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SCIENTIFIC OPINION

Modification of the terms of authorisation of the feed additive consisting of a preparation of canthaxanthin (CAROPHYLL® Red 10%) for breeder hens to include canthaxanthin produced with *Yarrowia lipolytica* CBS 146148 (DSM Nutritional Products Ltd.)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

Following a request from the European Commission, the EFSA was asked to deliver a scientific opinion on the proposed modification of the terms of the authorisation of the feed additive consisting of a preparation of canthaxanthin (CAROPHYLL® Red 10%), regarding the addition of a new production route, by the yeast *Yarrowia lipolytica* CBS 146148 and to modify the additive specifications by substituting ethoxyquin by 4.4% butylated hydroxytoluene (BHT) and increasing the limit for dichloromethane to 80 mg/kg. The additive is already authorised as zootechnical feed additive for breeder hens. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded. CAROPHYLL® Red 10% containing canthaxanthin produced by fermentation with *Yarrowia lipolytica* CBS 146148 is safe for the target species, consumer and environment under the current authorised conditions of use for CAROPHYLL® Red 10%. Regarding user safety, canthaxanthin is not irritant to skin and eyes and unlikely to be a skin sensitiser. No conclusion can be reached on the respiratory sensitisation of canthaxanthin. In the absence of data with the additive CAROPHYLL® Red 10%, no conclusions can be reached regarding the safety of the additive for the user. CAROPHYLL® Red 10%, containing canthaxanthin produced by fermentation with *Yarrowia lipolytica* CBS 146148, is efficacious in breeder hens at 6 mg/kg complete feed.

KEYWORDS

canthaxanthin, CAROPHYLL® Red 10%, efficacy, poultry for breeding purposes, safety, *Yarrowia lipolytica* CBS 146148, zootechnical additive

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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 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a request from DSM Nutritional Products Ltd.[2] represented in the EU by DSM Nutritional Products Sp. Z o.o, for the modification of the terms of the authorisation of the additive consisting of canthaxanthin (produced by chemical synthesis) (CAROPHYLL® Red 10%) for breeder hens (category: zootechnical additive; functional group: other zootechnical additives (stabilisation of reproductive performance)). The modification consists in the change of the manufacturing process, to include canthaxanthin produced by fermentation with *Yarrowia lipolytica* CBS 146148 and to modify the additive specifications by substituting ethoxyquin with BHT and increasing the limit for dichloromethane.

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 13(3) (modification of the authorisation of a feed additive). The dossier was received on 15 March 2021 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00150. The particulars and documents in support of the application were considered valid by EFSA as of 19 May 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, consumer, user and the environment and on the efficacy of the feed additive consisting of canthaxanthin produced with *Y. lipolytica* CBS 146148 (CAROPHYLL® Red 10%) when used under the proposed conditions of use (see Section 3.1.6).

1.2 | Additional information

The additive under assessment is authorised as zootechnical additive for use in feed for breeder hens (4d161g).[3] Canthaxanthin is also authorised as a sensory feed additive (functional group: colourants) for use in feed for chickens for fattening, minor poultry species for fattening, laying poultry, poultry reared for laying, ornamental fish, ornamental birds and ornamental breeder hens (2a161g).[4] Both authorisations refer to canthaxanthin produced via chemical synthesis, while canthaxanthin produced by fermentation is currently not authorised.

The FEEDAP Panel adopted two opinions on the use of canthaxanthin, one as zootechnical (EFSA FEEDAP Panel, 2013) and one as colourings (EFSA FEEDAP Panel, 2014).

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[5] in support of the authorisation request for the use of canthaxanthin produced with *Y. lipolytica* CBS 146148 (CAROPHYLL® Red 10%) as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 26 May 2021 to 26 August 2021; the comments received were considered for the assessment.

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' (elicitation) knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the canthaxanthin in animal feed are valid and applicable for the current application.[6]

[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]DSM Nutritional Products Ltd., represented in the EU by DSM Nutritional Products Sp. Z o.o., Tarczyńska 113, 96-320 Poland.

