SCIENTIFIC OPINION

Scientific opinion on the safety and efficacy of CAROPHYLL® Red 10% (preparation of canthaxanthin) for all poultry for breeding purposes (chickens, turkeys and other poultry)[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 17 January 2014, replaces the earlier version published on 8 January 2013.[4]

ABSTRACT

CAROPHYLL® Red 10 % (active substance canthaxanthin) is intended for use as an additive for all poultry species for breeding purposes at a concentration of 6 mg/kg complete feed. Canthaxanthin from CAROPHYLL® Red 10 % is safe for breeder hens at the proposed dose. The safety of the use level can be extrapolated to other minor poultry breeder hens. The maximum proposed canthaxanthin concentration in feed for breeder hens does not exceed that already authorised for pigmenting eggs of the same animal category. Consequently, the intake of edible tissues and products from canthaxanthin-treated poultry does not exceed the acceptable daily intake with respect to the established maximum residue limits for poultry tissues. No concerns regarding consumer safety would arise from the use of canthaxanthin in the additive in breeder poultry at the proposed dose. The use of lignosulphonate, the major constituent of the additive, as a carrier in CAROPHYLL® Red 10 % is considered to be safe for the consumer. CAROPHYLL® Red 10 % was not tested for potential as an irritant or sensitiser. In the absence of any information on lignosulphonate, it would be prudent to consider the additive as an irritant to skin and eyes and a skin sensitiser. The risk to users from inhalation toxicity is low. Considering the oxidative susceptibility of carotenoids, the use of CAROPHYLL® Red 10 % will not result in a risk to the environment. Canthaxanthin from CAROPHYLL® Red 10 % has the potential to stabilise the reproductive performance of breeder hens, particularly during phases when the hatchability of eggs from breeder hens fed canthaxanthin-free diets is reduced. An extrapolation of this conclusion to other breeder poultry species is not possible owing to inconsistencies in the timing of the effects observed and the absence of a scientifically sound theory on the mode of action.

KEY WORDS

zootechnical additive, CAROPHYLL® Red 10 %, canthaxanthin, poultry for breeding purposes, safety, efficacy

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[1] On request from the European Commission, Question No EFSA-Q-2011-00083, adopted on 12 December 2012.

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[4] Revision 1: This scientific opinion has been edited following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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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 an opinion on the safety and efficacy of CAROPHYLL® Red 10 % (preparation of canthaxanthin) for all poultry for breeding purposes (chickens, turkeys and other poultry). Canthaxanthin is a naturally occurring red carotenoid (β-Carotene-4,4'-dione).

Canthaxanthin from CAROPHYLL® Red 10 % was safe for breeder hens at the proposed dose of 6 mg/kg complete feed with a margin of safety of at least four. Safety of the canthaxanthin use level can be extrapolated to minor poultry breeder hens.

The maximum proposed canthaxanthin concentration in feed for breeder hens (6 mg/kg) does not exceed that already authorised for pigmenting eggs of the same animal category (8 mg/kg). Consequently, the intake of edible tissues and products from canthaxanthin treated poultry does not exceed the acceptable daily intake when respecting the established MRLs for poultry tissues. Eggs produced for breeding purposes are normally not consumed. Even given the unlikely case that all eggs for breeding would be consumed as table eggs, no concerns regarding consumer safety would arise from the use of canthaxanthin from CAROPHYLL® Red 10 % in breeding poultry at the dose of 6 mg/kg complete feed.

The FEEDAP Panel considered the use of lignosulphonate as a carrier in CAROPHYLL® Red 10 % to be safe for the consumer.

Canthaxanthin is not an irritant to skin and eyes and unlikely to be a skin sensitiser. CAROPHYLL® Red 10 % was not tested for irritancy or sensitisation potential. In the absence of any information on lignosulphonate, the major constituent of the additive, it would be prudent to consider the additive as an irritant to skin and eyes and a skin sensitiser. The exposure by inhalation of users, when handling CAROPHYLL® Red 10 %, was expected to be minimal. Consequently, the risk of inhalation toxicity is low.

Considering the oxidative susceptibility of carotenoids, the use of CAROPHYLL® Red 10 % at the proposed maximum concentration of 6 mg canthaxanthin/kg complete feed for breeder hens will not result in a substantial increase in the canthaxanthin concentration in the environment and consequently does not pose a risk to the environment.

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. An extrapolation of this conclusion to other breeder poultry species was not possible owing to inconsistencies in the timing of the effects observed and the absence of a scientifically sound theory on the mode of action.

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BACKGROUND

Regulation (EC) No 1831/2003[5] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company DSM Nutritional Products Spz.o.o.[6] for authorisation of the product Carophyll® Red 10 % (preparation of canthaxanthin) when used as a feed additive for turkeys for breeding purposes, other poultry for breeding purposes (category: zootechnical; functional group: d) other Zootechnical additive (improved reproductive performance of poultry breeders)) under the conditions mentioned in Table 1.

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). EFSA received directly from the applicant the technical dossier in support of this application.[7] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 31 March 2011.

Canthaxanthin (E161g) is authorised as a feed additive under Council Directive 70/524/EEC[8] as a sensory additive for poultry. Regulation (EC) No 775/2008[9] established the maximum residue limits for canthaxanthin when used as feed additive in poultry other than laying hens, laying hens and salmonids.

Canthaxanthin is authorised as food colour according to Directive 94/36/EC[10] (use in Saucisses de Strasbourg at an inclusion rate of 15 mg/kg).

The Scientific Committee on Food (SCF) issued opinions on canthaxanthin in 1984, 1989 and 2000 (EC, 1984, 1989, 2000). The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety of canthaxanthin for salmon and trout, laying hens, and other poultry (EC, 2002). EFSA issued an opinion on the Maximum Residue Limits for canthaxanthin in foodstuffs coming from animals fed with canthaxanthin used as a colouring feed additive (EFSA, 2007) and an opinion on the re-evaluation of canthaxanthin (E161g) as food additive (EFSA, 2010a).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 animal(s), consumer, user and the environment and the efficacy of the product Carophyll Red® (canthaxanthin), when used under the conditions described in Table 1.

[5] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[6] DSM Nutritional Products Spz.o.o., Ul. Tarcynska 113, PL 96-320 Mszczonow, Poland.
[7] EFSA Dossier reference: FAD-2010-0407.
[8] Directive 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs. OJ L 270, 14.12.1970, p.1.
[9] Commission Regulation (EC) No 775/2008 of 4 August 2008 establishing maximum residue limits for the additive canthaxanthin in addition to the conditions provided for Directive 2003/7/EC. OJ L 207, 5.8.2008, p.5.
[10] 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.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Canthaxanthin preparation
Registration number/EC No/No (if appropriate)	Not defined
Category(-ies) of additive	Zootechnical Additive
Functional group(s) of additive	(d) other zootechnical additive (Improved reproductive performance of poultry breeders)

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Active substance: Canthaxanthin	$C_{40}H_{52}O_2$	Min. 96 %	Spectrophotometric/HPLC
Preparation of canthaxanthin		Min. 10 %	Spectrophotometric

Trade name (if appropriate)	CAROPHYLL® Red
Name of the holder of authorisation (if appropriate)	DSM Nutritional Products Ltd.

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
Poultry for fattening Poultry for laying (table eggs) Poultry for laying (liquid eggs for processed food)	All poultry for breeding purposes: (chickens, turkeys, other poultry)	2	6	None

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	The use of CAROPHYLL® Red in breeder feed shall start 2 weeks before the start of lay and until the end of the laying period
Specific conditions or restrictions for handling (if appropriate)	None
Post-market monitoring (if appropriate)	None
Specific conditions for use in complementary feedingstuffs (if appropriate)	None

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of Animal	Target tissue(s) or Food products	Maximum content in Tissues

ASSESSMENT

1. Introduction

Canthaxanthin (E161g) is authorised without a time limit under Council Directive 70/524/EEC as a sensory additive at a maximum content of 8 and 25 mg/kg complete feedingstuffs for laying hens, and for poultry other than laying hens and salmon and trout, respectively.[11] Regulation (EC) No 775/2008[12] established the maximum residue limits (MRLs) for canthaxanthin when used as colouring feed additive in poultry other than laying hens, laying hens and salmonids.

