglets. The additive is composed by carvacrol (≥ 40 %) as an active substance encapsulated with high-melting lipids. Nimicoat® is intended to be used in feed for weaned piglets at a minimum recommended dose of 250 mg/kg complete feed and at a maximum recommended dose of 1,000 mg/kg complete feed corresponding to a minimum and maximum of 100 and 400 mg carvacrol/kg complete feed, respectively. Based on a tolerance study, the FEEDAP Panel concluded that the use of Nimicoat® in feed for weaned piglet at the maximum recommended dose of 1,000 mg/kg feed is safe for the target animal; however, a precise figure for the margin of safety cannot be defined. Nimicoat® used in feed for weaned piglets at the maximum recommended concentration does not pose a safety concern for consumers. The additive is corrosive to eyes, skin and the respiratory mucosae. Concerning safety for the environment a Phase II was required; taking into consideration the data provided, the FEEDAP Panel concluded that the additive does not pose any risk to the terrestrial and aquatic compartments and that bioaccumulation potential for carvacrol is low and risk for secondary poisoning for worm/fish eating birds and mammals is not likely to occur. Only one study positively supporting efficacy of the additive was available; therefore, the FEEDAP Panel is not in a position to conclude on the efficacy of Nimicoat®.

Keywords: zootechnical additives, other zootechnical additives, Nimicoat®, Carvacrol, Safety, Efficacy, piglets

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Question number: EFSA-Q-2016-00076

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Acknowledgements: The EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) wishes to thank the following for the support provided to this scientific output (in alphabetical order of last name): Montserrat Anguita, Jaume Galobart, Matteo L. Innocenti, Paola Manini, Jordi Tarrés-Call and Maria Vittoria Vettori.

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Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis V, Azimonti G, de Lourdes Bastos M, Christensen H, Dusemund B, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Finizio A, Focks A, Teodorovic I, Gropp J, Mantovani A and López-Gálvez G, 2020. Scientific Opinion on the safety and efficacy of Nimicoat® (carvacrol) as a zootechnical additive for weaned piglets. EFSA Journal 2020;18(4):6070, 25 pp. https://doi.org/10.2903/j.efsa.2020.6070

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Nimicoat® (carvacrol), as a zootechnical additive for weaned piglets. The assessment was performed based on the principles of Regulation (EC) No 429/2008 and the applicable Guidance of the FEEDAP Panel.

The additive Nimicoat® is composed of the active substance carvacrol (40 %) encapsulated with high-melting lipids; other components of the additive are amorphous silica and a surfactant. It is intended to be used as a zootechnical additive (functional group: other zootechnical additives) to enhance the growth in weaned piglets, at a minimum recommended dose of 250 mg/kg complete feed and at a maximum recommended dose of 1,000 mg/kg complete feed corresponding to a minimum and maximum of 100 and 400 mg carvacrol/kg complete feed.

Nimicoat® is considered safe for weaned piglets at a maximum content of 1,000 mg/kg complete feed (corresponding to 400 mg carvacrol/kg complete feed). However, owing to the uncertainties derived from necropsy data (only one gender examined, organ weight not reported), a precise figure for the margin of safety cannot be defined.

Carvacrol was shown to be not genotoxic. The available studies indicate that any carvacrol residue in edible tissues would be below the measurable levels, when the additive is used at the maximum intended dose. Consequently, and considering that carvacrol is authorised for food, the FEEDAP Panel concluded that the use of the additive in animal nutrition is considered safe for consumers of animal products under the proposed conditions of use.

Based on studies on eyes and skin and on the respiratory system, the FEEDAP Panel concluded that the additive is corrosive to eyes, skin and the respiratory mucosae.

Concerning safety for the environment, the Phase I calculations of the predicted environmental concentrations (PECs) in soil and groundwater showed that the values of the worst-case estimation were above the thresholds; therefore, a Phase II was required. Taking into consideration the physico-chemical properties of the additive, its fate and behaviour, the PECs, the ecotoxicity studies, the estimated risk characterisation for the terrestrial and aquatic species, the bioaccumulation potential and the risk for secondary poisoning, the FEEDAP Panel concluded that (i) the additive does not pose any risk to the terrestrial and to the aquatic compartment, and (ii) bioaccumulation potential for carvacrol is low and risk for secondary poisoning for worm/fish eating birds and mammals is not likely to occur.

Four efficacy studies were provided by the applicant to support the efficacy of the additive, from which only two were considered. Only in one of these studies, piglets' zootechnical performance was significantly improved with diets supplemented with Nimicoat® at the minimum recommended level of 250 mg/kg complete feed. Owing that three studies to positively support efficacy of the additive are needed, and only one was available, the FEEDAP Panel is not in a position to conclude on the efficacy of Nimicoat®.

The FEEDAP Panel further notes that the data provided do not support the proposal of the applicant to add Nimicoat® to compound feed at the maximum of 1,000 mg/kg of feedingstuffs during the first weeks after weaning to a maximum of 3 weeks, and at the minimum of 250 mg/kg thereafter for a minimum of 4 weeks.

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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 Techna France Nutrition[2] for authorisation of the product Nimicoat® (carvacrol), when used as a feed additive for weaned piglets (category: zootechnical additives; functional group: other zootechnical additives).

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 30 May 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 Nimicoat® (carvacrol), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The zootechnical additive Nimicoat® is composed by the active substance carvacrol encapsulated with high-melting lipids.

The FEEDAP Panel issued an opinion on the safety and efficacy of carvacrol as a flavouring feed additive for all animal species (EFSA FEEDAP Panel, 2012a), of an essential oil from *Origanum vulgare* containing about 78% carvacrol (EFSA FEEDAP Panel, 2019a) and of three zootechnical additives in which carvacrol is part of the composition (EFSA FEEDAP Panel 2015, 2019b, 2019c).

Carvacrol is authorised as a sensory feed additive with an specific entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural or corresponding synthetic chemically defined flavourings). Two zootechnical additives in which carvacrol is part of the composition are authorised in the European Union (EU), by Commission Implementing Regulation (EU) 2015/1490[3] and by Commission Implementing Regulation (EU) 2020/160.[4]

Carvacrol has been also assessed as food flavouring by JECFA (WHO, 2001) and the AFC Panel of EFSA (2008a), and is authorised as a flavouring substance for use in food.[5]

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[6] in support of the authorisation request for the use of Nimicoat® (carvacrol) as a feed additive. The technical

[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] Techna France Nutrition: Route de Saint Etienne Montluc, 44220 Coueron, France.
[3] Commission Implementing Regulation (EU) 2015/1490 of 3 September 2015 concerning the authorisation of the preparation of carvacrol, cinnamaldehyde and capsicum oleoresin as a feed additive for chickens for fattening (holder of the authorisation Pancosma France S.A.S.). OJ L 231, 4.9.2015, p. 4.
[4] Commission Implementing Regulation (EU) 2020/160 of 5 February 2020 concerning the authorisation of the preparation of oregano oil, caraway oil, carvacrol, methyl salicylate and L-menthol as a feed additive for weaned piglets (holder of authorisation Biomin GmbH). OJ L 34, 6.2.2020, p. 25.
[5] 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.
[6] FEED dossier reference: FAD-2016-0002.

dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[7] and the applicable EFSA guidance documents.

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 and other scientific reports 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 Nimicoat® (carvacrol) in animal feed. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Nimicoat® (carvacrol) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012b), 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, 2008b; EFSA FEEDAP Panel, 2019d), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c, EFSA FEEDAP Panel, 2017) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

The additive Nimicoat® is composed of carvacrol as the active substance, encapsulated with high-melting lipids. It is intended to be used as a zootechnical additive (functional group: other zootechnical additives) to enhance the growth in piglets.