[3]COMMISSION IMPLEMENTING REGULATION (EU) No 684/2014 of 20 June 2014 concerning the authorisation of canthaxanthin as a feed additive for breeder hens (holder of the authorisation DSM Nutritional products Ltd). OJ L 182, 21.6.2014, p. 20.

[4]COMMISSION IMPLEMENTING REGULATION (EU) 2015/1486 of 2 September 2015 concerning the authorisation of canthaxanthin as feed additive for certain categories of poultry, ornamental fish and ornamental birds, OJ L 229, 3.9.2015, pp. 5–8.

[5]Dossier reference: FAD-2021-0015.

[6]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/reports-and-technical-documentation/fad-2008-0048_en.

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the modification of the terms of the authorisation of the additive consisting of canthaxanthin produced with *Yarrowia lipolytica* CBS 146148 (CAROPHYLL® Red 10%) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: 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 FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018) and Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023).

3 | ASSESSMENT

The additive is currently authorised as a preparation containing 10% of canthaxanthin, produced by chemical synthesis, for use as a zootechnical additive (functional group: other zootechnical additives (stabilisation of reproductive performance)) in breeder hens.[8] The applicant is requesting a modification of the current authorisation, to include canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148 and to modify the additive specifications by substituting ethoxyquin with butylated hydroxy toluene (BHT) and increasing the limit for dichloromethane. For the current assessment, the additive will be referred to with its trade name CAROPHYLL® Red 10%.

3.1 | Characterisation

3.1.1 | Manufacturing process

The additional manufacturing process of the active substance canthaxanthin includes the fermentation step, the pasteurisation of the fermentation broth, the spray-drying of the biomass, the extraction of canthaxanthin from the biomass and the final crystallisation.[9]

For the manufacturing of the additive, the applicant declared that the process is the same as the one used for the formulation of canthaxanthin produced by chemical synthesis (EFSA FEEDAP Panel, 2013), with the exception of the replacement of ethoxyquin with BHT.

3.1.2 | Characterisation of the production organism

Canthaxanthin is produced by fermentation with a genetically modified strain of *Y. lipolytica* which is deposited [redacted] with deposit number CBS 146148.[10]

The taxonomic identification of the production strain as *Y. lipolytica* was confirmed using whole genome sequence (WGS) derived data [redacted].[11]

3.1.2.1 | *Characteristics of the parental microorganisms*

[redacted].[12]

[7]Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[8]Reg. (EU) No 684/2014. OJ L 182, 21.6.2014, p. 20.

[9]Technical dossier/Section II/ Sect_II_Identity.

[10]Technical dossier/Section II/Annex II.4.

[11]Technical dossier/Section II/Annex II.4.

[12]Technical dossier/Section II/Annex II.4.

3.1.2.2 | *Characteristics of the introduced sequences*

[redacted][13]

3.1.2.3 | *Structure of the genetic modification*

The structure of the genetic modification has been determined by comparing the WGS data of the production strain with [redacted] The absence of antimicrobial resistance genes or vector sequences in the production strain was demonstrated by the WGS analysis.

3.1.3 | Characterisation of the active substance

Canthaxanthin produced by fermentation is a crystalline powder of violet-brown colour and has a very low solubility in water (0.053 mg/L). The same purity is proposed for canthaxanthin produced by fermentation as specified in the current authorisation of the additive for the synthetic canthaxanthin, i.e. minimum 96%.

Compliance with this specification was shown in five batches with an average of 99% (range: 98%–100%).[14]

Impurities mainly consist of carotenoids, the amount of which is specified by the applicant as maximum 5% (chromatographic area). Analysis of five batches by high-performance liquid chromatography (HPLC) using spectrophotometric detection showed an average of 4% (range 4%–5%). Further chromatographic analysis indicated that the main carotenoids identified are metabolic precursors of canthaxanthin.[15] The most abundant are echinenone (around 2.5%) and 4-keto-gamma carotene (4-KGC; around 1.5%), followed by minor amounts of beta-carotene and gamma-carotene (below 0.4%), and traces of lycopene (around 0.05% or below). The sum of unknown carotenoid impurities (HPLC-UV/VIS) represents 0.1%–0.3% area, individually all below 0.2% area.