Canthaxanthin is authorised as a food colour according to Directive 94/36/EC[13] (use in saucisses de Strasbourg at an inclusion rate of 15 mg/kg).

The Scientific Committee on Food (SCF) issued opinions on canthaxanthin in 1984, 1989 and 2000 (EC, 1984, 1989, 2000). The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety of canthaxanthin for salmon and trout, laying hens and other poultry (EC, 2002). EFSA issued an opinion on the maximum residue limits for canthaxanthin in foodstuffs coming from animals fed with canthaxanthin used as a colouring feed additive (EFSA, 2007) and an opinion on the re-evaluation of canthaxanthin (E161g) as food additive (EFSA, 2010a).

This dossier seeks authorisation for a new use of a preparation of canthaxanthin (CAROPHYLL® Red 10 %) in poultry for breeding (2–6 mg/kg complete feedingstuff, later modified to a single concentration of canthaxanthin of 6 mg/kg complete feed[14]), within the category zootechnical additive, functional group others (the applicant proposed: enhancer of the productive performance of poultry breeders).

Canthaxanthin is marketed in Europe by the applicant in a stabilised form under the trade name CAROPHYLL® Red 10 %. Other trade names with different canthaxanthin concentrations are also used outside the European Union.

2. Characterisation

2.1. Identity of the additive

CAROPHYLL® Red 10 % contains the active substance canthaxanthin. The composition of CAROPHYLL® Red 10 % contains (w/w) 10 % canthaxanthin, 2.2 % ethoxyquin, 62.8 % lignosulphonate, 10 % dextrin (yellow) and 15 % corn starch.

Analysis of six batches of CAROPHYLL® Red 10 % indicated product consistency: mean canthaxanthin content was 10.3 % (range: 10.1–10.4 %; in a second set of five batches 10.1–11.1 %).[15,16,17] The loss on drying of these batches averaged 5.5 %.

The additive is a red-violet free-flowing powder. Analysis of three batches of CAROPHYLL® Red 10 %[18] by laser light diffraction showed that the product did not contain particles <100 μm (and only 0.05 % of the particles were between 100 and 150 μm; all other particles were >150 μm). The dusting potential of the three batches was measured (Stauber–Heubach test), with the result being 2.74 g/kg

[11] List of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ C 50, 25.2.2004, p. 1.
[12] OJ L 207, 5.8.2008, p. 5.
[13] OJ L 237, 10.09.1994, p. 13.
[14] Supplementary information/May 2012.
[15] Technical dossier/Section II/Annex 17.
[16] Technical dossier/Section II/Annex 20.
[17] Supplementary information/ May 2012.
[18] Technical dossier/Section II/Annex 20.

additive (corresponding to about 0.7 g/m^3). The active substance in dust amounted to only 3.2 % (compared with 10 % in the additive). The bulk density was 0.6 kg/L.

Three batches were analysed for heavy metals and arsenic.[19] The levels of arsenic, lead, mercury and cadmium were all <1 mg/kg. The level of the residual solvent dichloromethane (<10 mg/kg) was below the VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products) threshold (600 mg/kg). TPPO (insert full name) was not detected in three batches of crystalline canthaxanthin (limit of detection <50 mg/kg).[20]

2.2. Characterisation of the active substance

Canthaxanthin (β-carotene-4,4′-dione, CAS number 514-78-3) is a chemically synthesised substance. Its molecular formula is $C_{40}H_{52}O_2$, and its molecular weight is 564.85 g/mol. Its structural formula is given in Figure 1.

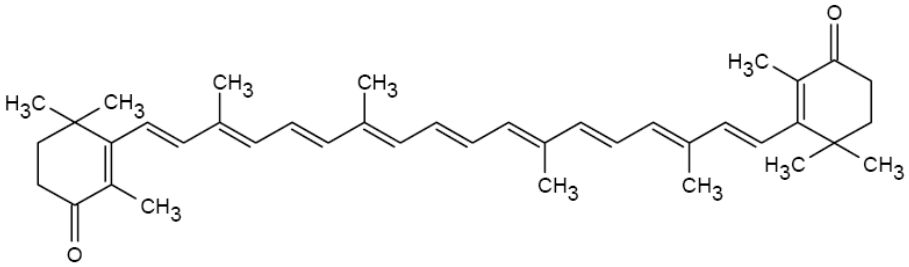

Figure 1: Structural formula of canthaxanthin

Canthaxanthin is rather insoluble in water (<0.053 mg/L at 20 °C) and in ethanol but soluble in chloroform. The calculated K_{ow} (K_d/K_{oc}) is 14.1.

The analysis[21] of three batches of canthaxanthin indicated a mean purity of 98 % and a mean subsidiary matter of 1.3 %. The mean values for acetone, petrol ether and methylene chloride were 0.2 %, 0.83 % and 1.5 %, respectively. The mean values for heavy metals (expressed as Pb) lead, mercury, cadmium and arsenic were <10, <2, <1, <1 and <1 mg/kg, respectively.

2.3. Manufacturing processes[22]

The manufacturing process of the product is fully described in the technical dossier.

2.4. Stability and homogeneity

2.4.1. Shelf-life of the additive

Shelf-life of the additive (two batches of CAROPHYLL® Red 10 %) was evaluated under two different sets of environmental conditions: 15 °C (relative humidity (RH) not specified) up to 36 months and 40 °C/75 % RH for 6 months. The data indicate a shelf-life under conventional conditions of 3 years (recovery of canthaxanthin 92 %). Recovery of canthaxanthin under accelerated conditions was 94.1 % after 6 months. In another three batches shelf-life was found to be 24 months (highest recovery 97 %) when kept at 15 °C.[23]

2.4.2. Stability in premixtures

The stability of one batch of CAROPHYLL® Red 10 % in a mineral–vitamin premixture for poultry was demonstrated over 3 months (recovery about 94 % of the initial canthaxanthin content) when kept

[19] Technical dossier/Section II/Annex 17.
[20] Supplementary information/May 2012.
[21] Technical dossier/Section II/Annex 14.
[22] This section has been edited following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[23] Supplementary information/May 2012.

at 25 °C. Another three batches were incorporated in vitamin–mineral premixtures (intended concentration of canthaxanthin 1.0 g/kg) and stored for 6 months in plastic bags at 25 °C. Essentially no losses were found.[24]

2.4.3. Stability in feed

The stability of CAROPHYLL® Red 10 % in complete feed for poultry and during processing (conditioning at 80, 85 and 90 °C, pelleting at 78, 85 and 87 °C) was studied in three batches at an intended canthaxanthin concentration of 20.5 mg/kg.[25] Samples were stored at 24 °C. Neither feed processing nor storage for 3 months affected the stability of canthaxanthin.

2.4.4. Homogeneity

The content uniformity of canthaxanthin in a pelleted broiler feed (intended concentration 5 mg/kg) was studied for three batches of CAROPHYLL® Red 10 %, in eight subsamples of each of the three compound feeds. The overall coefficient of variation of the analytical values (*X* mg/kg feed) was 5.9 %, indicating the capacity of the additive to distribute homogeneously in feed.[26]

2.5. Physicochemical incompatibilities in feed

Pure crystalline canthaxanthin is sensitive to oxidation, light and heat. Thus, it is mandatory that it is produced as a stabilised form of canthaxanthin, such as CAROPHYLL® Red 10 %. According to our current knowledge, no incompatibilities resulting from the use of canthaxanthin in compound feed are expected.

