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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of phenylcapsaicin (aXiphen) for chickens for fattening (aXichem AB)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of phenylcapsaicin (aXiphen®) as a zootechnical additive (functional group: physiological condition stabilisers) in feed for chickens for fattening. The additive under assessment, phenylcapsaicin, is safe for chickens for fattening up to the maximum proposed use level of 15 mg/kg complete feed. A margin of safety could not be established. Phenylcapsaicin is not genotoxic. The reference point for phenylcapsaicin derived from a 90-day repeated dose oral toxicity study in rats is 37.2 mg/kg body weight (bw) per day, the lowest of the model averaged $BMDL_{20}$ values for alanine aminotransferase increase in plasma. The metabolic similarity in the laboratory animals and the target species was not demonstrated and the identity of the marker residue could not be established. In the absence of such data, the safety for the consumers could not be evaluated. The inhalation exposure of phenylcapsaicin (as liquid) for the user was considered unlikely. The FEEDAP Panel considered the additive irritant to the eyes but not to the skin and it is not a dermal sensitiser. In the absence of appropriate data, the environmental risk assessment for phenylcapsaicin could not be performed. It is unlikely that phenylcapsaicin bioaccumulates in the environment and the risk of secondary poisoning is considered low. The FEEDAP Panel could not conclude on the efficacy of the additive in chickens for fattening at the proposed conditions of use.

KEYWORDS

aXiphen, efficacy, phenylcapsaicin, physiological condition stabilisers, safety, zootechnical additives

CONTENTS

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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from aXichem AB[2] for the authorisation of the additive consisting of phenylcapsaicin (aXiphen®), when used as a feed additive for chickens for fattening (category: zootechnical; functional group: physiological condition stabilisers).

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 20 of September 2022.

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, consumers, user and the environment and on the efficacy of the feed additive consisting of phenylcapsaicin (aXiphen®), when used under the proposed conditions of use (see **Section 3.1.6**).

1.2 | Additional information

The additive, which consists of phenylcapsaicin, has not been previously authorised as a feed additive in the European Union.

In 2019, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) adopted an opinion on the Safety of phenylcapsaicin as a novel food pursuant to Regulation (EU) 2015/2283 (EFSA NDA Panel, 2019). Phenylcapsaicin is currently authorised up to a maximum level of 2.5 mg/day as novel food (NF) in the EU.[3]

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[4] in support of the authorisation request for the use of phenylcapsaicin (aXiphen®) as a feed additive. The dossier was received on 21 November 2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00355.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 20 September to 20 December 2022 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[6] a non-confidential version of the dossier has been published on Open.EFSA.[7]

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[8] EFSA carried out a public consultation on the non-confidential version of the application from 25 September to 16 October 2022 for which no comments were received.

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

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

[2]aXichem AB; Södergatan 26, 21,134 Malmö, Sweden.

[3]Commission Implementing Regulation (EU) 2019/1976 of 25 November 2019 authorising the placing on the market of Phenylcapsaicin as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470 (Text with EEA relevance). OJ L 308, 29.11.2019, p. 40–43.

[4]Dossier reference: FEED-2022-4830.

[5]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[7]Available at: https://open.efsa.europa.eu/dossier/FEED-2022-4830

[8]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the phenylcapsaicin in animal feed.[9]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of phenylcapsaicin (aXiphen®) is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3 | ASSESSMENT

The additive aXiphen® consists of phenylcapsaicin and is intended to be used as a zootechnical additive (functional group: physiological condition stabilisers) in feed for chickens for fattening.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The applicant states that the feed additive consists of phenylcapsaicin (≥ 98%). Phenylcapsaicin is a chemical synthetic analogue of the naturally occurring capsaicin that is present in chillies (molecular formula: $C_{21}H_{23}NO_3$; chemical name: of *N*-[(4-hydroxy-3-methoxyphenyl)methyl]-7-phenylhept-6-ynamide; molecular weight: 337.41 g/mol, CAS number: 848127–67-3). The molecular structure of phenylcapsaicin is presented in Figure 1.

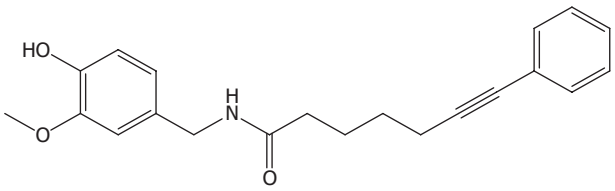

FIGURE 1 Molecular structure of phenylcapsaicin.

Proposed specifications for the feed additive are identical to phenylcapsaicin in the existing EU novel foods authorisation.[11] These specifications are ≥ 98% of phenylcapsaicin, ≤ 0.5% of moisture. Specifications are also set for impurities and contaminants as follows: ≤ 1 mg lead/kg, ≤ 0.5 mg cadmium/kg, ≤ 0.1 mg mercury/kg, ≤ 1.0 mg arsenic/kg, less than 10 colony forming unit (CFU) total plate count, yeasts and moulds or coliforms/g, absence of *Escherichia coli* and *Salmonella* in 10 g of sample, < 1% of total synthesis related production by-products, [redacted].[12]

Analytical data to confirm the specifications were provided for five batches of the additive showing the following average values:[13] 98.4% (98.1%–98.6%) of phenylcapsaicin and 0.14% (0.08%–0.23%) moisture.[14] The same batches of the additive were analysed for contaminants showing the following values: lead and cadmium were below the limit of quantification

[9]The full report is available on the EURL website: (https://joint-research-centre.ec.europa.eu/publications/feed-2022-3870-0_en) File name: finrep-feed-2022-4830-phenylcapsaicin.

