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Safety and efficacy of copper complexes of chlorophylls for ornamental fish, grain-eating ornamental birds and small rodents and of copper complexes of chlorophyllins for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

Copper complexes of chlorophylls are applied as colourants used for ornamental fish, grain-eating ornamental birds and small rodents (subgroup: substances which favourably affect the colour of ornamental fish or birds). Copper complexes of chlorophyllins are applied as a colourant to add colour to feedingstuffs of cats and dogs and all animal species and for all animal species for use in certain groups of feed materials. In the absence of adequate data on absorption, distribution, metabolism and excretion, genotoxicity, subchronic and chronic toxicity, carcinogenicity, and reproductive and developmental toxicity of copper complexes of chlorophyllins, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of this additive for the consumer. No data on the tolerance of target animals were provided. In the absence of adequate toxicological data that would allow deriving a no observed adverse effect level (NOAEL) and to exclude the genotoxic potential, the FEEDAP Panel could not conclude on the safety of copper complex of chlorophylls and copper complex of chlorophyllins for the target animals. In the absence of data the FEEDAP Panel cannot conclude on the safety for the user of the additives. No risks to the environment are expected from the use of copper complexes of chlorophylls or copper complexes of chlorophyllins in animal nutrition. The FEEDAP Panel is not in the position to conclude (i) on the efficacy of copper complexes of chlorophylls to colour ornamental fish, grain-eating ornamental birds and small rodents, and (ii) on the concentrations of copper complexes of chlorophylls and of chlorophyllins necessary to visibly colour feed of cats and dogs, ornamental birds, small rodents and certain feed materials for all animal species.

Keywords: copper complexes of chlorophylls, copper complexes of chlorophyllins, colourant, safety, efficacy

Requestor: European Commission

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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 copper complexes of chlorophylls and copper complexes of chlorophyllins.

Copper complexes of chlorophylls are applied as colourants used for ornamental fish, grain-eating ornamental birds and small rodents (subgroup: substances which favourably affect the colour of ornamental fish or birds). Copper complexes of chlorophyllins are applied as a colourant to add colour to feedingstuffs of cats and dogs and all animal species, and for all animal species for use in certain groups of feed materials.

In the absence of adequate data on absorption, distribution, metabolism and excretion, genotoxicity, subchronic and chronic toxicity, carcinogenicity, and reproductive and developmental toxicity of copper complexes of chlorophyllins, the FEEDAP Panel could not conclude on the safety of this additive for the consumer.

No data on the tolerance of target animals were provided. In the absence of adequate toxicological data that would allow deriving a no observed adverse effect level and to exclude the genotoxic potential, the FEEDAP Panel could not conclude on the safety of copper complex of chlorophylls and copper complex of chlorophyllins for the target animals.

In the absence of data, the FEEDAP Panel cannot conclude on the safety for the user of the additives.

No risks to the environment are expected from the use of copper complexes of chlorophylls or copper complexes of chlorophyllins in animal nutrition.

The FEEDAP Panel is not in the position to conclude (i) on the efficacy of copper complexes of chlorophylls to colour ornamental fish, grain-eating ornamental birds and small rodents, and (ii) on the doses of copper complexes of chlorophylls and of chlorophyllins necessary to visibly colour feed of cats and dogs, ornamental birds, small rodents and certain feed materials for all animal species.

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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. In particular Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC[2] for additives with a limited authorisation period.

The European Commission (EC) received a request from the company Phytone Ltd[3] for re-evaluation of copper complexes of chlorophyllins for cats and dogs, and for all species or categories of animals, with the exception of cats and dogs for use in certain groups of feed materials[4] (category: sensory additives; functional group: (a) colourants: (i) substances that add or restore colour in feedingstuffs). During the assessment, the applicant requested to include in the application the re-evaluation of copper complexes of chlorophylls under the same conditions of the current authorisation,[5] i.e. for ornamental fish, grain-eating ornamental birds and small rodents (category: sensory additives; functional group (a) colourants: (iii) substances which favourably affect the colour of ornamental fish or birds) as detailed in the European Union (EU) register of feed 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) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 14 June 2011.

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 additives comply 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 products copper complexes of chlorophylls and copper complexes of chlorophyllins, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

Copper complexes of chlorophylls and copper complexes of chlorophyllins are included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 with the same E number (E 141).