2.6. Conditions of use

The applicant proposes the use of CAROPHYLL® Red 10 % as a zootechnical additive (to enhance the productive performance of all poultry for breeding purposes) to be used in all poultry for breeding purposes (chickens, turkeys and minor poultry) from weeks before the onset of laying until the end of the laying period. The recommended inclusion level of canthaxanthin in feed was originally 2–6 mg/kg complete feed and was later modified to a single concentration of 6 mg/kg complete feed.[27]

2.7. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the active ingredient/marker residue in tissues. The executive summary of the EURL report can be found in the Appendix.

3. Safety

3.1. Safety for the target species

A total of 270 breeder hens (Lohmann) of 18 weeks of age were fed for 4 weeks a commercial pre-breeder canthaxanthin-free diet. At the age of 22 weeks, the hens (1 646 g average body weight) were distributed to five groups consisting of nine replicates (three cages of two hens each per replicate) and feed maize–soya bean-type mash diets containing 0, 6, 12, 24 or 60 mg canthaxanthin/kg from Carophyll Red 10 % for 29 weeks. The intended canthaxanthin concentrations in feed were analytically confirmed. Once per week the hens were artificially inseminated with semen from roosters then kept on a basal diet without addition of canthaxanthin. Body weight, feed conversion, laying and reproductive performance as well as the colour of the shanks of hens and their eggs (egg yolk) were determined in regular intervals. Furthermore, deposition of canthaxanthin in eggs (all treatments after eight weeks of supplementation) was measured. At the end of the trial, the canthaxanthin content in

[24] Supplementary information/May 2012.
[25] Technical dossier/Section II/Annex 26.
[26] Technical dossier/Section II/Annex 18.
[27] Supplementary information/ May 2012.

edible tissues (thigh muscle, liver, kidney, abdominal fat and skin) was analysed in two hens per replicate (groups fed 0 and 6 mg canthaxanthin/kg). Blood samples were taken from two hens per replicate of the groups fed 0, 6 and 60 mg canthaxanthin for determination of haematology and clinical chemistry parameters. Post-mortem evaluation and histopathological examination of livers and kidneys were performed. Chicks, hatched from eggs of hens after 14 weeks of canthaxanthin supplementation, were reared on a feed without canthaxanthin for seven days for analysis of performance as well as of blood and tissue canthaxanthin concentrations. The data were statistically evaluated by analysis of variance, linear contrast analysis and Duncan's multiple range test for group differences. The main performance parameters are summarised in Table 2.

Table 2: Performance of breeder hens treated with different dietary concentrations of canthaxanthin

	mg canthaxanthin/kg feed				
	0	6	12	24	60
Initial Body weight (g)	1633	1625	1670	1647	1656
Final body weight (g)	2064	1989	1991	2008	1997
Laying rate (%)	82.0	86.3	82.7	84.3	83.7
Egg weight (g)	56.7^{b}	58.8^{a}	57.6^{ab}	57.3^{b}	57.6^{ab}
Feed to egg mass ratio	2.16	2.07	2.08	2.09	2.12
mg canthaxanthin/kg egg yolk[1]	0.0^{e}	17.5^{d}	36.3^{c}	68.6^{b}	144.7^{a}
Fertility (%)[2]	89.4	88.3	91.5	90.5	84.5
Hatchability (%)[2]	75.2	73.6	76.1	75.5	61.7
Navel abnormalities (%)[2]	5.43^{b}	6.89^{b}	5.01^{b}	8.88^{a}	11.15^{a}

(1): Eight weeks after start of the trial
(2): Five incubations, four week intervals
$^{a, b, c, d, e}$: means in a row with different superscripts are significantly different ($P < 0.05$).

The canthaxanthin content of egg yolks showed a significant linear correlation with the dietary canthaxanthin concentrations.The final canthaxanthin content in liver (3.6 mg/kg), kidney (0.8 mg/kg), abdominal adipose tissue (0.9 mg/kg) and skin 0.2 mg/kg) in the group receiving 6 mg canthaxanthin/kg feed was significantly different from that in the unsupplemented control group (no canthaxanthin found).

Haematology (carried out only in groups receiving 0, 6 and 60 mg canthaxanthin/kg feed) did not show differences between the groups except a somewhat lower albeit significant erythrocyte count and haematocrit in the control group. Plasma creatinine values were significantly higher in the 6 mg canthaxanthin/kg group than in the control group and were also significantly higher in the 60 mg canthaxanthin group than in both the control and the 6 mg canthaxanthin/kg group. The plasma concentration of uric acid showed a tendency to be higher in the 60 mg canthaxanthin group. All other clinical chemistry parameters were unaffected by the treatment. No treatment-related adverse effects were seen at necropsy or histopathological examination.

Seven-day body weight gain and feed to gain ration of the progeny were not influenced by the treatment of the hens. Shank colour on day 1 was significantly higher in chickens from treated hens than in those from control hens. The canthaxanthin concentration in the liver of the progeny (one and seven days after hatch) was significantly correlated to the canthaxanthin supply of the hens, the tocopherol content of the livers being unaffected by the treatment.

3.1.1. Conclusion on the safety for breeding poultry

No toxic signs could be observed in breeder hens given the 10-fold of the recommended use level of canthaxanthin in feed for 29 weeks in terms of zootechnical parameters or haematological or clinical biochemical endpoints. However, a significant increase in navel abnormalities of hatched chickens and of plasma creatinine in the group receiving the high canthaxanthin dose together with an 18 %

reduction, although not significant, of hatchability could be regarded as indication of an intolerance at the highest canthaxanthin dose.

Canthaxanthin from CAROPHYLL® Red 10 % is safe for breeder hens at the proposed dose of 6 mg/kg complete feed with a margin of safety of at least four. Safety of the canthaxanthin use level can be extrapolated to minor poultry breeder hens.

3.2. Safety for the consumer

3.2.1. Metabolic and residue studies

3.2.1.1. Poultry

No new data have been submitted by the applicant concerning the metabolic fate and residues of canthaxanthin in poultry, reference being made to the FEEDAP Panel assessment (EFSA, 2007).

The main conclusions of that assessment were the following: (i) oral administration of canthaxanthin to poultry results in canthaxanthin deposition in tissues; (ii) the liver appears to be the target tissue in poultry; moreover, the skin/fat and the egg could be considered to be further target tissues; (iii) canthaxanthin is by far the major component of residues in target tissues and egg yolk; and (iv) despite the fact that canthaxanthin-related metabolites (reduced compounds 4′-hydroxyechinenone and zeaxanthin) occur in poultry tissues, the only adverse effects observed are directly related to canthaxanthin (the basis of the acceptable daily intake, ADI). Consequently, the FEEDAP Panel considered canthaxanthin to be the only residue of concern. Canthaxanthin (measured as the all-*trans* isomer) is the marker residue.

3.2.1.2. Laboratory animals

Most studies on canthaxanthin metabolism in laboratory animals (rat and cynomolgus monkey) submitted by the applicant have been assessed recently by the by the EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS Panel) in an opinion on the re-evaluation of canthaxanthin as a food additive (EFSA, 2010a). Absorption, distribution, metabolism and excretion (ADME) studies in the rat and monkey indicated limited absorption of canthaxanthin (3 % and 9 %, respectively), distribution to liver, spleen, adipose tissue and adrenal glands (in decreasing order) and slow elimination from the adipose tissue. Urinary metabolites in the rat contained some very polar compounds, which were present only in trace amounts in monkeys, while monkey urine contained some less polar compounds that were absent in rat urine. In the monkey retina, 4′-hydroxyechinenone and isozeaxanthin were identified as metabolites. No similar investigation was carried out in rats.