The comparison of the carotenoids profile of the active substance produced by fermentation and by chemical synthesis (EFSA FEEDAP Panel, 2013) showed that the two canthaxanthins are equivalent. The FEEDAP Panel concludes that the data submitted support the read across of the toxicological profile obtained with the synthetic canthaxanthin to the one produced by fermentation.

The applicant further investigated the presence of impurities other than carotenoids that could derive from the fermentation ingredients, such as amino acids, lipids and residues of the antifoaming agent. Proximate analyses on five batches indicate very low residual amounts of amino acids (0.01%–0.08%) and free fatty acids (0.9%–1.1%); mono-, di- or triglycerides of fatty acids were not detected. No residues of the anti-foaming agent were detected.[16]

Five batches of canthaxanthin produced by fermentation were analysed for cadmium, mercury, lead and arsenic, which were below their respective limit of quantification (LOQ).[17] The levels of dichloromethane and [redacted] were on average [redacted][18] [redacted]. The current authorisation sets a limit for dichloromethane for the additive of ≤ 10 mg/kg.

[13]Technical dossier/Section II/Annex II.4.

[14]Technical dossier/Section II/Annex_II_1. Sum of total carotenoids expressed as canthaxanthin – UV spectrophotometry.

[15]Technical dossier/Section II/Annex_II_3.

[16]Technical dossier/Section II/Annex II_8. The limit of detection (LOD) was not indicated.

[17]Technical dossier/Section II/Annex_II_1, and Supplementary information December 2022. LOQs in mg/kg were: 0.1 for lead cadmium and arsenic and 0.01 for mercury.

[18]Technical dossier/Section II/Annex_II_1.

No data were provided on the levels of mycotoxins and microbial contaminants. However, considering the raw materials and the manufacturing process, the FEEDAP Panel considers that microbial contamination/presence of mycotoxins is unlikely.

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

The presence of viable cells of the production strain was investigated in three batches of an intermediate concentrate from the pilot scale production in triplicate [redacted] No viable cells of the production strain were detected in the analysed samples.[19]

The presence of DNA from the production strain was investigated in three batches of the intermediate concentrate from the pilot scale. [redacted] No DNA was detected. [redacted].[20]

3.1.4 | Characterisation of the additive

The current authorisation specifies that the additive contains 10% canthaxanthin, ≤ 2.2% ethoxyquin and ≤ 10 mg dichloromethane/kg additive. The applicant requested the modification of these specifications by substituting ethoxyquin by 4.4% BHT and increasing the limit for dichloromethane to 80 mg/kg.

The updated composition of the additive CAROPHYLL® Red 10% is 10% canthaxanthin, 4.4% BHT, 10% dextrin (yellow), 15% corn starch and 60.6% lignosulfonate.

The applicant provided compositional data from five batches of the CAROPHYLL® Red 10%, containing canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148, which showed an average value of 10.9% canthaxanthin (range: 10.3%–11.5%).[21] The loss on drying was in the range of 5.3%–7.4%.[22]

Dichloromethane levels were in the range of 25–66 mg/kg.[23] The FEEDAP Panel notes that these levels are above the maximum levels set in the authorisation (≤ 10 mg dichloromethane/kg additive) but are well below both the new limit proposed (≤ 80 mg/kg) and the level of 600 mg/kg set in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL18(R2) (VICH, 2023), and are therefore considered to be of no concern.

CAROPHYLL® Red 10% appears as a red-violet free-flowing granulated powder ('beadlet'). Three batches of the additive CAROPHYLL® Red 10% were tested for its physical properties. The average loose density was 640 kg/m^3, the average tapped density was 750 kg/m^3. The particle size distribution was analysed by laser diffraction method; the results showed that the average particle size ranged from 333 to 381 μm and no particles below 150 μm were detected in any of the batches.[24] The dusting potential was determined using the Stauber-Heubach method and resulted in an average of 0.88 g/m^3 (range: 0.45–1.43 g/m^3) (mg airborne dust per m^3 of air).