3.1. Characterisation

3.1.1. Characterisation of the additive

Nimicoat® is a white to beige flowing powder.[9] The additive is specified to contain at least 40 % of carvacrol as active substance; the rest of the additive consists of lipids from vegetable oils (30–35 %),[10] amorphous silica (E 551a) (20–22 %) and surfactant (mono and diglycerides of fatty acids; 4–6 %). The analysis of five batches of the additive showed an average content of carvacrol of 40.7 % with values in the range of 38.5–42.5 %; the FEEDAP Panel notes that one batch did not meet the specification.[11]

Three batches of the additive were analysed for heavy metals. Values for arsenic (< 0.5 mg/kg), lead (< 0.7 mg/kg), cadmium (< 0.1 mg/kg) and mercury (< 0.01 mg/kg) did not give rise to safety concerns.[12] Total faecal coliforms and total fungal and yeast counts were measured in three batches of the additive with values < 10 colony forming units (CFU)/g and the absence of *Listeria monocytogenes* and *Salmonella* spp. in 25 g.[13]

Data on the content of aflatoxins and dioxins in three batches of the additive were submitted in the dossier.[14] Content of aflatoxins B1, B2, G1 and G2 were < 0.1 μg/kg, < 0.1 μg/kg, < 0.1 μg/kg and < 0.2 μg/kg, respectively. Dioxin content ranged from 0.154 to 0.161 ng WHO-PCDD/F-TEQ/kg and the sum of dioxins plus dioxin-like PCBs ranged from 0.236 to 0.247 ng WHO-PCDD/F-PCB-TEQ/kg. These values did not give raise to concern.

[7] 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.

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0002_carvacrol.pdf

[9] Technical Dossier/Supplementary Information (December 2016)/Section II.

[10] Commercial product containing minimum 97 % saturated fatty acids and maximum 3% mono unsaturated fatty acids, melting point 68−73°C. Technical Dossier/Supplementary Information (January 2020)/Annex 1.

[11] Technical Dossier/Section II/15.Annex II.11. The applicant argued that the variations in the amount of carvacrol in the additive are due to errors in sampling and analyses. Technical Dossier/Supplementary Information (April 2019).

[12] Technical Dossier/Section II/16.Annex II.12.

[13] Technical Dossier/Section II/17.Annex II.13.

[14] Technical Dossier/Supplementary Information (December 2016)/Section II/Annex_II_15.

The product has a tap density of 0.66 g/cm^3 and a bulk density of 580 kg/m^3 (average of three batches each).[15]

Particle size distribution of Nimicoat® was measured in three batches by laser diffraction. The percentage of particles below 10.5 and 104 μm were 11.1% and 30.2%, respectively, and about 70 % of the particles were between 100 and 750 μm.[16] Analytical data on dusting potential of three batches of the additive, performed according to Stauber–Heubach method, showed an average result of 768 mg/m^3 (range 640–940).[17]

3.1.2. Characterisation of the active substance

Carvacrol (2-methyl-5-(1-methylethyl)-phenol; synonyms *p*-cymene-2-ol, cymenphenol, 2-hydroxy-*p*-cymene, isopropyl-*o*-cresol, isothymol and iso-thymol; Chemical Abstracts Service (CAS) number 499-75-2; European Inventory of Existing Chemical Substances (EINECS) number 207-889-6, EU Flavour Information System (FLAVIS) number [04.031]; chemical formula $C_{10}H_{14}O$ and molecular weight 150.22 Da) is a colourless or pale yellow liquid with pungent odour. The structural formula of carvacrol is shown in Figure 1.

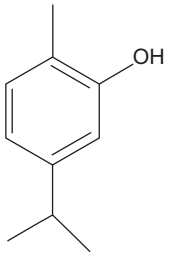

Figure 1: Structural formula of carvacrol

3.1.3. Manufacturing process

[redacted]

3.1.4. Stability and homogeneity

Five batches of the additive were stored in paper bags either at 25°C/60 % relative humidity (RH) or 40°C/75 % RH. The carvacrol content was monitored for a period of 6 months;[19] no losses were observed during this period.

Stability was studied in a complete feed for piglets in mash and pelleted form, stored at unspecified ambient conditions (intended carvacrol concentration 480 mg/kg) or under controlled conditions (25°C/60 % RH; intended carvacrol concentration 300 mg/kg) for 3 months. The loss of carvacrol after storage under both conditions ranged from 25 to 30 %.[20,21]

The applicant submitted a study to investigate the stability of the additive during feed pelleting at 60 or 85°C.[22] The intended carvacrol concentration of the mash feed was 3,600 mg/kg; the analysed value showed a loss of about 20%, independent of the pelleting temperature.

To assess the capacity of the additive to homogeneously distribute in feed, 12 samples of 100 g of a piglet mash feed were taken at random after mixing with the additive (intended carvacrol

[15] Technical Dossier/Supplementary Information (January 2020)/Annex 2.
[16] Technical Dossier/Supplementary Information (December 2016)/Section II/Annex _II_16.
[17] Technical Dossier/Supplementary Information (April 2019)/Section II/Annex _II_24.
[redacted]
[19] Technical Dossier/Section II/7.Annex II.3.
[20] Technical Dossier/Section II/8.Annex II.4.
[21] Technical Dossier/Section II/9.Annex II.5.
[22] Technical Dossier/Section II/Supplementary Information (May 2017)/Annex_II_18 and II_19.

concentration 300 mg/kg).[23] Each sample was divided in two portions and, subsequently, the 24 samples analysed for carvacrol content. The average coefficient of variation was 6.5 %.

3.1.5. Conditions of use

Nimicoat® is intended to be used in feed for weaned piglets at a minimum recommended level of 250 mg/kg complete feed and at a maximum recommended level of 1,000 mg/kg complete feed corresponding to a minimum and maximum of 100 and 400 mg carvacrol/kg complete feed.

The applicant further proposed to add Nimicoat® to compound feed at the maximum of 1,000 mg/kg of feedingstuffs during the first weeks after weaning to a maximum of 3 weeks, and at the minimum of 250 mg/kg thereafter for a minimum of 4 weeks.[24]

The additive should be directly incorporated in the complete feed. The applicant stated that the additive is not to be added to premixtures.

3.2. Safety

3.2.1. Safety for the target species

The applicant submitted a pilot study and a pivotal study on the safety of Nimicoat® in weaned piglets.

No information on the antimicrobial properties of carvacrol was submitted by the applicant; however, the antimicrobial activity of carvacrol is well known (Nostro and Papalia, 2012).

3.2.1.1. Pilot study on weaned piglets

A pilot study with a duration of 20 days was performed with a total of 64 early weaned crossbred piglets (4.8 kg body weight, 21 days old).[25] The animals were allocated to two treatments with four pens per treatment and eight piglets per pen. The treatments were a control diet without Nimicoat® and a test diet with 10,000 mg Nimicoat®/kg complete feed (corresponding to 4,000 mg carvacrol/kg, analytically confirmed). The diet consisted mainly of barley, wheat, soybean meal and whey and contained by calculation 18.2 % crude protein (CP), 1.22 % digestible lysine (Lys) and 10.8 MJ NE/kg. The piglets had *ad libitum* access to the feed. Body weight and feed intake were recorded at study start, after 7 days and at study end; average daily feed intake (ADFI), average daily gain (ADG) and feed:gain ratio (F:G) were calculated for the corresponding intervals. Data were analysed as a completely randomized block design by generalized linear model (GLM) the model included the diet effect and initial body weight was used as a covariate for performance.

Already after 7 days, Nimicoat® depressed significantly body weight and weight gain compared to the control group. At study end, body weight of the test group was 87% of the control (10.2 kg), ADFI 82% and ADG 75% of the corresponding control values (298 g/day and 269 g/day, respectively); all these differences were statistically significant.

Based on the results of the pilot study, the overdose of 5,000 mg Nimicoat®/kg complete feed (corresponding to fivefold concentration of the highest recommend dose) was selected to be applied in the tolerance study.