Copper complexes of chlorophylls (E 141(i)) are authorised for ornamental fish without limitation[6] and for grain-eating ornamental birds and small rodents with a maximum of 150 mg/kg.[7]

Copper complexes of chlorophyllins (E 141(ii)) are authorised for their use in cats and dogs as a colourant. The additive is also authorised for all species or categories of animals, with the exception of cats and dogs, for animal feedingstuffs only in products processed from: (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture. No maximum levels of copper complexes of chlorophyllins in feeds are established in the EU.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in
[2] Council Directive of 23 November 1970 concerning additives in feedingstuffs. OJ L 270, 14.12.1970, p. 1.
[3] Phytone Ltd, Third Avenue, Centrum 100, DE14 2WD, United Kingdom.
[4] (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture.
[5] Clarification letter March 2012 and supplementary information February 2015.
[6] In application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01).
[7] Commission Regulation (EC) No 358/2005 of 2 March 2005 concerning the authorisation without a time limit of certain additives and the authorisation of new uses of additives already authorised in feedingstuffs. OJ L 57, 3.3.2005, p. 3.

Copper complexes of chlorophylls and copper complexes of chlorophyllins are approved food colourants in the EU.[8] Maximum permitted levels (MPLs) of both colourants are defined in Annex II of Regulation (EC) 1333/2008 on food additives for use in food (authorised at *quantum satis* in 58 food categories).[9] The specific purity criteria concerning the use of these additives in foodstuffs are included in Commission Regulation (EU) No 231/2012.[10]

Copper complexes of chlorophyllins have been previously evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) in 1969 and 1974 (JECFA, 1970, 1975) which recommended a temporary group acceptable daily intake (ADI) of 15 mg/kg body weight (bw) for copper complexes of chlorophyll and of chlorophyllins and their sodium and potassium salts, on the basis of a no observed adverse effect level (NOAEL) of 1500 mg/kg bw per day from a long-term toxicity and reproduction study by Harrison *et al*. (1954). In 1975, the Scientific Committee for Food (SCF) evaluated copper complexes of chlorophylls and copper complexes of chlorophyllins (SCF, 1975) and set a group ADI of 15 mg/kg bw for the sum of both complexes. In 2002, the US Food and Drug Administration (FDA), by applying a safety factor of 200 instead of 100 used for JECFA and the SCF evaluations, allocated an ADI of 7.5 mg/kg per day for sodium copper chlorophyllins (FDA, 2002). In 2015, EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted an opinion on the re-evaluation of copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)) (EFSA ANS Panel, 2015) and recommended that the group ADI that had been set by the SCF should be withdrawn. The ANS Panel noted that the Harrison *et al.* study from 1954 was performed before the publication of the Organization for Economic Cooperation and Development (OECD) guidelines and was rather far from the current scientific standard. In particular, this study did not include the usual endpoints for reproductive and developmental toxicity and used a low number of animals. Furthermore, the ANS Panel considered that reliable data on absorption, distribution, metabolism and excretion (ADME), genotoxicity, (chronic) toxicity, carcinogenicity, and reproductive and developmental toxicity of copper complexes of chlorophylls and copper complexes of chlorophyllins were lacking.

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[11] in support of the authorisation request for the use of copper complexes of chlorophylls and copper complexes of chlorophyllins as feed additives. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003[12], Regulation (EC) No 429/2008[13] 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 and other expert bodies, 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 copper complexes of chlorophyllins in animal feed. The Executive Summary of the EURL report can be found in the Annex.[14] The FEEDAP Panel noted that methods which could be used for the control of copper complexes of chlorophylls could not be assessed by the EURL.

[8] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.09.1994, p. 13.
[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.
[10] Commission Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.
[11] FEED dossier reference: FAD-2010-0358.
[12] OJ L 268, 18.10.2003, p. 29.
[13] 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.
[14] The full report is available on the EURL_website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2010-0358?search&form-return

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of copper complexes of chlorophylls and copper complexes of chlorophyllins is in line with the principles laid down in Regulation (EC) No 429/2008[15] and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

Copper complexes of chlorophylls are applied for use in feed for ornamental fish, grain-eating ornamental birds and small rodents under the category sensory additives, functional group colourants, subgroup (iii), substances which favourably affect the colour of ornamental fish or birds.