[10]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.

[11]Commission Implementing Regulation (EU) No 2019/1976 (European Commission, 2019).

[12]The applicant stated that presence of total synthesis related production by-products and of other solvents should be according to the limits established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for residual solvents used in pharmaceutical products (ICH 2021).

[13]aXichem_Sect_II_Identification and characterisation Rev1 25 August 2022.

[14]Determined by high performance liquid chromatography. Based on international reference EP 2.26.46: Chromatographic Separation Techniques.

(LOQ),[15] while arsenic averaged 0.086 mg/kg (0.06–0.12 mg/kg), mercury averaged a value of 0.0047 mg/kg (ranging 0.0038–0.0056 mg/kg) in two of the batches whereas in the other three were below the LOQ.[15]

Impurities resulting from the production process were analysed in the same batches and averaged the following values: [redacted][16] [redacted] All the solvent impurity levels comply with the limits set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline (EMA/CHMP/ICH, 2021) for residual solvents used in pharmaceutical products.[17]

Microbiological contamination was analysed by determination of *Escherichia coli* and *Salmonella* spp. and were absent in 10 g of the additive, also the microbial analyses for total plate count, coliforms, yeasts and moulds were all below 10 CFU/g.

The analytical results demonstrated that the additive is produced in compliance with the proposed specifications.

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

To ease the inclusion of the additive in the diet, and to assure the expected concentration, the applicant states that the additive can be mixed with feed materials prior to the incorporation into the complete feed. [redacted] with hydrogenated glycerides from feed-grade fats/oils, [redacted][18]

3.1.2 | Manufacturing process

The additive is produced by chemical synthesis to reach a minimum concentration of 98% of phenylcapsaicin.[19] A complete description of the manufacturing process was provided by the applicant in the dossier.[20] [redacted]

3.1.3 | Physical properties of the additive

Phenylcapsaicin appears as a dark brown viscous liquid. It has a density of 1152 kg/m^3 (at 20°C), a pH of 6.12, a vapour pressure of 0.60 kPa (at 20°C).[21] The boiling point was defined to be at 231°C.

Phenylcapsaicin is insoluble[22] in water,[23] while it is freely soluble in dimethyl sulfoxide (DMSO), ethyl acetate and slightly soluble in *n*-heptane.[24] The *n*-octanol/water partition coefficient (K_{ow}) was reported to be 2.34.

It is noted that phenylcapsaicin can be solubilised into hydrogenated glycerides to obtain a solid [redacted] form. Particle size analysis of this product has been provided using laser diffraction method. Data showed an average geometric mean size of 391 μm with 0.6% of particles ≤ 100 μm and no particles ≤ 63 μm. The data provided from the applicant by laser diffraction are not suitable to conclude on the absence of (a fraction of) small particles including nanoparticles. However, the Panel considers that the solubility of phenylcapsaicin in lipids is sufficient to assure that any nano/small particles present in the formulation given in feed to target animals is expected to partition within the lipophilic cellular fraction after cellular internalisation in the gastrointestinal tract (GIT). Any absorbed phenylcapsaicin would be present in tissues and organs of the animals fully dissolved, thereby excluding any potential exposure of human consumers to nano/small particles.

3.1.4 | Shelf-life

The applicant sets the shelf-life of phenylcapsaicin at 2 years from the manufacturing date under the recommended storage conditions (well-closed, light resistant container under dry conditions at temperatures below 0°C).

[15] Limit of quantification (LOQ): lead = 0.05 mg/kg, cadmium = 0.01 mg/kg for cadmium and 0.003 mg/kg for mercury.

[16] Sum of 8 identified by-products; limits of quantification for each by-product was 0.05%.

[17] The Guideline for residual solvents Q3C(R8) suggests the following limits for the impurities mentioned above: [redacted]

[18] aXichem_Sect_II_Manufacturing Process.

[19] aXichem_Sect_II_Identification and characterisation Rev1 25 August 2022.

[20] aXichem_Sect_II_Manufacturing Process.

[21] Calculated according to the following equation: specific weight = density × acceleration of gravity (9.81 m/s^2).

[22] For solubility terms, see Table 2. of the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA SC, 2021).

[23] The solubility in water was reported to be 0.00193 g/L. Technical dossier/Section II/Annex_8.1.

[24] Based on results from solubility test: 200 g/L in DMSO, 167 g/L in ethyl acetate and < 10 g/L in *n*-heptane. Technical dossier/Section II/Annex_8.3 and 8.4.

The shelf-life of the feed additive (≥98% of phenylcapsaicin) was assessed in five batches stored at 25 ± 2°C, 60 ± 3% relative humidity (RH) in tightly sealed containers kept in dark conditions for 24 months. Phenylcapsaicin content after 24 months storage ranged between 97.95% and 98.31%. Shelf-life was also studied in five batches of phenylcapsaicin (98%) stored at 40 ± 2°C/75 ± 2% RH for 6 months. Phenylcapsaicin content after 0, 3 and 6 months storage was on average 98.5%, 98.3% and 98.1%, respectively.