A published study[28] concerning the identification of a major metabolite of canthaxanthin in the rat has been submitted. Rats (10 weeks old) were administered by gavage a single dose of 0.2–mg [6,7,6′,7′-^{14}C]-canthaxanthin. The cumulative urine output (at 4 days) as analysed (by HPLC) and a major metabolite was isolated and identified as 3-hydroxy-4-oxo-7,8-dihydroxy-β-ionone in the conjugated form (glucuronide), using gas chromatography–mass spectrometry and nuclear magnetic resonance spectroscopy. This metabolite results from the cleavage of the polyene chain at C9, hydroxylation at C3 and reduction at C7–C8. The result does not contradict the metabolic studies performed in poultry in which: (i) the use of canthaxanthin labelled on C15,C15′ did not allow the tracing of metabolites arising from splitting at C6,C6′; and (ii) conjugation of metabolites was not investigated.

3.2.1.3. Humans

ADME data in humans were assessed by the EFSA ANS Panel in 2010 (EFSA, 2010a). In humans, a part of the orally ingested canthaxanthin was absorbed (8–34 % of the dose), 60 % of the absorbed dose being transferred to fat tissue. No data on canthaxanthin biotransformation were reported in humans (EFSA, 2010a).

[28] Technical dossier/Section III/Annex 17.

In a pilot study submitted by the applicant, canthaxanthin 10 % beadlets were administered orally to one volunteer in tea at a concentration of 1 g beadlets per 100 mL tea (91.8 mg total canthaxanthin with a *cis*-isomer content of 20.4 %).[29] Plasma levels of canthaxanthin were recorded at frequent intervals over the following 168 hours. Canthaxanthin plasma base levels were 25 and 28 ng/mL. After approximately 0.75 hours, the same plasma level (30 ng/mL was measured). Thereafter, plasma canthaxanthin levels rose, reaching a peak concentration after approximately 4.92–7.67 hours (457–482 ng/mL). About half of this level was detected after 24–31.7 hours and canthaxanthin plasma level further declined to 96 ng/mL after 168 hours.

3.2.2. Toxicological studies on canthaxanthin

The toxicology of the active ingredient of canthaxanthin was recently reviewed by the EFSA ANS Panel (EFSA, 2010a). The FEEDAP Panel had access to some but not all of the full reports of studies that were considered as part of the ANS Panel's review. The data available to the ANS Panel covered many aspects of toxicology including acute toxicity, repeat-dose subchronic and chronic toxicity, carcinogenicity, mutagenicity, reproductive and developmental toxicity, immunotoxicology and special studies of effects on the eye. There are no outstanding issues regarding the toxicology of canthaxanthin that still need to be addressed.

The FEEDAP Panel had access to a few reports of studies that were not mentioned in the ANS Panel's opinion, but these were poorly reported, uninterpretable or added nothing to the evaluation. The exceptions to this were two mutagenicity studies which gave negative results and strengthened the ANS Panel's conclusion that canthaxanthin is not genotoxic. The findings of these studies are summarised below.

3.2.2.1. Genotoxicity studies including mutagenicity

Canthaxanthin crystalline (purity 97.1 %) was tested in the reverse mutation assay in *Salmonella enterica* subsp. *enterica* serovar Typhimurium (strains TA1535, TA1537, TA98 and TA100) and in *Escherichia coli* (strain WP2uvrA).[30] The test was performed in two independent experiments in the presence and absence of S9-mix (rat liver S9-mix induced by a combination of phenobarbital and β-naphthoflavone), in compliance with Organization for Economic Cooperation and Development (OECD) Guideline 471 (revised 1997). Some precipitation was observed on the plates at the start and at the end of the incubation period at concentrations of 3 330 and 5 000 μg/plate. The test substance did not induce a significant dose-related increase in the number of revertants at a concentration range of 100–3 330 μg/plate both in the absence and in the presence of metabolic activation. The negative and strain-specific positive controls performed as expected.

The possible clastogenicity and aneugenicity of canthaxanthin crystalline (purity 97.1 %) was tested in an *in vitro* micronucleus assay in cultured peripheral human lymphocytes.[31] The substance was tested in the presence and absence of a metabolic activation system (phenobarbital- and β-naphthoflavone-induced rat liver S9-mix) in two independent experiments, according to OECD Guideline 487. In the first experiment, canthaxanthin was tested up to 33 μg/mL for a three-hour exposure time with a 27-hour harvest time in the absence and presence of the S9-fraction. In the second experiment, the substance was tested up to 10 μg/mL for a 24-hour exposure time with a 24-hour harvest time in the absence of S9-mix. A precipitate was observed in the culture medium at 33 μg/mL and (slightly) at 10 μg/mL. The test item did not induce a statistically significant or biologically relevant increase in the number of mono- and binucleated cells with micronuclei in the absence and presence of S9-mix in either of the two independently repeated experiments, whereas the positive control chemicals had a significant effect.

[29] Technical dossier/Section III/Annex 11.
[30] Supplementary information/May 2012.
[31] Supplementary information/ May 2012.

3.2.2.2. Conclusion on genotoxicity of canthaxanthin

On the basis of a bacterial reverse mutation study and of an *in vitro* micronucleus assay, canthaxanthin is not considered to be of concern for genotoxicity.

3.2.3. Acceptable daily intake

The ANS Panel (EFSA, 2010a) set an ADI of 0.03 mg/kg body weight for canthaxanthin by applying an uncertainty factor of 10 to a NOAEL (no observed adverse effect level) of 0.25 mg/kg body weight per day for scotopic b-wave changes (without impairment of vision) in humans and a $BMDL_{05}$ (benchmark dose (lower confidence limit)) of 0.2–0.33 mg/kg body weight per day for crystal incidence in a meta-analysis of findings of crystals in the retina of humans exposed to canthaxanthin. The value of this ADI was identical to the value of ADIs previously set by the EU Scientific Committee for Food (SCF, 1997) and by the Joint World Health Organization/Food and Agriculture Organization Meeting on Food Additives (JECFA, 1995).

The FEEDAP Panel notes that it could be argued that a lower ADI of 0.02 mg/kg body weight could have been set for canthaxanthin if the lowest value of the $BMDL_{05}$ (0.02 mg/kg body weight/day) were used and if the NOAEL was rounded down to one significant figure (0.02 mg/kg body weight/day) rather than rounding up. Nevertheless, the FEEDAP Panel agrees that an ADI of 0.03 mg/kg body weight should be used for canthaxanthin in order to maintain international harmonisation with the ADI set by JECFA.

3.2.4. Toxicological studies of lignosulphonate

The FEEDAP Panel notes that the ANS Panel opinion (EFSA, 2010b) states that the consumer safety of lignosulphonate used as a carrier in vitamin and carotenoid preparations cannot be assessed from the available data. The ANS Panel considered that long-term toxicological studies are needed. However, the FEEDAP Panel also notes that lignosulphonate was non-genotoxic in mutagenicity tests and had a NOAEL of 2 000 mg/kg body weight per day (the highest dose tested) in a 90-day toxicological study in rats. The NOAEL is five orders of magnitude greater than the maximum estimate of consumer exposure (0.02 mg/kg body weight/day)[32] resulting from use of CAROPHYLL® Red 10 %, at the highest dose authorised for chickens for fattening or salmonids. Therefore, the FEEDAP Panel considers the use of lignosulphonate as a carrier in CAROPHYLL® Red 10 % to be safe for consumers.

3.2.5. Consumer safety

The maximum proposed canthaxanthin concentration in feed for breeder hens does not exceed that already authorised for pigmenting eggs of the same animal category. Consequently, the intake of edible tissues and products from canthaxanthin-treated poultry and salmonids does not exceed the ADI with respect to the established MRLs for poultry (and salmonid) tissues.[33]

Eggs produced for breeding purposes are not normally consumed. Even given the unlikely case that all eggs for breeding were consumed as table eggs, there would be no concern for consumer safety arising from the use of canthaxanthin from CAROPHYLL® Red 10 % in breeding poultry at the dose of 6 mg/kg complete feed.