Copper complexes of chlorophyllins are applied under the category sensory additives, functional group colourants, subgroups (i), substances that add or restore colour in feedingstuffs, for cats and dogs, and for all animal species for use in certain groups of feed materials.[16]

3.1. Characterisation

In the present assessment, the additive copper complexes of chlorophylls will be referred to as Cu-chlorophylls (active substances (major colouring principle) copper phaeophytin a and b[17]); the additive copper complexes of chlorophyllins will be referred to as Cu-chlorophyllins (active substances (major colouring principle) copper chlorophyllin a and b).

3.1.1. Copper-chlorophylls[18]

Cu-chlorophylls (synonyms CI Natural Green 3, copper chlorophyll, copper phaeophytin, CI 75810) are manufactured from fescue grass (*Festuca arundinacea*) using a process that consists in the extraction of the dried raw material using dichloromethane, with subsequent removal of the solvent, to yield a resin containing the non-polar components including chlorophylls, carotenoids, fats and waxes. The magnesium of the chlorophyll extract is then replaced by copper (coppering) using copper oleate, to enhance stability. To yield a product of constant dye content, the concentration of Cu-chlorophylls is standardised by addition of sunflower oil.

The additive is a blue green to dark waxy paste.

The specifications for copper chlorophylls are the same as for the food additive set by Commission Regulation (EU) No 231/2012[19] with the exception of the minimum content of copper phaeophytins (15% instead of 10%).

The concentrations of the active substance (copper phaeophytin a and b) were determined by spectrophotometry at wavelengths of 422 and 652 nm in a chloroform solution in comparison with the absorbance value of the pure active substance. Only three of five batches of the additive complied with the applicant's specification for the pigment content (15.2, 15.2, 15.1, 14.5 and 14.5%).[20] Data on the quantitative composition of the non-chlorophyll fraction of other five batches showed a range of 98.6–99.4% for dry weight, 95.8–98.1% for ether extract (including Cu-chlorophylls), 5.0–6.6% for crude protein (Nx6.25, notwithstanding the specific contribution of the four nitrogen atoms of Cu-

[15] OJ L 133, 22.5.2008, p. 1.
[16] (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture.
[17] Copper phaeophytin a and b are also called copper chlorophyll a and b; however, the correct name of chlorophyll without magnesium is phaeophytin.
[18] Technical dossier/Supplementary information March 2013.
[19] OJ L 83, 22.3.2012, p. 1.
[20] Technical dossier/Supplementary information March 2013/Annex 8.

chlorophylls) and 2.7–4.0% for crude ash (including copper from Cu-chlorophylls).[21] The fatty acid profile reflected the main components of grass lipids and added sunflower oil. No indication on the nature and quantity of carotenoids was given, nor on the eventual presence of other chlorophyll-derived compounds.

Cu-chlorophylls are insoluble in water, soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed waxes (JECFA, 2006).

Total copper concentrations in another five batches were between 0.6% and 0.9%.[22] These batches were analysed for heavy metals (arsenic, lead, mercury, cadmium, copper ions and total copper) and residual solvents (acetone, methyl ethyl ketone, methanol, hexane and dichloromethane).[23] All values were in compliance with the specifications for the food additive set by Commission Regulation (EU) No 231/2012.[24]

No data on other potential impurities (considering the source material, e.g. pesticides, mycotoxins, dioxins and dioxin-like polychlorinated biphenyls (PCBs)) were submitted.

The additive is a waxy paste, consequently, data on particle size distribution and dusting potential are not required.

Copper-phaeophytins

The main colouring substances of Cu-chlorophylls are copper phaeophytin a and copper phaeophytin b with a porphyrin (tetrapyrrole) ring as basic structure with a coordinated copper ion (Cu^{2+}) and a phytol ester side chain (Figure 1).

Copper phaeophytin a is [phytyl (13^2R,17S,18S)-3-(8-ethyl-13^2-methoxycarbonyl-2,7,12,18-tetramethyl-13′-oxo-3-vinyl-13^1-13^2-17,18-tetrahydrocyclopenta[at]-porphyrin-17-yl)propionate] copper (II), molecular formula $C_{55}H_{72}CuN_4O_5$, molecular weight 932.75, Chemical Abstracts Service (CAS) No 65963-40-8, European Inventory of Existing Commercial chemical Substances (EINECS) No 239-830-5.

Copper phaeophytin b is [phytyl (13^2R,17S,18S)-3-(8-ethyl-7-formyl-13^2-methoxycarbonyl-2,12,18-trimethyl-13′-oxo-3-vinyl-13^1-13^2-17, 18-tetrahydrocyclopenta[at]-porphyrin-17-yl)propionate] copper (II), molecular formula $C_{55}H_{70}CuN_4O_6$, molecular weight 946.73, CAS No 65963-40-8, EINECS No 246-020-5.