Further investigation was performed by the applicant on the presence of small/nanoparticles providing data on particle size/solubility in line with the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA SC, 2021).[25] [redacted].[26]

3.1.5 | Stability and homogeneity

The applicant provided stability and homogeneity studies with CAROPHYLL® Red 10% formulated with BHT and canthaxanthin from fermentation with *Y. lipolytica* CBS 146148. In addition, the shelf-life of the additive containing synthetic canthaxanthin was also studied in the same experiment.

[19]Technical dossier/Section II/Annex II_4.
[20]Technical dossier/Supplementary Information October 2021/DSM Canthaxanthin 0014 SIn – Annex.
[21]Technical dossier/Section II/Annex II.2.
[22]Technical dossier/Section II/Annex II.2.
[23]Technical dossier/Section II/Annex II.2.
[24]Technical dossier/Section II/Annex_II_11.
[25]Technical dossier/Supplementary information January 2023/DSM Canthaxanthin Art13 SIn x2 - Main Annex and Annex 4.
[26]Technical dossier/Supplementary information January 2023/Annex 3.

3.1.5.1 | *Shelf-life*

Stability testing was carried out at 25°C/60% relative humidity (RH) and at 40°C/75% RH with CAROPHYLL® Red 10% formulated with either synthetic (one batch) or fermentation-derived canthaxanthin (three batches). The recovery of canthaxanthin after 12-month storage was 90% (synthetic) and 95% (average of three batches, fermentation based) at 25°C/60% RH and 90% (synthetic) and 92% (average of three batches, fermentation based) at 40°C/75% RH.[27]

3.1.5.2 | *Stability in premixtures and feedingstuffs*

The stability of the additive CAROPHYLL® Red 10% (three batches) in a vitamin and mineral premixture (containing 5.4% choline chloride) was studied when supplemented at 1 g canthaxanthin/kg and stored at 25°C for 1, 3 and 6 months and at 30°C for 1 month. Recoveries of canthaxanthin after 3 months were on average 81% (range: 79%–85%) and after 6 months were on average 67% (range: 63%–70%) when stored at 25°C. Recoveries of canthaxanthin after 1 month were 91% in all batches when stored at 30°C.[28]

The stability of the additive CAROPHYLL® Red 10% (three batches) in two wheat-based compound feeds pelleted at 90°C was studied when supplemented at 20 mg canthaxanthin/kg feed, stored in plastic bags at 20—25°C with 50%–60% RH for 3 months. Recoveries of canthaxanthin after the end of storage period ranged from 82% to 102% in one feed and from 82% to 86% in the other feed. The pelleting process did not result in a loss of canthaxanthin content.[29]

3.1.5.3 | *Homogeneity*

The capacity for homogeneous distribution of CAROPHYLL® Red 10% was studied in 10 subsamples of the mash feed (one batch) and in 10 subsamples of the pelleted feed (two batches). The supplementation level was 20 mg canthaxanthin/kg feed. The coefficient of variation of the analysis of the canthaxanthin concentration resulted in 5.3% for the mash feed and ranged from 6.2% to 7.0% for the pelleted feed.[30] The Panel notes that the level of supplementation used in this study is more than three times the maximum authorised level.

3.1.6 | Conditions of use

The additive containing canthaxanthin (CAROPHYLL® Red 10%) is currently authorised to be used in feed for breeder hens at a minimum and maximum content of 6 mg canthaxanthin/kg complete feed.[31]

Maximum residue limits (MRLs) are also set in the authorising regulation as follows:

– 15 mg canthaxanthin/kg liver (wet tissue) and 2.5 mg canthaxanthin/kg skin/fat (wet tissue)

The applicant has not proposed any changes in the conditions of use linked to the modification of the current authorisation.