3.1.5 | Stability and homogeneity

No data was provided on the stability and homogeneity of the additive; to study the stability and homogeneity of the additive in feed, the applicant used [redacted] phenylcapsaicin (either, 1% or 1.5% phenylcapsaicin) described in Section 3.1.1.[25]

The stability of [redacted] phenylcapsaicin in feed (four batches; two as 1% and two as 1.5% formulations) was assessed in pelleted feed for chickens for fattening (pelleted at 70°C) supplemented to achieve 15 mg phenylcapsaicin/kg complete feed. Feed samples were stored for a 6-month period at 25 ± 2°C and 60 ± 2% RH in tightly sealed packages and dark conditions. The recovery of phenylcapsaicin relative to the initial concentration of the active substance led to 98.5% (98.0%–99.3%) and 96.9% (96.1%–98.1%), for 3 and 6 months, respectively.[26] No information was provided on the potential effect of feed processing on the stability of the additive.

The homogeneous distribution of the [redacted] phenylcapsaicin in feed was studied in 12 subsamples of one batch of the above feeds supplemented with [redacted] phenylcapsaicin (1% phenylcapsaicin). The coefficient of variation was 1.94%.

3.1.6 | Conditions of use

The additive is intended for use as a zootechnical additive in feed for chickens for fattening at a minimum content of 10 mg phenylcapsaicin/kg complete feed and a maximum content of 15 mg phenylcapsaicin/kg complete feed.

3.2 | Safety

3.2.1 | Safety for the target species

To support the safety of the additive the applicant provided a tolerance study in chickens for fattening in which the additive was tested as [redacted] phenylcapsaicin. The FEEDAP Panel considers that the [redacted]) of feed-grade quality will not add any safety concern and would not significantly modify the bioavailability of the additive and, therefore, the test item used is representative of the additive under assessment.

A total [redacted] 1-day-old male chickens (Ross 308) were distributed to 32 pens and randomly allocated to four treatment groups [redacted].[27] The study followed a two-phase feeding programme [redacted] both based on wheat, soyabean meal and maize. The diets were either not supplemented (control) or supplemented with the additive to provide 10 (0.67× maximum recommended level), 15 (1× maximum recommended level) or 150 (10× maximum recommended level) mg of phenylcapsaicin/kg feed (confirmed by analysis[28]). Feed and water were offered ad libitum, with starter and grower diets offered as pellets.

General health status of birds, mortality and litter quality were checked twice daily. Birds' weight and feed consumption were determined at weekly intervals and average daily weight gain, daily feed intake and feed to gain ratio were calculated. Litter quality was assessed (scale: 1 – wet to 10 – very dry) by three different assessors at days 14, 27 and 34 of the study. Foot pad lesions (Berg, 1998) and their severity (i.e. scale: 0, 1, 2) were determined in all birds in the pen at 34 days of age. Blood samples for haematology[29] and clinical biochemistry[30] were taken on day 35 from two birds per pen (birds close to the average weight in each pen). The same chickens were killed and necropsied.[31]

[25]aXichem_Sect_II__Physico-Chemical Technological Properties.

[26]aXichem_Sect_II__Physico-Chemical Technological Properties.

[27]Annex III-3.2 - Report efficacy and tolerance Phenylcapsaicin.

[28]Analysed phenylcapsaicin in the starter feeds were (control group not detected) were 8.8, 13.8 and 143.3 mg/kg complete feed for the 0.67×, 1× and 10× groups, respectively. Analysed phenylcapsaicin in the grower feeds were (control group not detected) were 8.6, 13.4 and 140.0 mg/kg complete feed for 0.67×, 1× and 10× groups, respectively (see Table 3).

[29]Packed cell volume (haematocrit), haemoglobin, mean corpuscular haemoglobin concentration (MCHC), and total and differential counts for leukocytes.

[30]Sodium, potassium, chloride, calcium, phosphate, magnesium, total protein, albumin, globulin, glucose, urea, cholesterol, creatinine, triglycerides, glutamate dehydrogenase, haemolyse-index, bilirubin, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, gammaglutamyltransferase, alkaline phosphatase and creatine kinase concentration.

[31]Liver, kidneys, spleen, lung, stomach, small intestine, colon, cecum, heart, pancreas, adrenal gland, thymus and thyroid gland.

The pen was the experimental unit for statistical purposes. The data were subject to analysis of variance (ANOVA) with treatment as a main factor and room and block (location within room) as random effects. Statistical significance was set at 0.05. Group means were assessed using the Fisher's unprotected least significant difference (LSD) test.

Mortality in all phenylcapsaicin treated groups was lower than that observed in the control group (Table 4; Section 3.3). No effects were observed in any of the performance parameters, except for a higher feed to gain ratio in the 10× group compared to the others, which is considered an adverse effect. In addition, litter quality and foot pad scores was not affected by treatment.

Most of the haematology and chemistry blood parameters under evaluation were unaffected by the use of phenylcapsaicin. The mean corpuscular haemoglobin concentration (MCHC) was 16.1, 15.0, 15.2 and 15.5 mmol/L for the control, 0.67×, 1× and 10× groups, respectively, being statistically lower in the 0.67× and 1× groups relative to the control group, but similar for the 10× group. Total protein in blood resulted in values of 32, 30, 31 and 30 g/L, sodium concentrations in blood were 153, 152, 152 and 151 mmol/L and creatine concentration in blood were 44.9, 48.0, 38.3 and 31.2 µmol/L for the control, 0.67×, 1× and 10× groups, respectively. Compared to the control group, phenylcapsaicin reduced total protein in blood in the 0.67× and 10× groups and sodium and creatinine only in the 10× group. All these variations were within the physiological ranges in birds and do not represent any safety concern. However, the Panel noted that some blood parameters were not analysed (i.e. thrombocytes, prothrombin, fibrinogen, acute phase proteins). No major macroscopic observations were observed in the necropsied birds at 35 days of age.