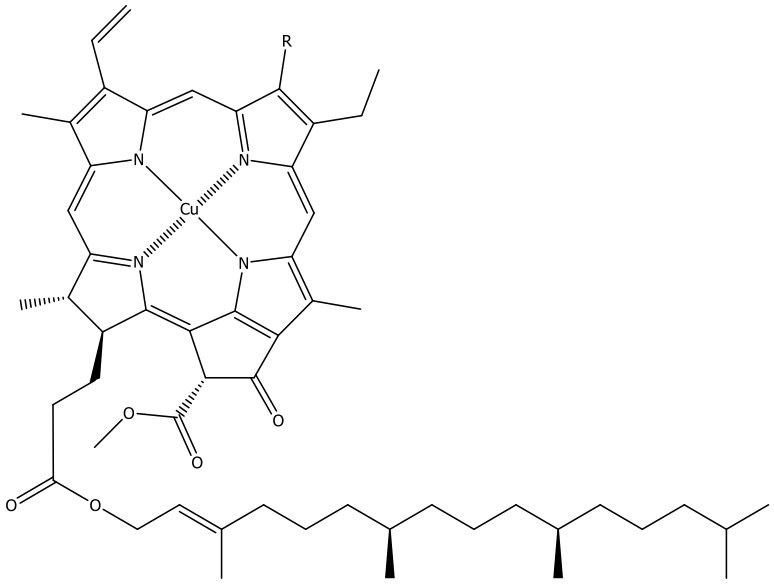

Figure 1: Structural formula of the major colouring principles of Cu-chlorophylls (copper phaeophytin a (R=CH_3) and copper phaeophytin b (R=CHO))

[21] Technical dossier/Supplementary information February 2015.
[22] Technical dossier/Supplementary information March 2013/Section II Copper Chlorophyll v2
[23] Technical dossier/Supplementary information March 2013/Annex 8.
[24] OJ L 83, 22.3.2012, p.1.

Cu-pyrophaeophytin a has been identified as a component of Cu-chlorophylls E 141(i) (EFSA, ANS Panel, 2015). No attempt was made by the applicant to identify and quantify copper phaeophytin derived compounds in the additive under assessment.

3.1.2. Copper-chlorophyllins

Cu-chlorophyllins (synonyms sodium copper chlorophyllin, potassium copper chlorophyllin, CI Natural Green 5, CI 75815) are manufactured from fescue grass (*Festuca arundinacea*) using a process that consists in the extraction of the dried raw material using dichloromethane, with subsequent removal of the solvent, to yield a resin containing the non-polar components including the chlorophylls, carotenoids, fats and waxes. The saponification of the resin removes the methyl and phytol ester groups and may partially cleave the cyclopentenyl ring, completed by hexane extraction of the less polar compounds. The magnesium of the treated extract is replaced by copper (coppering) using copper sulfate, to enhance stability, and finally the copper chlorophyllins are precipitated by the addition of sulfuric acid.

The additive is a green black free-flowing powder.

The applicant refers to the specifications of Commission Directive 2008/128/EC which are the same as those more recently established by Commission Regulation (EU) No 231/2012,[25] for solvent residues, arsenic, lead, mercury, cadmium, copper ions and total copper with the exception of the minimum content of copper-chlorines (100% instead of 95%), on a dried weight basis (100°C for 1 h). The determination of the additive was based on its optical density measured by spectrophotometry at wavelengths of 405 and 630 nm in aqueous phosphate buffer (pH 7.5), in comparison with the absorbance value of the pure active substance.

Salts of Cu-chlorophyllins are soluble in water, very sparingly soluble in ethanol, very slightly soluble in lower alcohols and ketones and diethyl ether, insoluble in chloroalkanes, hydrocarbons and fixed oils.[26]

The pigment content of five batches of the additive was considerably above the specified minimum content (116, 121, 126, 119 and 120%) questioning the accuracy of the method.[27]

Data from the same batches showed total copper concentrations comprised between 4.4% and 4.8% (Commission Regulation (EU) No 231/2012[28]: not more than 8.0%). The same batches were analysed for heavy metals (arsenic, lead, mercury, cadmium and copper ions) and residual solvents (acetone, methyl ethyl ketone, methanol, propan-2-ol, hexane and dichloromethane).[29] All values were in compliance with the specifications set by Commission Regulation (EU) No 231/2012.