3.2.1.2. Tolerance study in weaned piglets

For the tolerance study,[26] a total of 144 piglets (equal number of males and females, Stambo HBI Dalland 40), weaned at 24 days of age (average body weight 8.2 kg) was allocated to three groups approximately equal in body weight and litter origin. The groups received a basal diet (control group) or the same basal diet supplemented with the highest recommended (1,000 mg/kg; use-level group) or the fivefold of the highest recommended level (overdose group) of the additive, respectively, for 6 weeks. The corresponding intended carvacrol concentrations (0, 400 and 2,000 mg/kg) were analytically confirmed. Groups' size was 12 pens per treatment with four piglets each;[27] the pens were located in two different rooms and treatments evenly distributed between rooms. Main feed materials of the basal diet were barley, wheat (flaked and untreated), maize, soybean protein and whey, the diets were prepared

[23] Technical Dossier/Section II/10.Annex II.6. Technical Dossier/Supplementary Information (April 2019)/Section II/Annex _II_23.
[24] Technical Dossier/Supplementary Information (April 2019)
[25] Technical Dossier/Section III/Annex III.1
[26] Technical Dossier/Section III/2.Annex III.2a, 2b and 2c.
[27] A randomisation process was used to allocate piglets to replicates, such that each treatment consisted of an equal number of homogeneous replicates (with regard to weight, in order to have light, medium and heavy pens).

and given for *ad libitum* access as a prestarter (first 2 weeks, 18.3 % CP, 1.36 % Lys and 10.7 MJ NE/kg) and starter (until the end, 17.8 % CP, 1.34 % Lys and 10.4 MJ NE/kg). The animals were monitored for daily health status, and mortality was recorded. Body weight and feed intake was measured in biweekly intervals, ADG and F:G calculated correspondingly. Faecal consistency was assessed at 0, 1, 3, 7, 14, 21 and 42 days in each pen. Blood samples were taken from one piglet per pen at start and end of the study for haematology[28] and routine blood biochemistry[29] analyses.

At the end of the trial, four male piglets per treatment (from four different pens;[30] homogeneous weight) were killed and subjected to necropsy. The following organs were submitted to gross pathology: heart, liver, kidney, stomach, skeletal muscle and intestinal tracts (duodenum, jejunum, ileum, caecum and colon). Samples of intestine, heart, kidney, liver and muscle (*Longissimus dorsi*) were examined histologically.

In the statistical analysis of the performance data, the pen was considered as the experimental unit, while the animal for the haematology and biochemistry endpoints.[31] Data were analysed as a completely randomised block design by ANOVA; the model included the effects of treatment, time and their interaction. For body weight, biochemical and haematological parameters, the corresponding initial parameters were used as covariates.[32] Data from supplemented groups were compared with the control group using the Tukey's test (significance declared at $p < 0.05$).

No mortality was observed.[33] Average final body weight (22.2 kg for control, 23.7 for the use-level and 23.2 for the overdose group), ADG (334 g for control, 370 for the use-level and 357 for the overdose group) and ADFI (626 g for control, 650 for the use-level and 674 for the overdose group) did not show any significant difference among the treatments; feed to gain ratio showed a significant difference between the use-level and the overdose groups (1.77 vs. 1.91), however differences with the control group (1.88) were not significant.[34]

Faecal scores were not affected by the supplementation with Nimicoat®. Statistical analysis of haematological and blood biochemistry endpoints did not show significant effects for the treatment and the interaction time*treatment, but for time. Tukey's test identified anyway group differences for glucose (lower at the use level compared to the control) and urea (lower in the overdose group compared to the control). The effect on glucose was not dose-related and is considered not treatment-related; the decrease of blood urea, already seen but not statistically significant at the use-level group, could also be interpreted as an indication of better utilisation of dietary protein supported by better average daily gain. The macroscopic and histological evaluation of organs and muscle tissue did not identify any adverse effect of the tested carvacrol concentrations; the FEEDAP Panel notes that the organ weight was not reported.

In summary, the results show that Nimicoat® had no detrimental effect on piglets when administered at five times the recommended dose. No adverse effects were observed on general animal health, performance, serum biochemical and blood haematological parameters and histopathology of organs and tissues. However, owing to the uncertainties derived from necropsy data (only one gender examined, organ weight not reported), a precise figure for the margin of safety cannot be defined.

3.2.1.3. Conclusions on safety for weaned piglets

Nimicoat® is considered safe for weaned piglets at a maximum content of 1,000 mg/kg complete feed (corresponding to 400 mg carvacrol/kg complete feed). However, owing to the uncertainties derived from necropsy data (only one gender examined, organ weight not reported), a precise figure for the margin of safety cannot be defined.

[28] Red blood cells, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, haematocrit, white blood cells, platelets, neutrophils, eosinophils, basophils, lymphocytes and monocytes.
[29] Glucose, triglycerides, urea, creatinine, uric acid, total protein, albumin, sodium, potassium, chloride, calcium, phosphorus, alanine-aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl-transferase, bilirubin total/conjugated.
[30] Technical Dossier/Supplementary Information (January 2020)/Annex 3.
[31] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex 38a, 38b, 39a and 39b.
[32] Except bilirubin, uric acid, eosinophils and basophils; the data of these parameters were not covariate adjusted because the values of second time were too low compared to first time.
[33] Technical Dossier/Section II/Supplementary Information (December 2016)/Section III/Annex III_18.
[34] Technical Dossier/Section III/Supplementary Information (April 2019)/Annexes III_39a and 39b.

3.2.2. Safety for the consumer

The additive contains carvacrol as active substance. The FEEDAP Panel considers carvacrol as the compound of interest to assess safety for the consumers. The Panel considers that the rest of the components of the additive does not raise concern.

Carvacrol is authorised as a flavouring substance in food; it is also authorised as a component of several zootechnical additives in feed. Its main characteristics concerning absorption, distribution, metabolism and excretion are described by WHO (2001) and in several EFSA opinions (EFSA, 2006, 2008a; EFSA FEEDAP Panel, 2012a). Carvacrol is rapidly absorbed from the gastrointestinal tract, conjugated with sulfate and glucuronide and excreted primarily in the urine (WHO, 2001).

3.2.2.1. Residue studies

Residue study 1

A residue study was carried out in 30 male piglets of 21 days of age. Weaned piglets were assigned to one of the two treatment groups (15 piglets/treatment) based on their body weight at weaning and maternal origin: a control and an experimental group receiving Nimicoat® at 1,200 mg/kg complete feed (480 mg carvacrol/kg).[35] Analytical data on the dietary content of carvacrol were not reported. Experimental diets were provided *ad libitum* for 45 days and animals were individually weighed at 0, 21 and 45 days of the trial.

On day 45, ten piglets from each group were caged by pairs during 2 days. During this period, feed consumption and urine emission were recorded. Faeces were directly sampled while the animals were transferred to the slaughter room. Animals were slaughtered and samples from the adipose tissue and psoas major muscle (tenderloin) were taken. Content of the colon was also sampled in animals for which no faeces were collected during the transfer to the slaughter room. No liver or kidney samples were taken. Concentration of carvacrol was determined in fat, muscle, urine and faeces.[36]

In pigs from the control group, carvacrol was not detected in any of those tissues (below the limit of detection, LOD).[37] In pigs from the treated group, concentration of carvacrol in fat and muscle samples was below the LOD; carvacrol in faeces was on average 2.0 mg/kg (range: 0.90–3.26 mg/kg) and in urine 268.3 mg/L (range: 186.0–398.3 mg/L).

Residue study 2

An additional residue study was undertaken to investigate the content of carvacrol in liver and kidneys of piglets.[38] A total of 30 piglets of 21 days of age were fed diets containing 1,000 mg Nimicoat®/kg feed during 48 days. Ten piglets were slaughtered at the end of the feeding period, while the other two batches of ten piglets each were fed Nimicoat®-free diet for 2 and 7 days respectively, before slaughtering. The content of carvacrol in feed was analytically measured for the pre-starter and the starter feed, giving a recovery of 72% and 69%, respectively, while the Nimicoat®-free diet was not analysed.

Content of carvacrol in kidneys measured at day 48, day 50 and day 57 (total of 30 samples) was in all cases below the LOD (0.25 mg/kg).[39] Furthermore, to determine the eventual presence of a conjugated compound of carvacrol, an enzymatic hydrolisis was applied before the analysis; this procedure did not lead to quantifiable results (below the limit of quantification, LOQ).[40]

Content of carvacrol in liver measured at day 48 (total of 10 samples) was in all cases below the LOD (0.25 mg/kg).[41]

3.2.2.2. Toxicological studies

Reports of *in vitro* tests of the mutagenicity of carvacrol were provided in the technical dossier: two bacterial reverse mutation assays, two mammalian cell gene mutation assays (mouse lymphoma assay) and two mammalian cell micronucleus tests.