3.2 | Safety

The safety of CAROPHYLL® Red 10% (preparation of synthetic canthaxanthin) for all poultry for breeding purposes was previously evaluated by the FEEDAP Panel in 2013 (EFSA FEEDAP Panel, 2013). In that opinion, the FEEDAP Panel concluded that synthetic canthaxanthin from CAROPHYLL® Red 10% is safe under the proposed conditions of use for the target species, consumer and environment. In the same opinion, based on the data submitted, it was concluded that canthaxanthin is not an irritant to skin or eyes and it is unlikely to be a skin sensitiser. In the absence of any information, CAROPHYLL® Red 10% was considered to be as an irritant to skin and eyes and a skin sensitiser. Exposure by inhalation of users, when handling CAROPHYLL® Red 10%, was expected to be minimal.

For the present assessment, the applicant made reference to the previous evaluation done by the FEEDAP Panel in 2013 (EFSA FEEDAP Panel, 2013), provided a literature search covering the safety aspects of the additive related to the safety of canthaxanthin for the target animal and toxicology, submitted a new tolerance study in chickens for fattening and new

[27]Technical dossier/Section II/Annex_12.

[28]Technical dossier/Section II/Annex_11 and Supplementary information January 2023/Annex 2.

[29]Technical dossier/Section II/Annex_11 and Supplementary information January 2023/Annex 2.

[30]Technical dossier/Section II/Annex_11.

[31]The authorising regulation states under 'other provisions': (1) In the directions for use of the additive and premixture, indicate the storage conditions and stability to heat processing. (2) The mixture of different sources of canthaxanthin shall not exceed 6 mg canthaxanthin/kg of complete feedingstuff. (3) The mixture of this preparation with canthaxanthin and other carotenoids is allowed provided that the total concentration of the mixture does not exceed 80 mg/kg of complete feedingstuff.

4. For user safety: breathing protection, safety glasses and gloves should be worn during handling.

genotoxicity studies performed with CAROPHYLL® Red 10% containing the canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148. No new additional information was provided regarding user safety.

The tolerance study was not further considered in the present assessment as it was done in chickens for fattening.

The applicant has proposed the substitution of ethoxyquin with BHT in the formulation of the additive CAROPHYLL® Red 10%. The FEEDAP Panel notes that BHT is at present authorised for all animal species and it is under re-evaluation.

3.2.1 | Safety of the production strain

The production strain belongs to a species, *Y. lipolytica*, considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach when used for production purposes (EFSA BIOHAZ Panel, 2023). The identity of the strain has been confirmed and the genetic modifications do not introduce safety concerns. In addition, no viable cells or DNA of the production strain were detected in an intermediate concentrate representing the additive. Therefore, the use of *Y. lipolytica* CBS 146148 in the production of canthaxanthin is considered of no concern with regard to the production strain and/or potential fermentation residues that might be present in the final additive.

3.2.2 | Literature search

The applicant provided two literature searches covering the period 2014–2020. The first one was carried out in SCOPUS and PubMed and focused on the safety of canthaxanthin for the target species. A total of 58 papers were identified, of which 17 were considered relevant by the applicant.[32] The second one was conducted on MEDLINE, FSTA and TOXCENTER and focused on the toxicology of canthaxanthin. The safety for the environment was not included in the literature search. A total of 288 hits were retrieved of which 15 were considered relevant by the applicant.[33]

The FEEDAP Panel reviewed the relevant papers and concluded that none of them identified safety concerns of canthaxanthin for the target species or the consumer under the current conditions of authorisation.

3.2.3 | Genotoxicity studies

In the previous evaluations, no concern for genotoxicity was identified for the synthetic canthaxanthin (EFSA ANS Panel, 2010; EFSA FEEDAP Panel, 2013, 2014).