[32] In its lifetime a 2-kg broiler consumes 3 kg feed containing 25 mg canthaxanthin per kg. Total intake of canthaxanthin is then 75 mg, of CAROPHYLL® Red 10 % 750 mg and of lignosulphonate 471 mg (62.8 % of CAROPHYLL® Red 10 %). Assuming a 1 % retention of lignosulphonate (Becquet, 2012), 4.71 mg lignosulphonate would be retained in a 2-kg broiler, corresponding to 2.36 mg in a 1-kg broiler. This figure is considered to be edible tissue. An intake of 500 g edible tissue would provide the consumer with 1.18 mg daily, corresponding to 0.02 mg/kg body weight per day.

[33] OJ L 207, 5.8.2008, p.5.

3.3. Safety for the user

3.3.1. Effects on the respiratory system

The additive under application has a moderate dusting potential (0.7 g/m^3) and does not contain any particles of inhalable size (<100 μm); the canthaxanthin concentration in the dust is only about one-third of that in the additive. The exposure of the user by inhalation is expected to be minimal.

3.3.2. Effects on the eyes and skin

The applicant submitted one study that that conformed to OECD Test Guideline 404 and to good laboratory practice (GLP).[34] Canthaxanthin caused no primary irritation of rabbit skin when applied for 4 hours under a semiocclusive dressing.

The applicant submitted one study that conformed to OECD Test Guideline 405 and to GLP.[35] Canthaxanthin caused no irritation when instilled into the conjunctival sac of rabbit eyes.

The applicant submitted one pre-GLP study that did not conform to any OECD Test Guideline.[36] In the Maurer optimisation test in guinea pigs, no sensitising potential was detected.

The applicant submitted two reports[37] of observations in workers from two different plants: no cases of irritation or hypersensitivity following exposure to canthaxanthin during production were observed.

Considering (i) the absence of adverse findings in the Maurer optimisation test, (ii) the absence of observations of adverse effects in workers and (iii) the conclusion of the ANS Panel (EFSA, 2010a) that canthaxanthin had caused no biologically significant adverse effects in studies on its potential for allergenicity, hypersensitivity and intolerance, the FEEDAP Panel considers it unlikely that exposure to canthaxanthin as a result of the use of CAROPHYLL® Red 10 % would cause skin sensitisation.

3.3.3. Conclusions on user safety

The exposure by inhalation of users when handling the additive is expected to be minimal. Consequently, the risk of inhalation toxicity is low. Canthaxanthin is not an irritant to skin and eyes and unlikely to be a skin sensitiser.

CAROPHYLL® Red 10 % has not been tested for irritancy or sensitisation potential. In the absence of any information on lignosulphonate, the major constituent of the additive, it would be prudent to consider the additive as an irritant to skin and eyes and a skin sensitiser.

3.4. Safety for the environment

Based on the calculation method provided in EFSA technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008), assuming that 100 % of a maximum concentration (6 mg CAROPHYLL® Red 10 % per kg complete feed) will be excreted, the predicted environmental concentrations (PECs) for canthaxanthin in pore water and surface water were well below the trigger values. The PEC for canthaxanthin in soil was around the trigger value. However, considering the oxidative susceptibility of carotenoids, the use of CAROPHYLL® Red 10 % at the proposed maximum concentration in feed will not result in a substantial increase in the canthaxanthin concentration in the environment and consequently does not pose a risk to the environment.

4. Efficacy

The applicant provided a literature review on the possible mode of action of canthaxanthin on the reproductive performance of breeder hens.[38] In the view of the author of that review, results from the

[34] Technical dossier/Section III/Annex 82.
[35] Technical dossier/Section III/Annex 81.
[36] Technical dossier/Section III/Annex 48.
[37] Technical dossier/Section III/Annex 18, 66.

review present consistent evidence that canthaxanthin fed to poultry breeders is deposited in the egg yolk and then acts in eggs and embryo cells as a part of the antioxidant system. The antioxidative capacity protects the embryo from oxidative stress linked to the high metabolic activity as a result of rapid cell development and differentiation. Such a protective effect in the embryo could be linked to an improvement in the overall reproductive performance (measured by the number of chickens produced per breeder hen) of poultry breeders fed a diet containing canthaxanthin. Further, a wealth of data from non-breeder layers also confirms that deposited canthaxanthin exerts a protective antioxidant effect in the yolk of table eggs. In the view of the FEEDAP Panel, this hypothesis needs further research and supporting results, particularly from studies with other antioxidants.

The applicant submitted, defined by its own terminology, four core studies (raw data available) and two complementary studies (without raw data) with the aim of demonstrating the influence of canthaxanthin from CAROPHYLL® Red 10 % on the fertility of eggs from breeder hens. Among these studies, only the oldest trial was performed in Europe (Spain, published by Llaurado et al. as a conference paper in 1997); four trials were conducted in Brazil and one in China. The requirement of Regulation (EC) No 429/2008[39] ("such studies must permit the evaluation of the efficacy of the additive according to common farming practices in the EU") can be considered to be substantially met as the farming of poultry, and particularly of breeder hens, is largely harmonised at a global level.

4.1. The core studies

4.1.1. Experiment 1

Llaurado et al. (1997)[40] studied the effect of graded levels of canthaxanthin on hatchability in a total of 160 broiler breeder hens (Cobb; four treatment groups; 40 hens/treatment) housed in individual cages. The treatment started when the hens were 26 weeks of age and ended after 13 weeks. The hens were fed a basal diet in mash form consisting mainly of barley, wheat, maize and soybean with calculated values of 2 880 kcal ME/kg, 17 % CP, 0.65 % methionine + cysteine, 3.70 % calcium and 0.46 % inorganic phosphorus). The basal diet, supplemented with 10 000 IU vitamin A and 30 mg vitamin E/kg, was calculated to contain 2.24 mg xanthophylls/kg.[41]

The hens were allocated to random blocks, based on the laying rate in the 4 weeks before the start of the experiment. The experimental groups received the basal feed without canthaxanthin supplementation and supplemented with 2, 4 or 6 mg canthaxanthin from CAROPHYLL® Red 10 % per kg, respectively. The intended concentrations of canthaxanthin (2.0, 4.0 and 6.0 mg/kg) were not analytically confirmed. Only an indirect conclusion on the dietary content via the canthaxanthin concentration in egg yolks could be made using a linear regression proposed in the dossier[42] and derived from data published in a FEEDAP Panel opinion on canthaxanthin of (EFSA, 2007). The regression data confirmed the intended dietary levels.

The hens were artificially inseminated during four periods (in week 34, 35, 38 and 39), for which hatchability (expressed as number of chicks hatched per 100 fertile incubated eggs) was measured. Also the carotenoid concentration and pigmentation of the egg and of the shanks of 1-day-old chicks were analysed. Table 4 summarises the results of the study. Canthaxanthin improved overall hatchability by 3.5–6.5 %; however, the differences were significant ($P < 0.05$) only between the control and the highest supplementation group. Canthaxanthin supplementation also had a significant effect on the canthaxanthin concentrations of the egg yolk and shanks of the 1-day-old chickens. Also total xanthophylls in the egg yolk rose with increasing dietary canthaxanthin supplementation, although equal xanthophyll levels have to be assumed in the four diets.

[38] Supplemetary information/May 2012/Annex 2.
[39] 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.
[40] Supplemetary information/July 2011/Section IV/Annex 3.
[41] Supplementary information/May 2012.
[42] Supplemetary information/May 2012/Annex 1.