[35] Technical Dossier/Section III/3.Annex III.3.
[36] Technical Dossier/Section III/4.Annex III.4, 5, 6 and 7.
[37] LOD: fat 0.1 mg/kg; urine 0.75 mg/kg; muscle: 0.3 mg/kg ; faeces: 0.35 mg/kg.
[38] Technical Dossier/Section II/Supplementary Information (December 2016)/Section III.
[39] Technical Dossier/Section II/Supplementary Information (December 2016)/Section III/Annex III_20.
[40] Technical Dossier/Section II/Supplementary Information (December 2016)/Section III/Annex III_21.
[41] Technical Dossier/Section II/Supplementary Information (December 2016)/Section III/Annex III_22.

Bacterial reverse mutation assays

Two *in vitro* bacterial reverse mutation assays were provided. One of the studies was performed under good laboratory practices (GLPs) principles and according to the OECD Guideline 471,[42] while comparable quality indications were not found in the second study.[43] Both tests were performed in the presence and absence of metabolic activation by S9 derived from the livers of male Sprague–Dawley rats that had been treated with Aroclor 1254.

The GLP test was performed using *Salmonella* Typhimurium strains TA1535, TA1537, TA98, TA100 and TA102. Because of cytotoxicity, the maximum concentrations tested in the replicated Ames studies[43] were, depending on strain, 150–300 (no metabolic activation) and 300–500 (with metabolic activation) μg/plate. No significant increase in revertant colonies was detected in any tested strain in the presence or absence of metabolic activation.

The other study did not bear any formal GLP statement; nevertheless, it appeared to have been appropriately performed and reported, and it was carried out by the same laboratory as the GLP study previously described. Four *S.* Typhimurium strains were assayed (TA1537, TA98, TA100 and TA102). Due to marked toxicity, only concentrations up to 30 μg/plate were tested; no increase of revertant colonies was detected. The study provided supportive evidence to the lack of mutagenicity of carvacrol in the bacterial reverse mutation assay.3

In vitro *mammalian cell gene mutation assays*

Two *in vitro* mammalian cell gene mutation assays were provided. One of the studies was performed under GLP and according to the OECD Guideline 476,[44] while a comparable quality indicator was not found in the second study.[45] The replicated gene mutation assays used L5178Y mouse lymphoma cells at the TK locus and employed a microtitre cloning technique. In both tests, cells were exposed to various concentrations of carvacrol for 3 h in the presence of S9 or for 24 h in its absence.

In the GLP test, based on the detection of marked cytotoxicity at higher concentrations, the maximum concentrations used were 0.625 mM (93.9 μg/mL) (S9) and 0.31 mM (46.6 μg/mL) (no S9). Carvacrol did not produce an increased number of mutants (either small colonies or large colonies) when tested in the presence or absence of S9, indicating an absence of mutagenicity in this test.

The other study did not bear any formal GLP statement; nevertheless, it appeared to have been appropriately performed and reported, and it was carried out by the same laboratory as the GLP one. In this test the concentrations tested were up to 78.5 μg/mL. Also, in this test, no increase of mutants was observed, providing supportive evidence to the lack of mutagenicity of carvacrol in the *in vitro* mammalian cell gene mutation assay.

In vitro *mammalian cell micronucleus tests*

Two *in vitro* mammalian cell micronucleus tests were provided. One of the studies was performed under GLP and according to the OECD Guideline 487,[46] while a comparable quality indicator was not found in the second study.[47] The replicated micronucleus tests were performed using TK6 cell cultures derived from human lymphoblastoid cells. Cells were exposed to carvacrol concentrations for 3 h in the absence of S9 and for 2 and 27 h in the absence of S9

In the GLP test, based on the detection of marked cytotoxicity at higher concentrations, the maximum carvacrol concentrations used were 0.1 mM (15.0 μg/mL) (S9), 0.15 mM (22.5 μg/mL) (S9, 3 h) and 0.125 mM (18.8 μg/mL) (no S9, 27 h). None of the treatments produced an increased number of micronucleated cells, indicating an absence of genotoxicity in this test.

The other study did not bear any formal GLP statement; nevertheless, it appeared to have been appropriately performed and reported, and it was carried out by the same laboratory as the GLP study previously described. In this test, the concentrations tested were expressed as μg/mL and, based on detection of cytotoxicity, were up to up to 156.25 μg carvacrol/mL (3 hours, with and without S9) and 78.13 μg/mL (S9, 27 hours). Also, in this test no increase of micronucleated cells was observed, providing supportive evidence to the lack of genotoxicity of carvacrol in the *in vitro* mammalian cell micronucleus test.

[42] Technical Dossier/Section III/9.Annex III.12.
[43] Technical Dossier/Section III/6.Annex III.9.
[44] Technical Dossier/Section III/10.Annex III.13.
[45] Technical Dossier/Section III/7.Annex III.10.
[46] Technical Dossier/Section III/11.Annex III.14.
[47] Technical Dossier/Section III/8.Annex III.11.

Overall, the results of the available studies do not indicate that carvacrol may possess any potential for genotoxicity.

3.2.2.3. Assessment of the safety for consumers

Carvacrol is devoid of genotoxicity potential. Previous assessments clearly indicate carvacrol as a low toxicity substance (WHO, 2001; EFSA, 2006, 2008a; EFSA FEEDAP Panel, 2012a). Since the available data are insufficient for the setting of a health based guidance value, the FEEDAP Panel considered appropriate to use the threshold of toxicological concern (TTC) approach: carvacrol is a Cramer class I compound (EFSA Scientific Committee, 2019) and the corresponding daily safe exposure is 30 μg/kg body weight (body weight (bw).

Residue studies showed that the residues of carvacrol were not detectable or quantifiable in any of the tissues examined. Therefore, the consumer exposure was calculated following the methodology described in the most recent Guidance on the safety of feed additives for the consumers (EFSA FEEDAP Panel, 2017) by assuming, in a conservative exposure scenario, that residues were all present in tissues at concentrations corresponding to the LOD of the analytical method (0.1 mg/kg fat, 0.3 mg/kg muscle, 0.25 mg/kg liver and 0.25 mg/kg kidney).

For all the age classes (infants, toddlers, other children, adolescents, adults, elderly and very elderly), the highest estimate for chronic dietary exposure to carvacrol ranged between 1.0 (very elderly) and 2.6 (other children) μg/kg bw per day. When considering adults – likely the most exposed consumer class – the estimate for chronic exposure was 1.5 μg/kg bw per day, which corresponds to the 5 % of the TTC-derived safe intake; yet considering the highest exposure of 2.6 μg/kg bw per day, the percentage of the TTC would be 8.7 %. According to the FLAVIS database, the exposure as food flavour in the EU is only 14 μg/person per day (based on MSDI), which corresponds to 1 % of the threshold of the TTC (1,800 μg/person per day). Therefore, no concern for consumer safety was identified following the use of the additive at the proposed use level in piglets.

3.2.2.4. Conclusions on safety for the consumer

Carvacrol was shown to be not genotoxic. The available studies indicate that, when the additive is used at the maximum intended dose, any carvacrol residue in edible tissues would be below the measurable levels. Consequently, and considering that carvacrol is authorised for food, the use of the additive in animal nutrition is considered safe for consumers of animal products under the proposed conditions of use.

3.2.3. Safety for the user

3.2.3.1. Effects on eyes and skin

A skin irritation test was performed with Nimicoat® in rabbits following the OECD guideline 404.[48] Nimicoat® in fine particles (ground powder) induced signs of corrosion after a 3-minute application. Nimicoat® in its usual form (unground powder) induced signs of corrosion on the skin of the rabbit after a 4-hour application, which was not reversible 15 days after application in one animal. The additive should be considered as corrosive to skin, and consequently also corrosive to the eyes.