No data on other potential impurities (considering the source material, e.g. pesticides, mycotoxins, dioxins and dioxin-like PCBs) were submitted.

Particle size distribution of three batches was examined by laser diffraction.[30] The results indicate that all particles were < 63 µm, 50% (v/v) had a diameter < 20 µm and 10% (v/v) a diameter < 7.5 µm. No data on the dusting potential of the additive were provided.

Copper chlorophyllin a and b

The main colouring substances of Cu-chlorophyllins are copper chlorophyllin a and b, pigments with a porphyrin (tetrapyrrole) ring as basic structure with a coordinated copper ion (Cu^{2+}) (Figure 2).

Copper chlorphyllin a is the 3-(10-carboxylato-4-ethyl-1,3,5,8-tetramethyl-9-oxo-2-vinylphorbin-7-yl)propionate, copper complex, molecular formula $C_{34}H_{32}CuN_4O_5$, molecular weight 640.20, CAS number not given.

[25] OJ L 83, 22.3.2012, p.1.
[26] Technical dossier/Section II/Annex 3, 7 and 17.
[27] Technical dossier/Section II
[28] OJ L 83, 22.3.2012, p.1.
[29] Technical dossier/Section II/Annex 11.
[30] Technical dossier/Supplementary information January 2012/ Section II_Addendum.

Copper chlorophyllin b is the 3-(10-carboxylato-4-ethyl-3-formyl-1,5,8-trimethyl-9-oxo-2-vinylphorbin-7-yl)propionate, copper complex, molecular formula $C_{34}H_{30}CuN_4O_6$, molecular weight 654.18, CAS number not given.

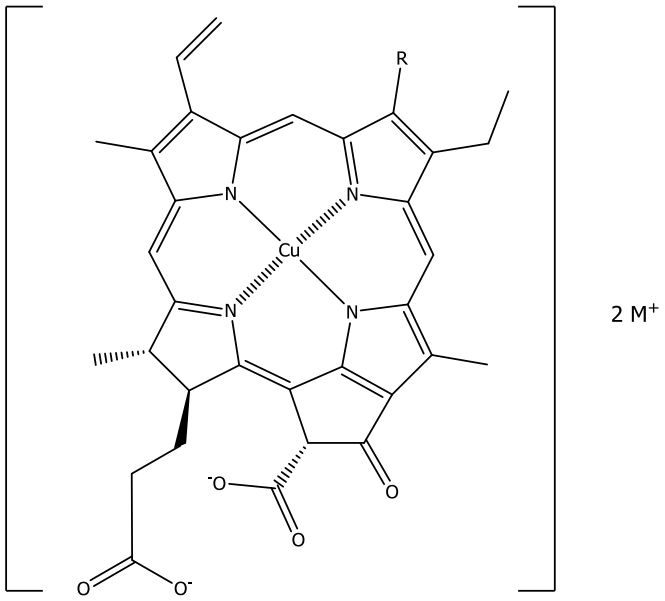

Figure 2: Structural formula of the major colouring principles of Cu-chlorophyllins (copper chlorophyllin a (R=CH_3) and copper chlorophyllin b (R=CHO), M=potassium and/or sodium)

Depending on the degree of hydrolysis the cyclopentenyl ring may be cleaved with the resultant production of a third carboxyl function. The analysis liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) of one commercial sample of copper chlorophyllins (Egner et al., 2000) showed similar quantities of copper chlorin e6 (31%) and copper chlorin e4 (33 %) (Figure 3).

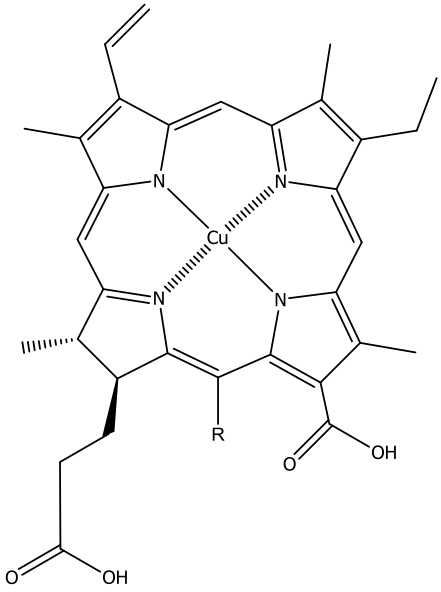

Figure 3: Structural formula of copper chlorin e6 (R=CH_2COOH) and copper chlorin e4 (R=CH_3)

No data were provided on the different components for the additive under assessment.