Table 4: Effect of canthaxanthin on the hatchability of fertile eggs and pigmentation of egg yolks and shanks of 1-day-old chickens (40 hens per group)

Canthaxanthin (mg/kg feed)[1]	Control	Canthaxanthin		
	0	2	4	6
Hatchability (%)[2,3]				
Week 34	85.4	90.2	89.3	92.1
Week 35	87.5	80.4	87.5	91.4
Week 38	81.6	91.8	88.7	90.3
Week 39	87.6	91.1	88.5	90.6
Average	85.5[b]	88.5[a,b]	88.5[a,b]	91.1[a]
Egg ratio (%)	78.6	78.3	76.1	77.4
Egg yolk – week 39 (*n*)	6	6	6	6
Canthaxanthi (mg/kg)[4]	0.0[c]	8.0[b]	10.6[b]	16.2[a]
Total xanthophylls (mg/kg)	4.7	11.1	18.4	25.6
Vitamin A (IU/kg)	17 086	17 383	17 633	18 750
Shanks of 1-day-old chicks (*n*)	5	5	5	5
Canthaxanthin (mg/kg)[4]	0.00[b]	0.95[a]	1.62[a]	1.62[a]
Lutein (mg/kg)	0.30	0.32	0.30	0.24
Zeaxanthin (mg/kg)	0.28	0.42	0.36	0.30
Roche colour fan units[5]	3.0[b]	12.6[a]	11.6[a]	14.8[a]

[1]Analysed concentration in the diets not given.
[2]Laying rate at 76 %, i.e. 840 eggs per treatment in 4 weeks.
[3]Means with different superscripts within a row are significantly different (P <0.05).
[4]Means with different superscripts within a row are significantly different (P <0.001).
[5]Means with different superscripts within a row are significantly different (P <0.01).

4.1.2. Experiment 2

The experiment of Rocha (2011)[43] was conducted in two sheds with the same batch of Cobb broiler breeders in a commercial operation. The animals were housed in five neighbouring sheds, each consisting of 8 000 females and 800 males. Between 40 and 45 weeks of age, fertility and hatching in terms of total incubated eggs and incubated fertile eggs from the five sheds was monitored for the selection of the two sheds used in the experiment. Over this period the selected sheds showed a mean total hatchability of 88.0 % for the group to be treated later with canthaxanthin and 86.5 % for the future control group. Feeding of the experimental diets in mash form started at the age of 46 weeks and ended at 60 weeks of age; feed was offered on a restricted basis.

Both sexes were fed an unsupplemented diet and a diet with added 6 mg canthaxanthin from CAROPHYLL® Red 10 % per kg feed; the analysed level in feed given when the birds were 50 weeks of age was 6.15 mg/kg layer diet (and 6.91 mg/kg cockerel diet). The basal diet consisted mainly of milled maize, wheat bran and soybean meal with small differences between the diets for female and male poultry. The basal diet (calculated contents of the layer diet: 2 720 kcal ME/kg, 14.4 % CP, 0.54 % methionine + cysteine, 3.2 % calcium and 0.60 % inorganic phosphorus) was supplemented with 9 000 IU vitamin A, 32 mg vitamin E/kg, and 75 mg zinc bacitracin/kg and calculated to contain 9.32 mg xanthophylls/kg layer diet (and 8.98 mg/kg cockerel diet).[44] Two experimental designs for different experimental questions were followed, a certain number of eggs being the experimental basis. Laying rate was not measured in the experimental weeks.

In trial 1 eggs were collected from both sheds when the poultry were 50, 51, 55, 59 and 60 weeks of age. All the eggs were from morning collections, and the eggs from the first collection were removed to prevent eggs laid on the previous day from affecting the results. Hatching eggs were classified by weight range and only eggs of 64–76.5 g were used in the experiment. For the fertility analysis, 10 trays with 84 eggs per treatment and time were used, the tray being regarded as the experimental unit

[43] Supplemetary information/July 2012/Section IV/Annex 5.
[44] Supplementary information/May 2012.

(replicate). Fertility was assessed at two points: at day 11 of incubation day and at day 21 of incubation. Infertile eggs and dead embryos were identified. Table 5 summarises the results.

Table 5: Fertility of eggs (%) from breeder hens treated with/without canthaxanthin from 46 weeks of age onwards

Canthaxanthin (mg/kg diet)	Age of breeder hens (weeks)					Average
	50	51	55	59	60	
0	94.9	95.4	90.8	87.3	88.9	91.5[b]
6	98.1	97.5	94.5	91.1	91.2	94.5[a]

Different superscript letters in the same column indicate a significant difference (P <0.0001).

Supplementation of a layer diet with 6 mg canthaxanthin/kg significantly improved the fertility of incubated eggs on average in five selected weeks between 50 and 60 weeks of age after a pre-feeding period of at least 4 weeks. A decline in broiler breeder fertility with increasing age was also observed.

In trial 2 (with hens of 59 weeks of age) the fertility parameters of the four treatments were studied in more depth than in trial 1. Eggs (14 replicates = trays of 84 eggs/treatment) were collected from both canthaxanthin groups and stored for 3 and 7 days, respectively, before incubation. The results are given in Table 6.

Fertility rate and hatchability (total and fertile eggs) were significantly improved by canthaxanthin. Canthaxanthin significantly reduced late embryonic mortality (days 15–21), whereas early embryonic mortality (days 0–7) was not influenced by the dietary treatment. The addition of canthaxanthin to the diet increased the concentrations of canthaxanthin and vitamin A and reduced the amount of vitamin E in egg yolk.

Table 6: The effect of canthaxanthin on hatchability and embryonic mortality

	Control			Canthaxanthin		
Canthaxanthin (mg/kg feed)	**0**			**6**		
Storage period (days)	**3**	**7**	**Mean**	**3**	**7**	**Mean**
Fertility (%)	88.9	87.7	88.3[b]	91.2	90.1	90.6[a]
Total hatching rate (%)[1]	80.0	77.0	78.5[b]	84.3	81.0	82.6[a]
Hatchability of fertile eggs (%)[2]	90.0	87.9	88.9[b]	92.4	89.9	91.1[a]
Early embryonic mortality (%)[3]	4.7	4.5	4.6	3.5	5.5	4.5
Late embryonic mortality (%)[3]	30.9	6.1	5.0[b]	30.2	3.6	3.4[a]

[1]Hatched chicks per total incubated eggs.
[2]Hatched chicks per fertile eggs.
[3]Early mortality = days 0–7; late mortality = days 15–21, including pipped eggs.
Means within a row with different superscripts are significantly different (P <0.05).

Storing eggs for 7 days reduced egg quality, delayed embryonic development, reduced hatchability and increased embryonic mortality after 15 days of incubation.

4.1.3. Experiment 3

Rosa and Weber (2010)[45] studied for 21 weeks the effect of canthaxanthin on the productive and reproductive development of broiler breeders (46 weeks old at the start of the experiment). A total of 360 Cobb 500 broiler breeder hens (and 36 males) were allocated to two treatments (six replicates/treatment, 30 hens/replicate).

The diet was a simple maize–soybean mixture containing by calculation 2 850 kcal ME/kg, 16 % CP, 0.55 % methionine + cysteine, 3.3 % calcium, 0.4 % available phosphorus, 0.69 mg selenium/kg and 40 mg oxytetracycline/kg. It also contained per kg 10 450 IU vitamin A, 54 mg vitamin E, 3.24 mg

[45] Supplemetary information/July 2011/Section IV/Annex 6.

total carotenes and 10.15 mg total xanthophylls (calculated values).[46] The control group was fed the unsupplemented basal diet, the treatment group the basal diet plus 6 mg supplemental canthaxanthin (from CAROPHYLL® Red 10 %) per kg feed (analysed 4.5 mg/kg). Feed in mash form was offered on a restricted basis.