3.2.3.2. Effects on the respiratory system

An acute inhalation study was performed following the OECD guideline 403.[49] Wistar rats were exposed to Nimicoat® for a 4-hour single exposure by nose-only inhalation. The mean achieved aerosol concentration was 0.113 mg/L. No mortality occurred during exposure or the post-exposure observation period. Body weight loss was observed in all but one animal on the day following treatment, but the body weight was soon recovered and body weights steadily increased from day 4 until termination on day 14. No other clinical signs or gross pathology alterations were observed.

The results indicate that there would be no concern for users following any inhalation exposure during handling of the additive. However, it should be noted that due to its corrosive properties the additive might damage the respiratory mucosae.

3.2.3.3. Conclusions on safety for the user

The additive is corrosive to eyes, skin and the respiratory mucosae.

[48] Technical Dossier/Section III/13.Annex III.16.
[49] Technical Dossier/Section III/12.Annex III.15.

3.2.4. Safety for the environment

Nimicoat® is intended to be used in feed for weaned piglets at a minimum recommended dose of 250 mg/kg complete feed and at a maximum recommended dose of 1,000 mg/kg complete feed corresponding to a minimum and maximum of 100 and 400 mg carvacrol/kg complete feed.

3.2.4.1. Phase I Assessment

3.2.4.1.1. Physico-chemical properties of the active substance

The physicochemical properties of carvacrol are summarised in Table 1.

Table 1: Physico-chemical properties of carvacrol

Property	Value	Unit
Molecular mass	150.2	g/mol
Water solubility (QSAR)	1,250	mg/L
Vapour pressure (QSAR)	3.09	Pa
Koc (QSAR)	1,050	L/kg
DT_{50} (from Thymol evaluation)	30	days

QSAR: quantitative structure–activity relationship; DT_{50}: Half-life.

The predicted environmental concentrations (PEC) in manure (PEC_{manure}), soil (PEC_{soil}) and ground water (PEC_{gw}) were calculated by the applicant proposing a total feed intake of 800 kg feed/year and a total nitrogen (N) excretion of 26.3 kg N/year, since the category 'piglets' was not considered in the EFSA Guidance of 2008. As posteriorly, the FEEDAP Panel updated the Guidance for the assessment of the safety of feed additives for the environment and revised the values of total feed intake and nitrogen excretion for target species including the category of piglets, these new values are considered more appropriate to calculate PEC_{soil} (EFSA FEEDAP Panel, 2019d). For piglets, total feed intake is 296 kg feed per year and total N excretion is 4 kg N/year. The input data used to calculate phase I PEC of carvacrol are summarised in Table 1.

The calculated PEC_{soil} and PEC_{gw}, considering an inclusion level of 400 mg carvacrol/kg complete feed, are reported in Table 2.

Table 2: Values of PEC soil and ground water in feed for weaned piglets (inclusion level 400 mg carvacrol/kg complete feed)

PEC	Calculated	Limit
Soil (μg/kg)	6,709	10
Ground water (μg/L)	79	0.1

PEC: predicted environmental concentration.

The Phase I calculations of PEC_{soil} and PEC_{gw} show that the values of the worst-case estimation are above the threshold for the soil and ground water. Therefore, a Phase II is required.

3.2.4.2. Phase II Assessment

3.2.4.2.1. Physico-chemical properties of the active substance

A solubility test was not provided by the applicant. From a quantitative structure–activity relationships (QSAR) evaluation, a solubility value of 1,250 mg/L was derived. Nevertheless, in the study on biodegradability presented by the applicant, carvacrol was reported as insoluble. Therefore, for further calculations, a value of 1 mg/L was considered.

The vapour pressure of carvacrol was evaluated through an experimental study; the experimentally determined vapour pressure is 350 Pa at 20°C.

The solubility and the vapour pressure of carvacrol used for PEC calculation in Phase II are summarised in Table 3.

Table 3: Physico-chemical properties of carvacrol

Property	Value	Unit
Water solubility[(a)]	1	mg/L
Vapour pressure[(a)]	350	Pa

(a): Technical Dossier/Supplementary Information (August 2019).

3.2.4.2.2. Fate and behaviour

3.2.4.2.2.1. Fate in soil: Adsorption

The adsorption and desorption properties of [^{14}C]-carvacrol were investigated according to the OECD Guideline 106, using a batch equilibrium method and following the direct approach. The adsorption coefficients (K_d and K_{oc}) and the Freundlich adsorption isotherm parameters, (K_f, K_{foc} and 1/n), were calculated for five soils (Table 4). The radioactive content of the adsorption and desorption supernatants was determined by liquid scintillation counting (LSC) and the radioactive content of the residues (at adsorption or desorption) was determined by combustion, followed by LSC. Adsorption and desortion parameters of carvacrol are reported in Table 4.

Table 4: Adsorption and desorption parameters of carvacrol

Soil	Mean partition coefficients		Freundlich coefficients			
	K_d (dm^3/kg)	K_{OC} (dm^3/kg)	K_f (dm^3/kg)	K_{foc} (dm^3/kg)	R^2	1/n
Refesol 02A	10.63	1,119	2.9758	313	0.9782	0.6590
Refesol 03G	15.65	518	5.9759	198	0.9724	0.7369
Refesol 06A	16.93	851	4.9204	247	0.9852	0.6635
LUFA 6S	7.32	411	3.5645	200	0.9940	0.7530
B2	1.84	163	1.4652	130	0.9958	0.8941
Mean	*10.47*	*612*	*3.7804*	*218*	*0.9851*	*0.7413*
Geomean	*8.24*	*506*	*3.4039*	*209*	*0.9851*	*0.7367*

From the data on adsorption and desorption isotherm experiments, carvacrol was classified (in accordance with the scheme proposed by McCall et al., 1981) as having low mobility. A geometric mean K_{oc} value of 506 dm^3/kg will be used for further refinement.

3.2.4.2.2.2. Fate in soil/Biodegradation

A biodegradability study performed according to the OECD Guideline 301 F was presented.[50] The degradation of the test item within 28 days was 81.4 % theoretical oxygen demand (ThOD, mean of three replicates) and the criteria for ready biodegradability were met (60% degradation of ThOD within a 10-day window). The study clearly showed the ready biodegradability of the substance. Following the Guidance of the European Chemical Agency (ECHA) on Biocidal Products Regulation (ECHA, 2017) a default DT_{50} in soil of 30 days at 12°C is obtained.

3.2.4.2.2.3. Conclusion on fate and behaviour

Carvacrol is characterised by a low mobility; the substance is not persistent. A DT_{50} in soil of 30 days at 12°C and a K_{oc} value of 506 dm^3/kg will be used for further refinement.

3.2.4.2.3. Predicted environmental concentrations (PECs)

The PEC in soil (PEC_{soil}), ground water (PEC_{gw}) and surface water (PEC_{sw}) were re-calculated, according to EFSA FEEDAP Guidance (EFSA, 2008b), considering the new experimental input values described in Table 3 as well as the DT_{50} and K_{oc} values described above. Moreover, since the highest recommended concentration of carvacrol in feed for piglets is 400 mg/kg feed for the first 3 weeks followed by 100 mg/kg feed for a minimum of 4 weeks, an average value of 240 mg/kg feed (round up) was considered a reliable carvacrol concentration in feed during the cycle. The PEC values for soil, ground water and surface water recalculated with all the above assumption are reported in Table 5.

[50] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_29.

Table 5: Initial PEC in soil, ground water and surface water of carvacrol considering practical use during the whole cycle (240 mg carvacrol/kg feed)

PEC in soil (μg/kg)	PEC in ground water (μg/L)*	PEC in surface water (μg/L)
4025	76	25

PEC: predicted environmental concentration,
*: The surrogate for the PEC groundwater in the Phase I is PECpore water.

3.2.4.2.3.1. Refinement based on metabolism

Data on the carvacrol content in urine of piglets, which had been fed with an increased dose of Nimicoat® from the weaning to the end of post-weaning period were presented by the applicant.[36] The study showed that 26% of the carvacrol intake is excreted via urine and hence, that 74 % is metabolised or degraded in the digestive tract.

Measurements of faeces showed very low carvacrol concentrations: about 2 μg carvacrol/g faeces of piglets fed with the recommended dose of Nimicoat® from weaning to post-weaning phase. Considering an average of 1 kg of faeces per day within the period, the amount of carvacrol excreted via the faeces is about 2 mg/day, which is less than 0.6 % of the intake.