3.1.3. Stability and homogeneity

Shelf-life data for Cu-chlorophylls[31] and Cu-chlorophyllins[32] were obtained after storage up to 32 and 43 months (storage conditions not given), respectively (one batch per time point). The maximum loss for Cu-chlorophylls was 8%, already seen after 11 months. For Cu-chlorophyllins, the losses observed after 24 to 25 and 41 to 43 months were about 4%.

The applicant stated that prolonged exposure to light may lead to colourless degradation products probably due to the breakdown of the porphyrine ring.

No data on stability or homogeneous distribution in premixtures or compound feed for Cu-chlorophylls and Cu-chlorophyllins were provided.

The FEEDAP Panel notes that stability and homogeneity data could not be generated in complex matrices by the use of the spectrophotometric method of analysis described in Commission Regulation (EU) No 231/2012.[33]

3.1.4. Conditions of use

Cu-chlorophylls are applied for use in feed for ornamental fish (without maximum content), grain-eating ornamental birds and small rodents (maximum content of 150 mg/kg) under the category sensory additives, functional group colourants, subgroup (iii), substances which favourably affect the colour of ornamental fish or birds.

Cu-chlorophyllins are applied without maximum content under the category sensory additives, functional group colourants, subgroup (i) substances that add or restore colour in feedingstuffs for cats and dogs, and for all animal species for use in certain groups of feed materials.[34]

The applicant reported[35,36] that the additives may be used at concentrations between 100 to 500 mg/kg in dog food and also up to 1000 mg/kg in feed for small rodents.

3.2. Safety

Cu-chlorophyllins (E 141(ii)) have been previously evaluated by JECFA in 1969 and 1974 (JECFA, 1970, 1975). In 1975, the SCF evaluated Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) (SCF, 1975). No new data have been provided by the applicant for the assessment of the safety for the target species and the consumer except the previous safety assessments from JECFA and the SCF.

In 2015, the EFSA ANS Panel (ANS Panel, 2015) adopted an opinion on the re-evaluation of Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)). In this opinion, the ANS Panel's re-assessed the toxicological studies previously considered by JECFA and the SCF.[37] No toxicological studies on Cu-chlorophylls were available. The studies of Cu-chlorophyllins and its derivatives that were taken into consideration in EFSA ANS Panel assessment included: ADME investigations in humans, an *in vitro* study of the digestion of a derivative of Cu-chlorophyllins, a study of the anti-tumour activity in mice of the sodium salt of Cu-chlorophyllins (Park and Surh, 1996), acute toxicity studies in mice (Worden *et al*., 1955) and rats (Harrison *et al*., 1954), several poor-quality short-term oral toxicity studies in rats and guinea-pigs, the long-term toxicity/reproduction study of Harrison *et al*. (1954), various studies of cancer initiation and promotion, a developmental toxicity study in mice that used the intraperitoneal route of administration (García-Rodríguez et al., 2002), and a poorly designed reproduction study in rats (Reber and Willigan, 1954).

[31] Technical dossier/Supplementary information March 2013/Annex 7.
[32] Technical dossier/Section II/Annex 9.
[33] OJ L 83, 22.3.2012, p.1.
[34] (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture.
[35] Technical dossier/Supplementary information January 2012/Section IIa addendum.
[36] Technical dossier/Supplementary information February 2015.
[37] Full reports of some of these studies were not available to the ANS Panel, so the summaries of other committees were used. No additional information on toxicological or biological effects was submitted in response to an EFSA public call for data. Nevertheless, a few new reports of relevant studies were found in the published scientific literature and were used in the evaluation by EFSA ANS Panel.

The ANS Panel concluded that 'adequate data on ADME, genotoxicity, (chronic) toxicity, carcinogenicity, and reproductive and developmental toxicity of Cu-chlorophylls (E 141(i)) and of Cu-chlorophyllins (E 141(ii)) were lacking. Therefore, their safety of use as food additives cannot be assessed and the current ADI should be withdrawn'.

The FEEDAP Panel endorses the above conclusions of the ANS Panel. Consequently, no conclusions on the safety of both additives for the consumer can be drawn.