3.2.1.1 | *Conclusions on safety for the target species*

The additive under assessment, phenylcapsaicin, is safe for chickens for fattening up to the maximum proposed use level of 15 mg/kg complete feed. A margin of safety could not be identified.

3.2.2 | Safety for the consumer

3.2.2.1 | *Absorption, distribution, metabolism and excretion (ADME) and residues*

ADME

Two comparative ADME studies of phenylcapsaicin and capsaicin in rats were submitted.[32] These studies were already assessed by the EFSA NDA Panel in its opinion on the use of phenylcapsaicin as a novel food (NF) (EFSA NDA Panel, 2019) and the main conclusions were that: (1) both compounds are extensively absorbed, metabolised in the liver, with major metabolites being excreted through the bile and eliminated in the faeces; (2) phenylcapsaicin is not metabolised to capsaicin and the metabolic fate of the two compounds is different: among the numerous metabolites separated and tentatively identified, most phenylcapsaicin metabolites harbour the phenyl-structure (7-phenylhept-6-ynamide); the hydrolysis of the amide bond generates a limited number of identical metabolites related to the common chemical structure part of the molecules of both compounds; the dominant metabolic pathway for phenylcapsaicin is oxygenation and glucuronidation, and glucuronidation for capsaicin; (3) excretion is rapid and no accumulation is noted in tissues; the incomplete metabolic balance of the radioactivity attached to the amide carbon indicates that an incorporation of the label in biological components (e.g. fatty acids) and an exhalation of radioactive carbon dioxide (not measured) is likely. The FEEDAP Panel evaluated the studies and endorsed the above conclusions.

In the current dossier, no experimental studies were submitted to investigate the ADME in the target species (chickens for fattening). To support the metabolic similarity of phenylcapsaicin between the laboratory animals and the target species the applicant made reference to literature data and submitted an *in silico* analysis.[33]

The FEEDAP Panel noted that the data retrieved from the literature (Chaiyasit et al., 2009; Rollyson et al., 2014; Suresh & Srinivasan, 2010; Surh & Lee, 1995) were related to the metabolism of capsaicin in the rats and, therefore, of limited value for the current assessment.

An *in silico* analysis aimed at demonstrating the phenylcapsaicin metabolic similarity among rat, chicken (*Gallus gallus*), and human was carried out. The degree of shared sequence homology of some CYPs enzymes (1A1, 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4) involved in the oxidation of capsaicin in humans was evaluated (Reilly & Yost, 2006). Comparison of the genomics of CYP P450 enzymes in the human/rat and rat/chicken (*Gallus gallus*) was done. The FEEDAP Panel concludes that the study results support the existence of genes encoding for CYP enzymes in chickens (*Gallus gallus*) that were proved to metabolise capsaicin in humans. However, it was noted that this commonality does not imply these genes are expressed and functional in the three species considered. In addition, comparable data were not available for phenylcapsaicin.

[32]Annex III-6 Tissue distribution capsaicin 2012a and Annex III-7 Tissue distribution phenylcapsaicin 2012b.

[33]Human_CYP450_against_gallus_gallus-HitTable, Human_CYP450_against_rattus-HitTable, Human_CYP450_against_rattus-Alignment and Human_CYP450_against_gallus_gallus-Alignment.

Residues

The applicant assumed that phenylcapsaicin is the marker residue in the tissues of chickens for fattening and measured its content at the end of the experimental period of the combined tolerance/efficacy study.[34] Tissue samples (including liver, abdominal fat and breast muscle) were collected from two birds per treatment group (0, 10 and 15 mg/kg complete feed) and the phenylcapsaicin content in the samples was analysed using an HPLC/MS–MS method.[35] Phenylcapsaicin concentration was low at the proposed conditions of use of the additive, the liver being the tissue with higher concentration (7.09 μg/kg) for the highest dose.[36] The reliability of these results is questionable since the requirements for a marker residue study, as indicated in the FEEDAP guidance on the safety of the additives for the consumer (EFSA FEEDAP Panel, 2017a), are not fulfilled (e.g. limited number of animals, lack of data for kidney).

3.2.2.2 | *Toxicological studies*

For the present evaluation, the applicant submitted two genotoxicity tests (a bacterial reverse mutation test,[37] an in vitro mammalian cell micronucleus test[38]) and a 90-day repeated-dose oral toxicity study in rats[39] that were already evaluated by the NDA Panel in 2019 (EFSA NDA Panel, 2019). In its assessment, the EFSA NDA Panel concluded as follows: 'The Panel considers that there is no concern with respect to genotoxicity of the NF. The applicant provided a 90-day study where there were several changes related to effects in the gastrointestinal tract and the liver. The Panel considers both the effects observed as critical effects related to the compound. The RP (reference point – as mentioned in the NDA opinion) derived based on the critical effects of phenylcapsaicin lowest of the model averaged $BMDL_{20}$ values for the increase in plasma alanine aminotransferase (ALAT) levels was 37.2 mg/kg body weight (bw) per day (females).' The FEEDAP Panel evaluated the studies and endorsed the above conclusions.

3.2.2.3 | *Conclusion on safety for the consumer*

The Panel considers that there is no concern with respect to genotoxicity of phenylcapsaicin. The reference point for phenylcapsaicin derived from a 90-day repeated dose oral toxicity study in rats is 37.2 mg/kg bw per day, the lowest of the model averaged $BMDL_{20}$ values for increase in plasma alanine aminotransferase.