The excretion via faeces is considered negligible and only the fraction excreted via urine (26% of the dose administered) will be considered in the PEC refinement. The outcome of the new PEC calculations considering this refinement based on metabolism is presented in Table 6.

Table 6: PEC refined in soil, ground water and surface water of carvacrol, considering a refinement based on metabolism

PEC in soil (μg/kg)	PEC in ground water (μg/L)	PEC in surface water (μg/L)
1046	20	7

PEC: predicted environmental concentration.

3.2.4.2.3.2. Refinement based on degradation in soil

Refinement of PEC_{soil} based on soil degradation data is possible when is realistic to assume that manure is spread in more than one application. It is reasonable to assume that manure would be applied at least twice per year on arable soils. Considering the soil DT_{50} of 30 days and a time interval of 6 months between the two applications, the resulting PEC_{soil} is 531 μg/kg.

3.2.4.2.3.3. $PEC_{groundwater}$ refined with metamodel

According to the applicable EFSA guidance, a metamodel may be applied to estimate leaching concentrations without running a FOCUS scenario (EFSA, 2008b).[50] This metamodel is based on a relation between K_{om} and DT_{50}. Considering a DT_{50} of 30 days and a K_{oc} of 506 dm^3/kg (corresponding to a K_{om} of 294 dm^3/kg), the expected concentration of carvacrol in groundwater is expected to be in the range of 0.01 μg/L. Therefore, no concerns for groundwater are expected from the use of this additive.

3.2.4.2.3.4. PECs for assessment in Phase II

The PEC values reported in Table 7 are used for risk assessment.

Table 7: PEC refined in soil and surface water and sediment of carvacrol in feed for piglets

PEC in soil (μg/kg)	PEC in surface water (μg/L)	PEC sediment(μg/kg)
531	7	805

PEC: predicted environmental concentration.

3.2.4.2.4. Ecotoxicity studies

3.2.4.2.4.1. Terrestrial compartment

3.2.4.2.4.1.1. Toxicity to microorganisms

The potential effects of carvacrol on soil microflora under aerobic conditions were investigated in a GLP study conducted according to the OECD Guideline 216 (Nitrogen Transformation Test).[51] The test item was dissolved in acetone and applied to fine quartz sand. After complete evaporation of the solvent, the test item was mixed into sieved field soil (Agricultural Research and research institute (LUFA) Speyer standard soil type 2.3 amended with powdered lucerne meal) at a concentration of 5.0 g/kg soil dry weight (28 days, 18.5−20.8°C, in the dark). Six test item concentrations (ranging from 1.26 to 500 mg/kg soil dry weight (dw)), a solvent control and an untreated control were tested in four replicates. Since the maximum variation in the control was below 15 %, the validity criterion was fulfilled.

The results of this study indicate that even 10 × the PEC did not result in ≥25 % difference between nitrate concentrations in control and treated soil samples.

3.2.4.2.4.1.2. Terrestrial plants

A GLP-compliant study following the OECD Guideline 208 was performed to investigate the effect of carvacrol on terrestrial plants.[52] Seeds of two monocotyledonous species (*Allium cepa* (onion) and *Avena sativa* (oat)), as well as four dicotyledonous species (*Brassica napus* (rape), *Cucumis sativus* (cucumber), *Pisum sativum* (pea) and *Solanum lycopersicum* (tomato)) were planted in a natural sandy loam soil (standard soil LUFA Sp 2.3) immediately after test item application (test duration: 14 days following 50 % emergence of the control plants). Test item concentrations (five) ranged from 12.6 to 500 mg /kg soil dw for all species. The measured concentrations of carvacrol in the stock solutions ranged between 85 % (*A. sativa, B. napus, C. sativus* and *S. lycopersicum*) and 109 % (*A. cepa*) of the nominal concentrations. The validity criteria were fulfilled (seedling emergence in the control ≥ 70.8 %; seedling survival in the control = 100 % for all species). Seedling emergence was significantly reduced at test item concentrations of 500 mg/kg soil dw in *A. sativa, B. napus, P. sativum* and *S. lycopersicum*. Plants growing in treated soil did not differ in their visual appearance from plants growing in non-treated control soil and did not exhibit any visible test item related damages except for *A. cepa, B. napus* and *C. sativus*. Typical damages of these seedlings were chlorosis (*B. napus* and *C. sativus* when treated with 32 mg test item/kg soild dw) and deformation (*A. cepa*, when treated with 500 mg test item/kg soil dw). Shoot fresh weight was significantly reduced at test item concentrations of 500 mg/kg soil dw in *A. cepa, B. napus, C. sativus, P. sativum*, at ≥ 200 mg/kg soil dw in *A. sativa,* and at ≥ 80 mg/kg soil dw in *S. lycopersicum*. The lowest no-observed-effect concentration (NOEC) and the lowest 50 % effect concentration (EC_{50}) were 32 mg/kg soil dw and 103.2 mg/kg soil dw, respectively, and were observed for shoot fresh weight of *S. lycopersicum*.

3.2.4.2.4.1.3. Earthworm acute test

The acute toxicity of carvacrol (14 days) to the earthworm *Eisenia fetida* was assessed in a GLP study conducted in accordance with the OECD Guideline 207.[53] Carvacrol was tested at five concentrations (15.4, 27.8, 50, 90 and 162 mg/kg of soil dry weight) with four replicates (ten worms per replicate) for the test item and four replicates for each control group (solvent treated, water treated). No mortality occurred in the solvent control and, as such, the study is considered valid. After 14 days, there were no effects on behaviour or morphology of the earthworms. No mortality was observed in the treatments up to 50.0 mg test item/kg soil dw. At the concentrations of 90.0 and 162.0 mg test item/kg soil (dw) mortality of 60.0 and 100.0 % was observed, respectively. The calculated LC_{50} value was 86.8 mg test item/kg soil dw.

[51] Technical Dossier/Supplementary Information (April 2019)/Section III.
[52] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_33.
[53] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_35.

3.2.4.2.4.2. Freshwater compartment

3.2.4.2.4.2.1. Effect on algae

The effect of carvacrol on the green algal species *Raphidocelis subcapitata* was investigated in a 72 h GLP study following the OECD Guideline 201.[54] Algal organisms were exposed to carvacrol under static conditions. During the test, carvacrol was stable in the treated solutions, the measured concentrations ranged from 97 to 104 % of the nominal concentrations. Therefore, the endpoints were expressed based on nominal concentrations. Five test concentrations (ranging from 1.0 to 16.0 mg carvacrol/L) and an untreated control were tested, with three replicates per tested concentration and six replicates in the control. The test met the validity criteria (mean biomass increase in the control cultures factor of at least 16 within the 72-hour test period; mean coefficient of variation for section-by-section specific growth rates in the control cultures up to 35 %; coefficient of variation of average specific growth rates during test period in replicate control cultures up to 7 %). The results showed an inhibitory effect on the growth rate at 72 h E_rC_{50} of 8.92 mg/L and E_rC_{10} of 2.67 mg/L.

3.2.4.2.4.2.2. Effect on crustaceans

The acute toxicity of carvacrol to the crustacean species *Daphnia magna* was investigated in a 48-hour static test in accordance with the OECD Guideline 202.[55] The measured concentrations of carvacrol throughout the whole exposure period ranged from 92 to 104 % of the nominal concentrations. Therefore, the endpoints were expressed based on nominal concentrations. Five test concentrations (ranging from 1.0 to 16.0 mg carvacrol/L) and an untreated control were tested with four replicates (five daphnids per replicate) per treatment. The validity criteria were fulfilled (immobilised daphnids in the control $<$ 10 %; dissolved oxygen concentration in control and test vessels at the end of the test $\geq$ 3 mg/L). The acute effect concentration of carvacrol was determined as 48-h EC_{50} with the value of 8.99 mg/L.