Considering that the purity of canthaxanthin from *Y. lipolytica* CBS 146148 is comparable with the purity of synthetic canthaxanthin (Section 3.1.3), the conclusions reached for synthetic canthaxanthin in previous FEEDAP Panel assessments can be extended to the canthaxanthin produced by *Y. lipolytica* CBS 146148. Moreover, the strain under assessment qualifies for the QPS approach and is considered safe.

In addition, the applicant submitted genotoxicity studies performed with CAROPHYLL® Red 10% containing 10% canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148, which are reported below.

3.2.3.1 | *Bacterial reverse mutation test*

In order to investigate the potential of the test item (CAROPHYLL® Red 10%) to induce gene mutations in bacteria, an Ames test was performed in Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA in accordance with OECD Guideline 471, in a study claimed to be compliant with good laboratory practices (GLP).[34] Two independent experiments were performed applying the plate incorporation and pre-incubation methods both in the presence and absence of metabolic activation. Six concentrations were tested ranging from 33 to 5000 μg/plate (corresponding to 3.3–500 μg canthaxanthin/plate). No toxicity was observed in any experimental condition. All positive control chemicals induced significant increases in number of revertant colonies confirming the sensitivity of the test system and efficacy of the metabolic activation system. No increase in the mean number of revertant colonies was observed in any tested condition in any tester strain. The FEEDAP Panel concludes that the test item did not induce gene mutations in bacteria under the experimental conditions applied in this study.

3.2.3.1.1 | *In vitro mammalian cell micronucleus test*

To evaluate the potential of the test item (CAROPHYLL® Red 10%) to induce chromosomal damage, an in vitro micronucleus test was carried out in human peripheral blood lymphocytes according to OECD Test Guideline 487 (2016), in a study claimed to be compliant with GLP.[35] The highest concentration tested was limited by the occurrence of precipitation at 213

[32]Technical dossier/Section III/Annex III-1 and Annex III-1bis.

[33]Technical dossier/Section III/Annex-III-4_literature-toxicology_FIZ33996.

[34]Technical dossier/Section III/Annex III_6.

[35]Technical dossier/Section III/Annex III_7.

μg/mL after short treatment (4 h + 16 h of recovery) and 107 μg/mL after continuous treatment (20 h + 20 h of recovery), corresponding to 21.3 and 10.7 μg/mL of canthaxanthin, respectively. Cytochalasin B (Cyt B) was added 20 h after the beginning of treatment and the cells were cultured for additional 20 h (in total 88 h of culturing). Therefore, in the short-term treatment, a 16-h recovery period in the absence of CytB was applied. The Panel noted that this is a deviation from the OECD TG 487, recommending the addition of CytB at the end of treatment. The long-term test was performed in line with OECD TG 487. No cytotoxicity and no increase of the frequency of micronuclei were observed in binucleated cells after treatment with the test item in any experimental condition. The FEEDAP Panel concludes that the test item did not induce structural and numerical chromosomal aberrations in human peripheral blood lymphocytes under the experimental conditions applied in this study.

Conclusions on genotoxicity

The FEEDAP Panel noted that the two in vitro studies were performed with the formulated additive containing only 10% of the active substance canthaxanthin and, thus, considered the results of limited relevance.

In addition, the FEEDAP Panel took into account that the conclusions reached for synthetic canthaxanthin in previous opinions raising no concern for genotoxicity (EFSA ANS Panel, 2010; EFSA FEEDAP Panel, 2013, 2014) could be extended to canthaxanthin produced by *Y. lipolytica* CBS 146148 since the purity of canthaxanthin from *Y. lipolytica* CBS 146148 is comparable with the purity of synthetic canthaxanthin (Section 3.1.3). Moreover, the FEEDAP Panel noted that the strain under assessment qualifies for the QPS approach and is considered safe. Based on all the available data, the FEEDAP Panel concludes that canthaxanthin produced with *Y. lipolytica* CBS 146148 does not raise concern for genotoxicity.

3.2.4 | Assessment of the safety of the additive

The use of *Y. lipolytica* CBS 146148 in the production of canthaxanthin is considered of no safety concern.