No tolerance studies were provided to support the safety for the target species. According to the FEEDAP Technical Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), in the absence of tolerance studies for the target species, the maximum safe feed concentration for the target animals could be derived from the lowest NOAEL (or by benchmark dose procedure) of appropriate substance-specific toxicological studies[38]. In the absence of adequate toxicological data that would allow deriving a NOAEL and to exclude the genotoxic potential, the FEEDAP Panel cannot conclude on the safety of Cu-chlorophylls and Cu-chlorophyllins for the target animals.

3.2.1. Safety for the user

No information on user safety was provided by the applicant, other than a description of the physical characteristics of the additives.

The additive Cu-chlorophylls is a waxy paste, and as such users are likely exposed to minimal amounts of the additive by inhalation. In the absence of any data on irritancy to skin and eyes or skin sensitisation, the Panel cannot conclude on the potential of the additive to be skin/eye irritant or a skin sensitiser.

The additive Cu-chlorophyllins is a powder with 50 % of particles (v/v) below a diameter of 20 µm. Although data on dusting potential were not submitted, the small particle size indicates that users may be exposed by inhalation. In the absence of any data on inhalation toxicity, irritancy to skin and eyes, or skin sensitisation, the FEEDAP Panel cannot conclude on the safety of this additive for users.

3.2.2. Safety for the environment

The use of additives in pet animals has no environmental impact on agricultural land.[39] Consequently Regulation (EC) No 429/2008 does not require an environmental assessment of substances used in non-food-producing animals only.

Cu-chlorophylls are applied for ornamental fish, birds and small rodents only. Environmental assessment therefore is not necessary.

Cu-chlorophyllins are applied for cats and dogs; for that use an environmental assessment is also not necessary.

Cu-chlorophyllins are also applied for use in certain groups of feed materials in all animal species.[40] However, chlorophyllins show the same porphyrinic structure as chlorophylls, which are physiological plant substances and abundant in nature, and do not need environmental risk assessment.

Cu-chlorophyllins contain 4.4–4.8% total copper. Assuming a supplementation rate of 1000 mg/kg feed material (Section 3.1.4., Conditions of use) and use level of certain feed materials in complete feeds of 50% the copper supplementation of complete diets would reach 24 mg/kg, which is near to the maximum content in complete feed for most animal species/categories.[41] Provided that maximum contents for total copper are respected also when adding copper chlorophyllins to feed, no particular concern for the environment would arise.

The FEEDAP Panel concludes that the use of Cu-chlorophylls and Cu-chlorophyllins as feed additives would not pose a risk to the environment.

[38] These should include at least a 90 day oral toxicity study
[39] OJ L 268, 18.10.2003, p. 29.
[40] (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture.
[41] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.

3.3. Efficacy

Cu-chlorophylls are applied for use in feed for ornamental fish, grain-eating ornamental birds and small rodents in order to favourably affect their colour. No data were provided to support this claim; therefore, the FEEDAP Panel cannot conclude on the efficacy of the additive.

Cu-chlorophyllins are intended to add or restore colour in feedingstuffs for cats and dogs, and for all animal species when used in certain groups of feed materials.[42]

Where the function requested for feed is the same as that used in food, no further demonstration of efficacy might be necessary (Regulation (EC) No 429/2008).[43] However, considering the wide variety of feedingstuffs used in complete and complementary feed for cats and dogs, ornamental fish and birds, and the uncertainty which concentration of the additives would result in a visible effect, a demonstration of a dose-effect in a typical feed material and compound feedingstuff appeared necessary.

Visual demonstration of the efficacy in a few commercial feeds for dogs, small rodents and ornamental birds was provided. The data does not allow a clear conclusion on the colour source, Cu-chlorophylls or Cu-chlorophyllins. The pictures allow the conclusion that the additives used colour effectively feed for dogs (treats and extruded food particles) and for ornamental birds. No demonstration is considered acceptable for rodents (packaging pictures only). The reported doses range from 68 to 500 mg/kg feed. The higher doses may apply to complementary feed. [44]

Although the ability of the additives to colour feed has generally been demonstrated, no precise conclusion on the additive and the related effective concentration could be made.

4. Conclusions

In the absence of adequate data on absorption, distribution, metabolism and excretion, genotoxicity, subchronic and chronic toxicity, carcinogenicity, and reproductive and developmental toxicity of copper complexes of chlorophyllins, the FEEDAP Panel cannot conclude on the safety of this additive for the consumer.