The 21-day chronic exposure to carvacrol in *D. magna* was investigated in a semi-static test following the OECD Guideline 211.[56] Daphnids of less than 24 hours old were used and the measured endpoints were reproduction, survival of parental daphnids and length of parental daphnids. The time weighted measured concentrations of carvacrol throughout the whole exposure period were $>$ 90 % of the nominal concentrations. Therefore, the endpoints were expressed based on nominal concentrations. Five concentrations of carvacrol (ranging from 0.1 to 1.6 mg carvacrol/L) and an untreated control were tested with four replicates per treatment and six replicates for the control. At the highest concentration of exposure (1.6 mg/L) 50 % survival was recorded; however, no mortality was recorded at the lower concentrations. After 21 days, reproduction was reduced at exposure concentration of 1.6 mg/L (the percent of decrease was 67 %). In addition, at 1.6 mg/L, the length of daphnids was decreased, by 14.2 %. However, even if the reduction in length at 1.6 mg/L was statistically significant, the small decrease is not considered to have any ecological consequences. The lack of biological significance is also supported by the absence of effects on the other considered endpoints. The validity criteria were fulfilled (the mortality of the parental daphnids in the controls $\leq$ 20 %; the mean number of live offspring produced per parent animal in the controls survived at the end of the test $\geq$ 60). The chronic effect concentration of carvacrol was determined as 21-day EC_{10} (based on reproduction) with the value of 0.843 mg/L.

3.2.4.2.4.2.3. Effects on fish

The acute toxicity of carvacrol on rainbow trout *Oncorhynchus mykiss* was investigated in a study following OECD Guideline 203.[57] Since not all of the test concentrations were stable within the requested range of 80−120 % throughout the exposure period, results are related to the geometric mean measured test item concentrations in mg/L of carvacrol. In the 96-h static test five concentrations (ranging from 1.12 to 12.8 mg carvacrol/L) and an untreated control were tested in one replicate (seven fish per replicate). The validity criteria were fulfilled (mortality in the control $<$ 10 % or one out of seven fish; dissolved oxygen concentration in control and test vessels $\geq$ 60 % of air saturation value). The acute effect concentration of carvacrol was determined as 96-h LC_{50} with the value of 2.18 mg/L.

[54] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_31.
[55] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_30.
[56] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_37.
[57] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_32.

3.2.4.2.4.2.4. Effect on sediment dwelling organisms

The toxicity of carvacrol to *Chironomus riparius* was investigated in a GLP study using spiked sediment in accordance to OECD Guideline 218.[58] First instar larvae were exposed to five test concentrations (nominal) ranging from 5.0 to 80 mg/kg dry sediment, solvent control and an untreated control were tested in static test. Test concentrations were tested in four replicates, solvent control in six and control in four replicates. The pH of the overlying water varied between 7.4 and 7.9. The validity criteria were fulfilled (mean emergence in the control > 70 %; mean emergence in the solvent control > 70 %; emergence of adults in the controls between 14 and 25 days after addition to the test vessels; dissolved oxygen concentration in all test vessels at the end of the test > 60 % of the air saturation value; pH of the overlying water in all test vessels at the end of the test ranged from 6 to 9; water temperature difference not more than ± 1.0°C throughout the test).

No concentration–response relationship was observed for the emergence ratio after 28 days of exposure, and for the development rate. Therefore, an EC_{10} could not be determined and NOEC values were determined based on statistical evaluation of results based on measured concentrations. The measured initial concentrations were 37.9 % of nominal. The highest tested concentration of 80 mg/kg dry sediment corresponds to 30.34 mg/kg dry sediment. The NOEC was > 30.34 mg/kg dry sediment based on both emergence and development rate.

3.2.4.2.4.3. Conclusion on effect assessment for the soil, surface water and sediment compartment

For the terrestrial compartment, data are available for micro-organisms, earthworms and plants. Toxic concentrations resulted in EC_{50} of 86.8 mg/kg of dry soil for earthworms and in NOEC of 32 mg/kg of dry soil for plants. For the aquatic compartment, data are available for algae, aquatic invertebrates and fish. The lowest toxicity value of 21-day EC_{10} of 0.843 mg/L for the aquatic compartment was found in a study on the long-term effect on daphnids. Ecotoxicological data for sediment-dwelling invertebrate *Chironomus riparius* is provided for the sediment compartment resulting in NOEC > 30.34 mg/kg.

3.2.4.3. Risk characterisation for terrestrial and aquatic species

In the following tables (Tables 8, 9 and 10) the risk characterisation for terrestrial and aquatic species is proposed.

Table 8: Risk characterisation (PEC/PNEC ratio) of carvacrol for terrestrial compartment

Taxa	PEC_{soil} (µg/kg)	LC_{50} / NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Earthworm	531	86.8*	100	868	0.6
Plants		32**	10	3,200	0.2

PEC_{soil}: predicted environmental concentrations in soil; LC_{50}: lethal concentration, median; NOEC: no-observed-effect concentration. AF: Assessment factor; PNEC: Predicted No Effect Concentration.
*: LC_{50}.
**: NOEC.

Table 9: Risk characterisation (PEC/PNEC ratio) of carvacrol for freshwater compartment

Taxa	$PEC_{surfacewater}$ (µg/L)	E(L)C_{50}/EC_{10} (mg/L)	AF	PNEC (µg/L)	PEC/PNEC
Algae *Raphidocelis subcapitata*	7	2.67* 8.92**	50	16.9	0.4
Aquatic invertebrates *Daphnia magna*		0.843* 8.99**			
Fish *Brachydanio rerio*		2.18***			

PEC_{soil}: predicted environmental concentrations in soil; LC_{50}: lethal concentration, median; EC_{50}: 50% effect concentration; EC_{10}: 10% effect concentration; NOEC: no-observed-effect concentration; AF: Assessment factor; PNEC: Predicted No Effect Concentration.
*: E_rC_{10}.
**: E(L)C_{50}.
***: LC_{50}.

[58] Technical Dossier/Supplementary Information (April 2019)/Section III/Annex III_36.

TABLE 10: Risk characterisation (PEC/PNEC ratio) of carvacrol for sediment-dwelling invertebrates

Taxa	$PEC_{sediment}$ (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Chironomus riparius	805	> 30.3	10	3,030	0.3

PEC_{soil}: predicted environmental concentrations in soil; LC_{50}: lethal concentration, median; NOEC: no-observed-effect concentration; AF: Assessment factor; PNEC: Predicted No Effect Concentration.

3.2.4.3.1. Bioaccumulation and risk for secondary poisoning

In order to assess bioaccumulation and the risk for secondary poisoning, the method proposed in the relevant Guidance from the European Medicines Agency (EMA) has been considered (EMA, 2015). Based on log K_{ow} of 3.49 (data from PUBCHEM) carvacrol has a potential for bioaccumulation in the food chain. The data provided based on QSAR of the compound showed that the fish bioconcentration factor (BCF; 185 L/kg)[59] is higher than the threshold for bioaccumulation potential (BCF ≥100 L/kg) (ECHA, 2017). However, the fish BCF of carvacrol calculated with EPI Suite model is below the threshold (93.3 L/kg). Besides this, 74 % of carvacrol is metabolised or degraded in the digestive tract, and we can expect that the experimental fish BCF would be in fact lower than predicted with QSAR models. Beside this, applicant provided evidence, that for similar substance, thymol, experimental values for fish BCF are between 7.8 and 48 L/kg.[60]

Based on the above presented facts, the FEEDAP Panel can conclude that bioaccumulation potential for carvacrol is low and risk for secondary poisoning for worm/fish eating birds and mammals is not likely to occur.

3.2.4.4. Conclusions on safety for the environment

The FEEDAP Panel concluded that Nimicoat® does not pose any risk to the terrestrial and aquatic compartments. Bioaccumulation potential for carvacrol is low and risk for secondary poisoning for worm/fish eating birds and mammals is not likely to occur.

3.2.5. Efficacy

Three efficacy studies were provided by the applicant to examine the effects of the additive at the lowest supplementation level of 250 mg Nimicoat®/kg complete feed on the growth performance of weaned piglets. From these three studies, one of them showed a high percentage of diarrhoea in the control group (80 %), including a 3.3 % of dead piglets, being these animals treated with antibiotic. In another study there was a high incidence of diarrhoea in the pre-starter phase – veterinary treatments not reported – and a high mortality of the piglets was reported during the study (not treatment related; group means ranging from 6 to 11 %, being more than 9 % in three experimental groups). Owing to these reasons, these studies were not further considered in the efficacy assessment. In addition, the tolerance study was assessed for efficacy, although only diets supplemented with Nimicoat® at the highest recommended level (1,000 mg/kg complete feed) were used.