Considering that canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148 is equivalent to the canthaxanthin produced by chemical synthesis, the safety of canthaxanthin produced by fermentation can be based on the read across of the metabolic/residues and toxicological profile obtained with the synthetic canthaxanthin without the need of additional studies.

No new studies were found in the literature that would change the previous conclusions on the safety of the canthaxanthin for the target species and consumer.

The FEEDAP Panel considers that the conclusions previously reached for synthetic canthaxanthin with regard to target animals, consumer and environment, also apply to canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148.

3.2.5 | Conclusions on the safety of the additive

The FEEDAP Panel concludes that using canthaxanthin produced with *Y. lipolytica* CBS 146148 in the formulation of CAROPHYLL® Red 10% is considered safe for the target species, the consumer and the environment under the current authorised conditions of use for synthetic canthaxanthin.

Canthaxanthin is not irritant to skin and eyes and unlikely to be a skin sensitiser. No conclusion can be reached on the respiratory sensitisation of canthaxanthin. In the absence of data with the additive CAROPHYLL® Red 10%, no conclusions can be reached regarding the safety of the additive for the user.

3.3 | Efficacy

In the opinion adopted by the FEEDAP Panel in 2013, it was concluded that canthaxanthin from CAROPHYLL® Red 10% at a concentration of 6 mg/kg complete feed has the potential to stabilise the reproductive performance of breeder hens as measured by hatchability and related parameters after incubation of eggs, particularly in phases of reduced hatchability of eggs from breeder hens fed canthaxanthin-free diets.

Canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148 has been shown to have the same purity as canthaxanthin produced by chemical synthesis, and it is considered by the FEEDAP Panel as equivalent.

Consequently, the conclusions reached in the previous opinion on CAROPHYLL® Red 10% containing synthetic canthaxanthin are considered also applicable to CAROPHYLL® Red 10% containing canthaxanthin produced by fermentation with *Y. lipolytica* CBS 146148, without the need for additional studies.

The FEEDAP Panel concludes that the additive is efficacious in breeder hens at 6 mg/kg complete feed.

4 | CONCLUSIONS

CAROPHYLL® Red 10% containing canthaxanthin produced by fermentation with *Yarrowia lipolytica* CBS 146148 is safe for the target species, consumer and environment under the current authorised conditions of use for CAROPHYLL® Red 10%.

Regarding user safety, canthaxanthin is not irritant to skin and eyes and unlikely to be a skin sensitiser. No conclusion can be reached on the respiratory sensitisation of canthaxanthin. In the absence of data with the additive CAROPHYLL® Red 10%, no conclusions can be reached regarding the safety of the additive for the user.

CAROPHYLL® Red 10%, containing canthaxanthin produced by fermentation with *Yarrowia lipolytica* CBS 146148, is efficacious in breeder hens at 6 mg/kg complete feed.

5 | RECOMMENDATIONS

The FEEDAP Panel recommends that:

- The provision 'The mixture of different sources of canthaxanthin shall not exceed 6 mg canthaxanthin/kg of complete feedingstuff' should be amended in the current authorisation for synthetic canthaxanthin and in the eventual authorisation of the product under assessment as follows: 'The mixture of different sources of the active substance canthaxanthin shall not exceed 6 mg canthaxanthin/kg of complete feedingstuff.'
- Considering that xanthophylls is a subgroup of carotenoids, the provision 'The mixture of this preparation with canthaxanthin and other carotenoids is allowed provided that the total concentration of the mixture does not exceed 80 mg/kg of complete feedingstuff' should be amended in the current authorisation for synthetic canthaxanthin and in the eventual authorisation of the product under assessment as follows: 'The mixture of this additive with other additives containing canthaxanthin and other carotenoids is allowed provided that the total concentration of the mixture does not exceed 80 mg total carotenoids/kg of complete feedingstuff.'

ABBREVIATIONS

ADI	acceptable daily intake
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
MRL	maximum residue limit
QPS	Qualified Presumption of Safety
RH	relative humidity

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REQUESTOR

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