Based on the available data, the FEEDAP Panel concluded that the ADME of phenylcapsaicin is well established in rats. However, metabolic similarity between the laboratory animals and the target species is not demonstrated and it is not possible to establish the identity of the marker residue in the target animals.

In the absence of such data, the Panel cannot estimate the exposure of the consumer to residues and, therefore, cannot conclude on the safety for the consumer.

3.2.3 | Safety for the user

3.2.3.1 | *Effect on respiratory system*

Phenylcapsaicin is in liquid form; therefore, exposure through inhalation is considered unlikely.

The applicant submitted an acute inhalation toxicity study (nose exposure only) performed with the liquid form of phenylcapsaicin (98.3%) in rats, following the Organisation for Economic Co-operation and Development (OECD) Testing Guideline (TG) 403.[40] On the basis of this study, it was concluded that the lethal concentration 50 (LC_{50}) of phenylcapsaicin (98.3%) is greater than 5.65 mg/L.

3.2.3.2 | *Effect on eyes and skin*

The skin irritation potential of phenylcapsaicin (97%) was tested in an in vivo study performed according to OECD TG 404, which showed that it is not a skin irritant.[41]

The eye irritation potential of phenylcapsaicin (97%) was tested in a valid study performed according to OECD TG 405, which showed that it is irritant to the eye.[42]

In a skin sensitisation study following OECD TG 406, phenylcapsaicin (98.21%) did not show any skin sensitisation potential.[43]

[34]Annex III-3.2 - Report efficacy and tolerance Phenylcapsaicin.
[35]Annex II-11.1 - Method Validation-phenylcapsaicin, Annex II-11.2 - Measurement of Phenylcapsaicin in tissue and Annex II-11.3 - Method of analysis.
[36]aXichem_ScientificSummary_Phenylcapsaicin, aXichem_Sect_III_Safety Consumers.
[37]Annex III-8 reverse mutation assay 2015.
[38]Annex III-9 mammalian micronucleus 2016.
[39]Annex III-1 90-day oral tox rat 2016.
[40]Annex III-102015 Acute Inhalation Tox.
[41]Annex III-12.1 - Acute Dermal.
[42]Annex III-11.1 - 25 Acute Eye.
[43]Annex III-132015 Skin Sensitisation.

3.2.3.3 | *Conclusions on safety for the user*

Since phenylcapsaicin is in liquid form, the exposure through inhalation is unlikely. Based on the studies submitted, the FEEDAP Panel concluded that the additive under assessment is irritant for the eyes but not for the skin and it is not a dermal sensitiser.

3.2.4 | Safety for the environment

The additive is intended to be used in chickens for fattening at minimum of 10 mg phenylcapsaicin/kg complete feed and a maximum of 15 mg phenylcapsaicin/kg complete feed. The environmental risk assessment has been performed assuming that all the phenylcapsaicin ingested is excreted as such in the environment.

3.2.4.1 | *Phase I*

Physico-chemical properties of phenylcapsaicin

The physico-chemical properties of phenylcapsaicin are summarised in Table 1.

TABLE 1 Physico-chemical properties of phenylcapsaicin.

Property	Value	Unit
Molecular weight	337.41	g/mol
Octanol/water partition coefficient (log K_{ow})[44]	2.34 (EPIWIN 3.8, Vega QSAR 4.33, ACD/Labs 3.73)	–
Water solubility at 20°C[45]	1.93	mg/L
Dissociation constant pK_a[46]	10.01	–
Vapour pressure at 20°C[47]	600 (EPIWIN 2.2×10^{-10})	Pa

Fate and behaviour

Fate in soil

Adsorption

No adsorption studies were submitted. The applicant calculated the adsorption coefficient through a correlation with the K_{ow} of phenylcapsaicin (2.34) based on Vowles and Mantoura (1987).[48] The estimated Koc value is 0.161 L/kg. Considering the very low value obtained, the FEEDAP Panel considers that a K_{oc} of 0 L/kg has to be considered for Phase I assessment.

Degradation

No degradation studies were submitted. The applicant estimated a DT_{50} in soil at 12°C of ~ 34 days using the BioWin3 tool in the EpiWin v4.11 suite.[49] The Panel noted that, in line with the requirements of the technical guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), this value can be considered acceptable just for Phase I assessment.

Conclusion on fate and behaviour

A K_{oc} of 0 mL/g and a DT_{50} of 34 days at 12°C can be used just for Phase I assessment.

Predicted environmental concentrations (PECs)

The predicted environmental concentrations (PECs) were calculated according to the above-mentioned guidance (EFSA FEEDAP Panel, 2019). The input values used for initial PEC calculations were: phenylcapsaicin dose of 15 mg/kg feed, molecular weight of 337.41 g/mol, vapour pressure of 600 Pa, solubility of 1.93 mg/L, DT_{50} of 34 days (at 12°C) and K_{oc} of 0 L/kg. The initial PEC values are reported in Table 2.

[44] Annex II-8.

[45] aXichem – Sect_III_Safety_Environmental.

[46] Annex III-22.1 EFSA-2023-00016439 IWTN-W000015567RL001 pKa study.

[47] Annex II-8.5 - Vapour pressure.

[48] K_{ow} of phenylcapsaicin (2.34) was undertaken using Annex 4 of OECD TG 106 (Soil Adsorption/Desorption) using the correlation $K_{oc} = -2.53 + 1.15 \log P_{ow}$ (Vowles & Mantoura, 1987; OECD, 2000a).

[49] aXichem - Sect_III_Safety_Environmental.