The FEEDAP Panel further notes that the applicant did not provide any study to support the proposal of to add Nimicoat® to compound feed at the maximum of 1,000 mg/kg of feedingstuffs during the first weeks after weaning to a maximum of 3 weeks, and at the minimum of 250 mg/kg thereafter for a minimum of 4 weeks.

3.2.5.1. Study 1

In total, 300 weaned female piglets of 26 days-of-age (crossbreed PIC Large White x Landrace, average body weight 6.65 ± 1.32 kg) were used in this experiment.[61] The gilts were assigned to one of the three experimental groups based on body weight to have homogeneous replicates. The experimental set up was a randomised block design with three dietary treatments: control (non-supplemented diet), supplementation with Nimicoat® at 250 or 1,000 mg/kg complete feed. Concentrations of carvacrol were analytically tested; analysis of feed for the active compound suggested that recovery was about 15% and 30% lower than the intended values in the phase I and phase II diets, respectively. There were ten replicates (pens) per treatment with ten piglets per replicate. Diets were composed of maize, barley and soybean meal and were fed as mash *ad libitum* at

[59] Technical Dossier/Supplementary Information (September 2019).
[60] Technical Dossier/Supplementary Information (January 2020).
[61] Technical Dossier/Supplementary Information (April 2019)/Section IV/Annex IV_5a and IV_5b.

phase I (1–28 days; 17 % CP, 1.30 % Lys and 10.01 MJ NE/kg) and phase II (29–56 days; 16 % CP, 1.15 % Lys and 10.1 MJ NE/kg).

General health of the animals and mortality/culling were monitored daily. Piglets were weighed individually at 1, 28 and 56 days of the trial; weight gain and feed intake were measured and feed to gain ratio was calculated per replicate (pen) at 28 and 56 days of the trial. Data were subjected to analysis of variance (ANOVA) with the pen as the experimental unit; treatments were compared using the Tukey's test and statistical significance was declared at $p < 0.05$.

There was no mortality of piglets during the whole trial period.[50] There were no differences among treatments in feed intake, final weight, total weight gain at 56 days or average daily body weight gain. Feed to gain ratio was better in the groups receiving the additive at 250 and 1,000 mg/kg complete feed (Table 11).

3.2.5.2. Study 2

The tolerance study (see Section 3.2.1.) has been also submitted to demonstrate the efficacy of the additive on the zootechnical performance of weaned piglets. In this study, only the supplementation level of 1,000 mg Nimicoat®/kg diet was tested.

However, the results of the study showed that there were not significant differences for average final bw, ADG, ADFI and feed to gain ratio between control and the Nimicoat® fed group (Table 11).

Table 11: Effects of Nimicoat® on the performance of weaned piglets from the two studies described in the efficacy section (Duration: Study 1, 56 days; Study 2, 42 days)

Study	Nimicoat® mg/kg feed	Body weight	Daily weight gain (g/day)	Daily feed intake (g/day)	Feed to gain ratio
1	0	25.7	365	800	2.20[a]
	250	27.1	396	786	1.99[b]
	1,000	27.0	400	796	1.99[b]
2	0	22.2	334	626	1.89
	1,000	23.7	370	650	1.76

a,b: For a given study, means within a column with different superscript letters are significantly different ($p \leq 0.05$).

3.2.5.3. Conclusions on efficacy

Efficacy of the additive for piglets was shown only in one study with diets supplemented at the minimum recommended dose of 250 mg Nimicoat®/kg complete feed, corresponding to 100 mg carvacrol/kg complete feed. With the available data the FEEDAP Panel is not in a position to conclude on the efficacy of Nimicoat®.

3.3. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[62] and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel concludes that the use of Nimicoat® in feed for weaned piglets at the maximum recommended dose of 1,000 mg/kg complete feed (corresponding to 400 mg carvacrol/kg complete feed) is safe for the target animals. A precise figure for the margin of safety cannot be defined.

Carvacrol was shown to be not genotoxic. The available studies indicate that any carvacrol residue in edible tissues would be below the measurable levels, when the additive is used at the maximum intended dose. Consequently, and considering that carvacrol is authorised for food, the use of the additive in animal nutrition is considered safe for consumers of animal products under the proposed conditions of use.

The additive is corrosive to eyes, skin and the respiratory mucosae.

[62] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

The additive does not pose any risk to the terrestrial and aquatic compartments. Bioaccumulation potential for carvacrol is low and risk for secondary poisoning for worm/fish eating birds and mammals is not likely to occur.

The FEEDAP Panel is not in a position to conclude on the efficacy of Nimicoat® for weaned piglets.

Documentation provided to EFSA/Chronology

Date	Event
04/01/2016	Dossier received by EFSA. Nimicoat® (Carvacrol) for piglets (weaned). Submitted by Techna France Nutrition
19/01/2016	Reception mandate from the European Commission
30/05/2016	Application validated by EFSA – Start of the scientific assessment
22/06/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer, safety for the environment and efficacy*
30/08/2016	Comments received from Member States
02/08/2016	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
22/12/2016	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issue: Characterisation*
10/05/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
08/06/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the environment and efficacy*
07/07/2017	Clarification teleconference during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products"
10/04/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
21/06/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the environment and efficacy*
27/08/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
28/08/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issue: safety for the environment*
27/09/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
19/11/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the environment and efficacy*
21/11/2019	Clarification teleconference during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products"
23/01/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AF	Assessment Factor
ANOVA	analysis of variance
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CP	crude protein
dw	dry weight
EC_{50}	50 % effect concentration
ECHA	European Chemical Agency
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	EU Flavour Information System
GC-FID	gas chromatography coupled with flame ionisation detection
GLM	generalized linear model
GLP	good laboratory practice
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC_{50}	lethal concentration, median
LOD	limit of detection
LOQ	limit of quantification
LSC	liquid scintillation counting
Lys	lysine
NOEC	no-observed-effect concentration
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzo-*p*-dioxins and dibenzofurans
PEC	predicted environmental concentration
PEC_{gw}	predicted environmental concentrations in ground water
PEC_{manure}	predicted environmental concentrations in manure
PEC_{soil}	predicted environmental concentrations in soil
PNEC	Predicted No Effect Concentration
QSAR	quantitative structure–activity relationship
RH	relative humidity
RSDip	relative standard deviation for *intermediate precision*
RSDr	relative standard deviation for *repeatability*
TEQ	toxic equivalent
ThOD	theoretical oxygen demand
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 Carvacrol

In the current application authorisation is sought under article 4(1) for *Carvacrol* under the category/functional group 4(d) 'zootechnical additives'/'other zootechnical additives' according to the classification system of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for weaned piglets. The *feed additive* is to be marketed as a colourless solid product ("*Nimicoat*") consisting of 40 to 42 % *Carvacrol* (active substance) and a carrier containing fatty acids, silica and a surfactant. The *feed additive* is intended to be incorporated directly into *feedingstuffs* (not through *premixtures*) to obtain a dosage ranging from 250 to 1000 mg "*Nimicoat*"/kg complete *feedingstuffs,* which is equivalent to a *Carvacrol* content ranging from 100 to 420 mg/kg *feedingstuffs*.

For the quantification of *Carvacrol* in the *feed additive* and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on gas chromatography coupled with flame ionisation detection (GC-FID), and reported the following performance characteristics: - a relative standard deviation for *repeatability* (RSDr) ranging from 0.8 to 3.8 %; - a relative standard deviation for *intermediate precision* (RSDip) ranging from 1.9 to 9.6 %; - a *recovery* rate (Rrec) ranging from 78 to 108 %; and - a limit of quantification (LOQ) of 19 mg *Carvacrol*/kg *feedingstuffs*. Based on the experimental evidence available the EURL recommends for the official control this GC-FID method for the quantification of *Carvacrol* in the *feed additive* 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) is not considered necessary.