Reproductive performance was measured each week (from 46 to 66 weeks of age). The following incubation endpoints were determined: total hatchability, hatchability of fertile eggs, fertility rate, embryonic mortality, pecked eggs and weight (and quality) of chicks. All eggs, collected on any given day and considered as suitable for incubation, were used after a maximum storage period of 7 days.

No significant differences in broiler breeder weight and laying performance were seen in the pre-period (2–4 weeks) and during the trial period. Egg weight, albumen and yolk weight and specific gravity were not significantly influenced by the dietary treatment, whereas the coloration of the egg yolks in the canthaxanthin group was at any time point significantly higher (P <0.001) than in the control group.

Canthaxanthin significantly improved the average values of the 21-week trial period for total hatchability (83.0 % vs. 86.2 %; P <0.05), hatchability of fertile eggs (91.3 % vs. 93.7 %; P < 0.0005) and fertility (91.0 % vs. 92.1 %; P <0.05), and reduced embryonic mortality (5.5 % vs. 3.7 %; P <0.01); the greatest effect on embryonic mortality was observed in the first 48 hours and between days 15 and 21 of incubation.

It should be noted that the favourable effect of canthaxanthin on incubation parameters (on fertility) was not consistent over the whole trial period. Considering the weekly results, the effect of canthaxanthin on hatchability of fertile eggs reached significance only in 7 (weeks 9, 10, 13, 16, 17, 19 and 21) out of 21 weeks. Table 7 shows the mean values for the weeks with and without a significant canthaxanthin effect. From the mean values it can be concluded that a canthaxanthin effect becomes evident when the fertility of the control group is reduced.

Table 7: Hatchability of fertile eggs in weeks with or without a significant effect of canthaxanthin

	7 weeks with a significant canthaxanthin effect		**14 weeks without a significant canthaxanthin effect**	
Canthaxanthin (mg/kg)	0	6	0	6
Hatchability (%)	89.6	93.8	92.1	93.4

The number of contaminated eggs was also reduced by canthaxanthin supplementation (P < 0.001). While egg production and percentage of incubatable eggs per bird were not different between the treatment groups, the number of chicks per hen was significantly improved with canthaxanthin from 59.5 to 64.9 (P < 0.05). Canthaxanthin reduced the levels of thiobarbituric acid reactive substances (TBARS) in the egg yolks; the differences between the treatment groups became more pronounced with storage time (13.53 vs. 10.70 µg malondialdehyde (MDA)/mg protein at 0 days, P < 0.05; 28.97 vs. 16.86 µg MDA/mg protein at 12 days, P < 0.001). However, in incubated eggs that antioxidative effect of canthaxanthin was only observed until day 7 of incubation.

4.1.4. Experiment 4

The study by Santos et al. (2012)[47] was largely similar in design and conduct to that of Rosa and Weber (2010). After a pre-period of 2 weeks to allow allocation of the hens to groups similar in body weight, the effect of canthaxanthin on the productive and reproductive development of broiler breeders (29 weeks of age at the start) was studied for 34 weeks. A total of 264 Cobb 500 broiler breeder hens (and 24 males) was allocated to two treatments (six replicates/treatment, 22 hens/replicate).

[46] Supplementary information/May 2012.
[47] Supplementary information/May 2012/Annex 16.

The diet was a simple maize–soybean mixture with small differences between two phases (25–40 weeks of age, week 41 until the end of the study) containing by calculation 2 860 (phase 1)/2 850 (phase 2) kcal ME/kg, 16 % CP, 0.55 % methionine + cysteine, 3.0 % (phase 1)/3.3 % (phase 2) calcium, 0.45 % (phase 1)/0.4 % (phase 2) available phosphorus, 0.69 mg selenium/kg and 40 mg oxytetracycline/kg. It contained also per kg 10 450 IU vitamin A, 54 mg vitamin E, 3.2 mg total carotenes and 10.25 mg total xanthophylls (calculated values). The control group was fed the unsupplemented basal diet, the treatment group the basal diet plus 6 mg supplemental canthaxanthin (from CAROPHYLL® Red 10 %) per kg feed (analysed 5.8 mg/kg). Feed in mash form was offered on a restricted basis.

Laying rate was calculated weekly and body weight measured at 4-week intervals. The reproductive performance was measured each week (29–62 weeks of age). The following incubation endpoints were determined: total hatchability, hatchability of fertile eggs, fertility rate, embryonic mortality, pecked eggs and weight (and quality) of chicks. All eggs, collected on any given day and considered as suitable for incubation, were used after a maximum storage period of 7 days.

No significant differences in broiler breeder weight and laying performance were seen during the trial period. Egg weight, albumen and yolk weight and specific gravity were not significantly influenced by the dietary treatment, whereas the coloration of the egg yolks in the canthaxanthin group was at any time point significantly higher ($P < 0.001$) than in the control group.

Canthaxanthin significantly ($P > 0.05$) improved the average values over the 34-week trial period for total hatchability (86.2 % vs. 89.2 %), hatchability of fertile eggs (91.3 % vs. 92.4 %) and fertility (94.6 % vs. 96.9 %). The reduction in embryonic mortality (5.8 % vs. 5.0 %) failed to reach significance ($P = 0.0534$).

It should be noted that the favourable effect of canthaxanthin on incubation parameters (on fertility) was not consistent over the whole trial period. Considering the weekly results, the effect of canthaxanthin on hatchability of fertile eggs reached significance only in 5 (weeks 35, 38, 41, 48 and 49) out of 34 weeks. Table 8 shows the mean values for the weeks with and without a significant canthaxanthin effect. From the mean values it can be concluded that a canthaxanthin effect became evident when the fertility of the control group was reduced.

Table 8: Hatchability of fertile eggs in weeks with or without a significant effect of canthaxanthin

	5 weeks with a significant canthaxanthin effect		**29 weeks without a significant canthaxanthin effect**	
Canthaxanthin (mg/kg)	0	6	0	6
Hatchability (%)	89.9	95.1	91.6	91.9

No effect of the dietary treatment on the number of contaminated or pecked eggs was observed.

4.2. The complementary studies

In a field trial (Souza et al., 2008)[48], 5 848 females and 560 males received a control diet (sorghum/soybean meal/corn/wheat; 0 mg/kg analysed canthaxanthin) and 6 128 females and 559 males were fed the same basal diet supplemented with 6 mg/kg canthaxanthin per kg feed (4.45 mg/kg analysed canthaxanthin) for 8 weeks. After 4 weeks on the respective diets the eggs were collected and incubated. Significant differences were observed for all production parameters (Table 9).

[48] Supplemetary information/July 2011/Section IV/Annex 7.

Table 9: The effect of canthaxanthin on the production parameters of breeder hens in a commercial hatchery

	Control	Canthaxanthin
Canthaxanthin (mg/kg feed)	**0**	**96**
Infertility (%)	3.33[b]	1.81[a]
Embryonic mortality (%)	12.66[a]	10.81[b]
Hatchability (%)	82.66[b]	86.24[a]
Rate of incubation (%)	85.98[b]	88.05[a]
High-quality chickens (%)	79.61[b]	83.17[a]

Means within a row with different superscript letters are significantly different ($P < 0.05$)

Zhang et al., 2011[49] investigated in a total of 270 Chinese broiler breeder hens (two treatment groups, five replicates of 27 hens each) the effect of canthaxanthin on the performance of the hens and post-hatch neonatal chickens for 24 weeks (from 23 to 47 weeks of age). The hens received a basal diet (maize–soybean meal) with 0 (control) or 6 mg/kg canthaxanthin per kg feed (CAROPHYLL® Red 15 %) (analysed value 3.4 mg/kg). Fertility and hatchability were recorded every 3 weeks. At 24 weeks, 100 hatched chicks from both treatments (five replicates of 20 chicks each) were fed a commercial broiler diet for 21 days.