TABLE 2 Initial predicted environmental concentrations (PECs) of phenylcapsaicin in soil (μg/kg) and groundwater (μg/L).

Compartment	PEC
Soil	227
Ground water	9.39

The Phase I trigger values were exceeded. Therefore, a Phase II assessment is considered necessary.

3.2.4.2 | *Phase II*

Exposure assessment

The applicant did not provide experimental data for K_{oc} and DT_{50}, providing just estimated data, referring to Appendix D of the technical guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

The FEEDAP Panel notes that Appendix D suggests that fate properties can be estimated using non-testing approaches, such as quantitative structure–activity relationship models (QSARs) or read-across procedures, only in Phase I. Generally, experimental data from good laboratory practice (GLP)-accredited studies should be available for Phase II for substances like phenylcapsaicin.

Therefore, since no experimental data are available, no exposure assessment for surface water and sediment can be performed.

Considering groundwater, since no refinement is possible, a concern is highlighted from the calculation in Phase I.

The FEEDAP Panel notes that the applicant submitted a study evaluating the aerobic degradation potential of phenylcapsaicin in chicken manure.[50] The study was conducted according to the principles of GLP and was designed to comply with the European Medicines Agency (EMA) guideline on Determining the Fate of Veterinary Medicinal Products in Manure (EMA, 2011). Radiolabelled (^{14}C) phenylcapsaicin was added at a rate of 45 mg/kg wet weight to chicken manure and incubated at 20°C ± 2°C in the dark. The mass balances were in the range of 91.2%–104% AR for all samples until day 30. Samples were analysed by scintillation counting and high-performance liquid chromatography to quantify the parent compound. The degradation time (DT_{50}) was 29 days according to SFO kinetics. According to EMA guideline (EMA, 2011), the DT_{50} value was corrected to 25°C for chicken manure using the Arrhenius equation. The resulting value is 18 days.

In the absence of experimental data for K_{oc} and DT_{50}, no refinement of the exposure in environmental compartments is possible.

Ecotoxicity studies

Toxicity to terrestrial compartment

Effects on plants

The applicant submitted a study in plants claimed to be performed according to OECD 208.[51] The FEEDAP Panel noted that the study shows a number of major limitations in design and reporting: (i) the report does not contain any data (even historical) on the sensitivity of the tested species to reference substance, (ii) the concentration ranges used were the same, for all the species tested, which resulted with the non-monotonous dose response curves that did not fulfil the requirement of OECD 208 guideline to have the response between 20% and 80% inhibition; therefore, in most cases, the EC_{50} could not be precisely expressed, (iii) the concentrations tested are not properly selected in terms of getting the precise NOEC values while EC_{10} values have not been calculated, (iv) EC_{50} calculations and NOEC values are not convincingly presented; in particular, for NOEC values, there is insufficient evidence of proper statistical analysis. Based on the above, the FEEDAP Panel concluded that the study and the results cannot be used for the evaluation.

Effects on earthworms

An earthworm (*Eisenia foetida*) acute toxicity test was conducted according to OECD Guideline 207.[52] The concentration levels of phenylcapsaicin were 1.0, 10, 30, 100, 300 and 1000 mg/kg dry soil. A blank control and the solvent control were also tested. Four replicates of 10 individuals were included with each test concentration and controls. After 14 days of exposure, no mortality was observed either in controls or in any of the treatment groups. Consequently, the acute toxicity of phenylcapsaicin to the earthworm (*Eisenia foetida*) is LC_{50}(14 days) > 1000 mg/kg dry soil.

[50]Annex III-15165601177_TRAMA_Final_Report_OCR.

[51]Annex III-23.23522030011 OECD208 Terresterial Plant Seedling Emergence and Growth Test.

[52]Technical dossier/Safety/Annex III-18.

Effects on soil microorganisms

The applicant submitted a nitrogen transformation study claimed to be performed in line with OECD 216.[53] The FEEDAP Panel noted that the concentrations tested (lower than the PEC/10 × PEC) and the study duration (terminated on day 42) are not in compliance with the requirements of FEEDAP guidance to assess the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019). In addition, a positive control was missing in the study. Based on the above, the FEEDAP Panel concluded that the study and the results cannot be used for the evaluation.

Toxicity to aquatic organisms

Effect on algae

To investigate the effect of phenylcapsaicin on green algae (*Pseudokirchneriella subcapitata*), a GLP compliant study was performed claimed to follow the OECD guideline 201 (OECD, 2011a).[54] The green algal species was exposed to a nominal concentration range of 0.14, 0.37, 1.66, 5.35 and 16.73 mg/L (test duration 72 h). To assess the stability of the test item, the concentration of phenylcapsaicin in the test media was determined at the start and after 96 h in all concentrations. Phenylcapsaicin was stable over the exposure period with concentrations at the end of exposure between 80% and 120% of the nominal values. Consequently, the evaluation of biological endpoints was performed using nominal concentrations. The validity criteria of the study were met. Therefore, under the conditions of the study, the 72-h E_rC_{50} for the green algae *Pseudokirchneriella subcapitata* was 4.533 mg/L. The FEEDAP Panel noted that some concentrations considerably exceed the phenylcapsaicin water solubility reported in Table 1. Therefore, the acceptability of the study is questionable.

The Panel noted that the algal growth inhibition study in *Phaeodactylum tricornutum*[55] cannot be used for the evaluation since it is performed with a marine algal species, not a freshwater species.