No data on the tolerance of target animals were provided. In the absence of adequate toxicological data that would allow deriving a NOAEL and to exclude the genotoxic potential, the FEEDAP Panel cannot conclude on the safety of copper complex of chlorophylls and copper complex of chlorophyllins for the target animals.

In the absence of data, the FEEDAP Panel cannot conclude on the safety for the user of the additives.

No risks to the environment are expected from the use of copper complexes of chlorophylls or copper complexes of chlorophyllins in animal nutrition.

The FEEDAP Panel is not in the position to conclude (i) on the efficacy of copper complexes of chlorophylls to colour ornamental fish, grain-eating ornamental birds and small rodents, and (ii) on the concentrations of copper complexes of chlorophylls and of chlorophyllins necessary to visibly colour feed of cats and dogs, ornamental birds, small rodents, and certain feed materials for all animal species.

5. Recommendations

The FEEDAP Panel would draw attention to the opinion of the EFSA ANS Panel on the re-evaluation of copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)) as food additives (EFSA ANS Panel, 2015) in which a revision of the current specification is recommended. The FEEDAP Panel strongly recommends to adapt these specifications also for the feed additives.

[42] (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture.
[43] OJ L 133, 22.5.2008, p.1.
[44] Technical dossier/Supplementary information January 2012/Section IIa addendum.

6. Remark

It should be noted that the copper content of copper complex of chlorophyllins should be considered when respecting the maximum content of total copper in complete feed for animal species/categories set by the legislation.

Documentation provided to EFSA

1. Copper Complexes of Chlorophyllins. November 2010. Submitted by Phytone Ltd.
2. Copper Complexes of Chlorophyllins. Supplementary information. January 2012. Submitted by Phytone Ltd.
3. Copper Complexes of Chlorophylls and Chlorophyllins. Supplementary information. October 2012. Submitted by Phytone Ltd.
4. Copper Complexes of Chlorophylls and Chlorophyllins. Supplementary information. March 2013. Submitted by Phytone Ltd.
5. Copper Complexes of Chlorophylls and Chlorophyllins. Supplementary information. February 2015. Submitted by Phytone Ltd.
6. Evaluation report of the European Community Reference Laboratory for Feed Additives on the methods(s) of analysis for Copper Complexes of Chlorophyllins.
7. Comments from Member States.

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANS	Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
EC	European Commission
EINECS	European Inventory of Existing Commercial chemical Substances
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	liquid chromatography
MPL	maximum permitted level
MS	mass spectrometry
NOAEL	no observed adverse effect level
OECD	Organization for Economic Cooperation and Development
PCB	polychlorinated biphenyl
SCF	Scientific Committee for Food
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 Copper Complexes of Chlorophyllins

In the current application authorisation is sought under articles 4(1) and 10(2) for *Copper complexes of chlorophyllins – E 141(ii)* – under the 'sensory additives', functional group 2(a) 'colourants', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all species and categories.

Copper complexes of chlorophyllins – E 141(ii) is readily water-soluble dark green/black free-flowing powder, extracted from *Festuca arundinacea* plants. The active substance of the additive has a minimum purity of 95%. The *additive* is intended to be incorporated directly in dry or moist *feedingstuffs*, with no recommended minimum or maximum levels.

For the determination of *Copper complexes of chlorophyllins* in the *feed additive*, the Applicant proposed the internationally recognised Food and Agriculture Organization of the United Nations (FAO) Joint FAO/WHO Expert Committee on Food Additives (JECFA) monograph for food additives. Identification is based on a spectrophotometric analysis at 405 and 630 nm in aqueous phosphate buffer and a test for copper presence, while quantification of *Copper complexes of chlorophyllins* in the *feed additive* is based on spectrophotometry at 405 and 630 nm in aqueous phosphate buffer, as recommended by Commission Directive 2008/128/EC laying down specific purity criteria concerning colours for use in foodstuffs. Even though no performance characteristics are provided, the European Union Reference Laboratory (EURL) recommends for official control the JECFA monograph based on spectrophotometry for the quantification of the *Copper complexes of chlorophyllins* in the *feed additive*.

The Applicant did not provide any experimental method or data for the determination of *Copper complexes of chlorophyllins* in *premixtures*, *feedingstuffs* and *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *Copper complexes of chlorophyllins* in *premixtures*, *feedingstuffs* and *water*.

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.