During weeks 13–16 an outbreak of colibacillosis resulted in a culling rate of 3.1 % in the control group and 10.4 % in the canthaxanthin group (overall: 3.7 % vs. 10.4 %).

There were no treatment-related effects on laying performance. Fertility, average hatchability, chick quality and percentage of healthy chicks were not influenced by the dietary treatment. However, canthaxanthin increased the egg yolk ratio ($P \leq 0.05$), yolk colorimetric score ($P \leq 0.001$) and shank colour of the newly hatched chicks ($P \leq 0.001$). Neither the performance, nor the cumulative mortality of the chickens was significantly influenced by the canthaxanthin supplementation over 21 days. TBARS values in the egg yolk and serum of the hens, reflecting antioxidative status, were not affected by the treatment, whereas the overall total antioxidative capacity (T-AOC) was significantly increased in both the serum (13.17 vs. 16.58 U/mL; $P < 0.05$) and egg yolk (1.87 vs. 3.16 U/g; $P \leq 0.001$). In the chickens, canthaxanthin reduced serum TBARS at days 1 and 7, but had no significant effect on T-AOC.

4.3. Conclusions on efficacy

Experiment 1 is considered to be a dose range-finding study, showing a significant improvement in the of hatchability of fertile eggs from supplementation with 6 mg canthaxanthin/kg feed for the average of four selected experimental weeks after a feeding pre-period of at least seven weeks. The main study in experiment 2 showed significant improvement in fertility rate and hatchability (total and fertile eggs) in older breeder hens (59 weeks of age) from supplementation with 6 mg canthaxanthin/kg only over 1 week. The results of both studies cannot be considered to be a convincing demonstration of the efficacy of canthaxanthin on the reproduction of breeder hens because of the short duration (four weeks and 1 week out of 35–40 production weeks). Trial 1 of experiment 2 was poorly designed and reported and covered only a very limited time span of five weeks.

Two trials with broiler breeder hens (experiments 3 and 4) fed diets with 6 mg supplemental canthaxanthin from CAROPHYLL® Red 10 % per kg feed and a duration of 21 and 34 weeks showed, as an average of the entire experimental period, a significant improvement of total hatchability, hatchability of fertile eggs and fertility.

From a comparison of the weekly data it can be concluded that a canthaxanthin effect on reproduction became evident when the fertility of the control group was reduced. The reasons for this reduction remain unknown. The small, however significant, differences calculated for the entire experimental period are driven by large differences in a few weeks.

[49] Supplemetary information/July 2011/Section IV/Annex 9.

It should be noted that in experiments 2, 3 and 4 diets containing antibiotics were used (zinc bacitracin in experiment 2, oxytetracycline in experiments 3 and 4). As a consequence, these studies could not have been performed in Europe. However, there is no suggestion of an interaction between these antibiotics and canthaxanthin. The FEEDAP Panel concludes that non-compliance with European feed safety regulations in three of the efficacy studies is not a reason for not considering their results.

The complementary studies essentially did not contribute to any new aspect of the role of canthaxanthin in the reproductive performance of breeder hens.

In summary, the FEEDAP Panel concludes 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 during phases when the hatchability of breeder hens fed canthaxanthin-free diets is reduced.

Owing to the inconsistent effect of canthaxanthin from CAROPHYLL® Red 10 % in studies with an adequate duration and the absence of a scientifically sound theory on the mode of its action, the potential of canthaxanthin to stabilise reproductive performance in breeder hens cannot be extrapolated to other poultry breeders.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for a specific post-market monitoring plan other than those requirements established in the Feed Hygiene Regulation[50] and good manufacturing practice.

CONCLUSIONS

Canthaxanthin from CAROPHYLL® Red 10 % is safe for breeder hens at the proposed dose of 6 mg/kg complete feed with a margin of safety of at least four. Safety of the canthaxanthin use level can be extrapolated to minor poultry breeder hens.

The maximum proposed canthaxanthin concentration in feed for breeder hens does not exceed that already authorised for pigmenting eggs of the same animal category. Consequently, the intake of edible tissues and products from canthaxanthin-treated poultry and salmonids does not exceed the ADI with respect to the established MRLs for poultry (and salmonid) tissues. Eggs produced for breeding purposes are not normally consumed. Even given the unlikely case that all eggs for breeding were consumed as table eggs, there would be no concerns regarding consumer safety arising from the use of canthaxanthin from CAROPHYLL® Red 10 % in breeding poultry at a dose of 6 mg/kg complete feed.

The FEEDAP Panel considers the use of lignosulphonate as a carrier in CAROPHYLL® Red 10 % to be safe for the consumer.

Canthaxanthin is not an irritant to skin or eyes and is unlikely to be a skin sensitiser. CAROPHYLL® Red 10 % has not been tested for potential as an irritant or sensitiser. In the absence of any information on lignosulphonate, the major constituent of the additive, it would be prudent to consider the additive as an irritant to skin and eyes and a skin sensitiser. Exposure by inhalation of users, when handling CAROPHYLL® Red 10 %, is expected to be minimal. Consequently, the risk for inhalation toxicity is low.

Considering the oxidative susceptibility of carotenoids, the use of CAROPHYLL® Red 10 % at the proposed maximum concentration of 6 mg canthaxanthin/kg complete feed for breeder hens will not result in a substantial increase in the concentration of canthaxanthin in the environment and consequently does not pose a risk to the environment.

[50] 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.

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 during phases when the hatchability of eggs from breeder hens fed canthaxanthin-free diets is reduced. It is not possible to extrapolate from this conclusion to other breeder poultry species owing to inconsistencies in the timing of the effects observed and the absence of a scientifically sound theory on the mode of action.

DOCUMENTATION PROVIDED TO EFSA

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2. CAROPHYLL® Red (canthaxanthin). Supplementary information. July 2011. Submitted by DSM Nutritional Products Spz.o.o.

3. CAROPHYLL® Red (canthaxanthin). Supplementary information. May 2012. Submitted by DSM Nutritional Products Spz.o.o.

4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for CAROPHYLL® Red (canthaxanthin).

5. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Carophyll® Red[51]

In the current application authorisation is sought under Article 4(1) for a *Canthaxanthin preparation, Carophyll® Red*, under the category/functional group 4(d) 'zootechnical additives'/'other zootechnical additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *Carophyll® Red* for turkeys and other poultry for breeding purposes. The *feed additive* is intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs*. The Applicant suggested 2 and 6 mg/kg respectively as minimum and maximum *Canthaxanthin* concentration in *feedingstuffs*.

For the determination of the *active substance*, *Canthaxanthin*, in the *feed additive*, the Applicant submitted a ring trial validated method based on spectrophotometry. The following performance characteristics were reported:

– a standard deviation for *repeatability* (RSD_r) ranging from 0.2 to 0.8 %;

– a standard deviation for *reproducibility* (RSD_R) ranging from 1.3 to 4.0 %, and

– a *recovery* rate (R_{Rec}) ranging from 96.2 to 105 %.

Based on the performance characteristics presented the EURL recommends for official control the ring trial validated spectrophotometric method, submitted by the Applicant, to determine *Canthaxanthin*, in the *feed additive*.

For the determination of *Canthaxanthin* in *premixtures* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified method based on Normal Phase High-Performance Liquid Chromatography coupled to VIS detection (NP-HPLC-VIS). The following performance characteristics were reported:

– RSD_r ranging from 1.4 to 15 %;

– a standard deviation for *intermediate precision* (RSD_{ip}) ranging from 2.1 to 14.8 %, and

– R_{Rec} ranging from 85.5 to 107 %.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified NP-HPLC-VIS method, submitted by the Applicant, to determine Canthaxanthin in premixtures and feedingstuffs.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[51] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0407.pdf