Effect on crustaceans

The acute toxicity of phenylcapsaicin to *Daphnia magna*, under static conditions, was claimed to be conducted in a GLP-compliant study (OECD guideline 202).[56] Daphnids were exposed to phenylcapsaicin concentrations of 5.04, 7.41, 9.57, 11.78, 14.72 and 18.66 mg/L (test duration 48 h). An untreated control was also included in the test. Five *D. magna* neonates were added to each test vessel (four replicates for treated or control medium). The mortalities and immobilisation conditions of daphnia were inspected and recorded at 24 h interval during the 48 h. Validity criteria of the test were met. Under the conditions of the study, the 48h-EC_{50} of phenylcapsaicin to *D. magna* was established as 12.25 mg/L. The FEEDAP Panel noted that the concentrations tested are up to one order of magnitude higher than the phenylcapsaicin water solubility reported in Table 1. Therefore, the acceptability of the study is questionable.

Effect on fish

The acute toxicity of phenylcapsaicin to Zebra fish (*Brachydanio rerio*) was investigated in a GLP-compliant study claimed to follow the OECD guideline 203 (96 h, static exposure regime).[57] According to the results of the preliminary test, the definitive test was conducted as limit test at saturated concentration of 17.65 mg/L. Blank control was run in addition (three replicates for control and tested concentration, with 10 fish in each replicate). Since the exposure concentrations remained within 80%–120% of the initial measured concentration, the test results were expressed in terms of the nominal concentration of phenylcapsaicin. Validity criteria of the test were met. No mortality occurred at saturated concentration of phenylcapsaicin during the test duration (96 h), and thus, the LC_{50} value for phenylcapsaicin was > 17.65 mg/L. The FEEDAP Panel noted that the concentrations tested are up to one order of magnitude higher than the phenylcapsaicin water solubility reported in Table 1. Therefore, the acceptability of the study is questionable.

Conclusions on ecotoxicity studies

The dataset available to evaluate the toxicity of phenylcapsaicin for the terrestrial compartment is not complete (the plant study and the study on the toxicity to soil microorganisms present major limitations). In principle, the studies on algae, crustacean and fish could be used for the evaluation; however, the FEEDAP Panel noted that the concentrations used in the tests are up to one order of magnitude higher than the phenylcapsaicin water solubility. Therefore, the acceptability of these studies is questionable.

[53]Supplementary information/Annex III-23.13522030012 OECD 216 Nitrogen Transformation Test.

[54]Supplementary information/Annex III-19 freshwater Algae, OECD 201.

[55]Technical dossier/Annex III-19.

[56]Annex III-20 Daphnia acute immobilisation 2014.

[57]Annex III-21 acute tox zebra fish 2014b.

Bioaccumulation and secondary poisoning

The log K_{ow} of phenylcapsaicin is less than 3, indicating that the substance is unlikely to bioaccumulate, and the risk of secondary poisoning is considered low.

3.2.4.3 | *Conclusions on safety for the environment*

In the absence of appropriate data, the FEEDAP Panel cannot conclude on the safety of phenylcapsaicin for the environment.

3.3 | Efficacy

The additive is intended to be used as a zootechnical additive, functional group: physiological condition stabiliser. The Panel notes that the applicant did not make a specific claim on the expected effect of the additive as a physiological condition stabiliser.

To support the efficacy of the additive, the applicant provided in vivo efficacy studies in which the additive was tested in the [redacted] form. The Panel considers that the [redacted] would not significantly modify the bioavailability of the additive and therefore, the test item used is representative of the additive under assessment.

A total of three in vivo trials (two efficacy studies and the tolerance/efficacy study discussed above) and a large-scale field trial were submitted. The latter was not further considered in the assessment of the efficacy due to the inadequacy of the design (lack of replicates and experimental endpoints) and the poor detail in the description of the study.[58]

The details of the tolerance-efficacy study (Trial 1) are given in Section 3.2.1.[59] In trials 2 and 3, 1-day-old male Ross 308 chickens were fed diets (based on maize, wheat and soybean meal) that were either not supplemented (control) or supplemented with phenylcapsaicin [redacted] with hydrogenated glyceride) at 10 mg or 15 mg/kg of complete feed (confirmed by analysis; see Table 3).[60,61] In both trials, chickens were distributed in collective pens and randomly allocated to the three dietary treatments. Diets were offered ad libitum as pellets for 35 days.

An overview of the details on the study design of each of the three trials and the main results are provided in Tables 3, 4, respectively.

TABLE 3 Trial design and use level of phenylcapsaicin in the efficacy trials performed in chickens for fattening.

Trial	Total no of animals (animals per replicate) replicates per treatment	Breed sex (duration)	Composition feed (form)	Phenylcapsaicin (mg/kg complete feed)	
				Intended	Analysed
1[62]	[redacted]	Ross 308	Wheat–maize–soybean meal	0	ND
	[redacted]	♂	(pellet)	10	8.8–8.6
	[redacted]	(35 days)		15	13.8–13.4
				150	143.3–140.0
2[63]	[redacted]	Ross 308	Maize–wheat–soybean meal	0	ND
	[redacted]	♂	(pellet)	10	10.3–9.0
	[redacted]	(35 days)		15	15.4–15.4
3[64a]	[redacted]	Ross 308	Maize–wheat–soybean	0	NA
	[redacted]	♂	Meal–rapeseed (pellet)	10	9.5–9.4
	[redacted]	(35 days)		15	15.2–13.3

Abbreviations: NA, not analysed; ND, not detected.

[a] [redacted] phenylcapsaicin supplementation from day 8 to day 42 of life.

In trial 2, birds followed a two-phase feeding programme with starter (1–14 days) and grower (15–35 days) diets. The additive was given from day 1 to day 35. Birds were weighed at start of the trial. Thereafter, body weight and feed consumption were determined per pen at 14 and 35 days. Average daily feed intake, daily weight gain and feed to gain ratio were calculated and corrected for mortality for the complete period. Health status and mortality were checked daily. The data were subjected to analysis of variance (ANOVA) and the Tukey test used for the multiple comparison of means. Significance was declared at 0.05.

[58] Annex IV-1 aXichem 2021.
[59] Annex III-3.2 - Report efficacy and tolerance Phenylcapsaicin.
[60] Annex III-4 efficacy phenylcapsaicin broilers 2020.
[61] Annex III-5.1 Floor pen trial Broiler 2020.
[62] Annex III-3.2 - Report efficacy and tolerance Phenylcapsaicin.
[63] Annex III-4 efficacy phenylcapsaicin broilers 2020.
[64] Annex III-5.1 Floor pen trial Broiler 2020.

TABLE 4 Effects of phenylcapsaicin in the efficacy trials on the performance of chickens for fattening.

Trial	Groups (mg/kg complete feed)	Daily feed intake (g)	Final body weight (g)	Average daily weight gain (g)	Feed to gain ratio	Mortality and culling (%)
1[65]	0	96.2	2556	71.9	1.34[b]	7.0[a]
	10	97.0	2590	72.9	1.33[b]	3.1[b]
	15	95.9	2565	72.2	1.33[b]	0.6[b]
	150	96.5	2538	71.4	1.35[a]	3.1[b]
2[66]	0	97.4	2416	67.8	1.44	2.2
	10	97.5	2434	68.3	1.43	0.0
	15	97.7	2437	68.4	1.43	1.7
3[67c]	0	140.5	2515	91.9	1.53	6.3
	10	137.5	2542	94.1	1.46	4.9
	15	136.4	2494	92.0	1.49	3.5

[a,b]Mean values within a trial and within a column with a different superscript are significantly different $p < 0.05$.

[c]Body weight refers to 42 days of age and daily feed intake or average daily weight gain refers to the 8–42 days period (period of additive administration).

In trial 3, all birds received a basal (un-supplemented) starter diet for the first 7 days of life. Then, birds received the starter (8–14 days) and grower (15–42 days) diets. Feed and birds (individually) were weighed on a weekly basis. Health status and mortality were checked daily. Performance data were subjected to analysis of variance (ANOVA) with bird as the experimental unit for growth parameters and pen for the other variables. Group means were compared with a Bonferroni test with significance being declared at 0.05.

No effects on the performance parameters were observed in any study when phenylcapsaicin was supplemented at the proposed conditions of use, with the exception of a reduced mortality in treated groups with respect to the control in Trial 1 (Table 4). The Panel notes that mortality in the control group was relatively high in Trial 1. The Panel notes that, in Trial 3, the age of the animals at start was 8 days and that the statistical analysis was done considering the individual animal as the experimental unit and not the pen. Considering the absolute values recorded for these parameters and the fact that no differences were observed, the Panel considers it unlikely that the use of the pen as the experimental unit would modify the statistical significance of the differences among groups. In trial 1, footpad lesions and litter quality were not affected by the additive under assessment. The Panel notes that no other parameters that could be linked to an effect on the physiological condition of the animals were measured in the studies.

In the absence of positive results, the FEEDAP Panel is not in the position to conclude on the efficacy of aXiphen for chickens for fattening.

3.3.1 | Conclusions on efficacy

Considering the absence of positive effects on the performance or on other parameters relevant to the improvement of the physiological condition of the birds, the FEEDAP Panel cannot conclude on the efficacy of phenylcapsaicin as a zootechnical additive, functional group physiological condition stabilisers, in chickens for fattening.

3.4 | 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[68] and good manufacturing practice.

4 | CONCLUSIONS

The additive under assessment, phenylcapsaicin, is safe for chickens for fattening up to the maximum proposed use level of 15 mg/kg complete feed. A margin of safety could not be established.

Phenylcapsaicin is not genotoxic. The averaged $BMDL_{20}$ value for phenylcapsaicin derived from a 90-day repeated dose oral toxicity study in rats is 37.2 mg/kg bw per day based on an increase in plasma alanine aminotransferase.

The metabolic similarity in the laboratory animals and the target species is not demonstrated and the identity of the marker residue not established. In the absence of such data, the safety for the consumer of products of animals fed phenylcapsaicin cannot be evaluated.

[65]Annex III-3.2 Report efficacy and tolerance phenylcapsaicin.

[66]Annex III-4 efficacy phenylcapsaicin broilers 2020.

[67]Annex III-5.1 Floor pen trial Broiler 2020.

[68]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 inhalation exposure of phenylcapsaicin is unlikely. The FEEDAP Panel considers the additive irritant to the eyes but not to the skin and it is not a dermal sensitiser.

In the absence of appropriate data, the FEEDAP Panel cannot conclude on the safety of phenylcapsaicin for the ground water, the terrestrial and aquatic compartments. It is unlikely that phenylcapsaicin bioaccumulates in the environment and the risk of secondary poisoning is considered low.

The FEEDAP Panel cannot conclude on the efficacy of the additive in chickens for fattening at the proposed conditions of use.

ABBREVIATIONS

BW	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FCR	feed conversion ratio
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification
Log K_{ow}	logarithm of octanol–water partition coefficient
MCHC	mean corpuscular haemoglobin concentration
MRL	maximum residue limit
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity
WHO	World Health Organisation

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

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