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Safety and efficacy of saponified paprika extract, containing capsanthin as main carotenoid source, for poultry for fattening and laying (except turkeys)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Gabriele Aquilina, Georges Bories, Jürgen Gropp, Derek Renshaw, Orsolya Holczknecht, Maria Vittoria Vettori and Maryline Kouba

Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of saponified paprika extract, containing capsanthin as main carotenoid source, for poultry for fattening and laying (except turkeys). The saponified paprika (*Capsicum annuum*) extract contains various carotenoids at a concentration of 25–90 g/kg of which capsanthin being the major one with quantity specified as > 35% of total carotenoids (TC). The maximum recommended use level of 40 mg TC/kg feed is safe for chickens for fattening and laying hens. The margin of safety is at least 6. This conclusion is extrapolated to minor poultry species for fattening and laying. The saponified paprika extract is not genotoxic. Based on the no observed effect level (NOEL) of the 90-day study in rat and the exposure estimates, the Panel considered that there would be an adequate margin of exposure (between 700 and 2000) to conclude that the level of exposure to residues of the saponified paprika (*C. annuum*) extract (capsanthin not less than 35% of TCs) in animal tissues and products does not raise concern for the safety for the consumer. The saponified paprika extract is a viscous paste and as such users will not be exposed by inhalation. The applicant recognises that the extract may be irritant to skin and eyes. The FEEDAP Panel cannot conclude on the potential of any preparation to be toxic by inhalation, skin/eye irritant or skin sensitiser since no data were submitted. The use of saponified paprika extract in poultry feed raised no concern for the environment. Saponified paprika extract has the potential to pigment broiler skin and egg yolk. This conclusion is extrapolated to minor poultry species for fattening and laying.

Keywords: capsanthin, extract of *Capsicum annuum*, paprika extract, sensory additives, colouring agents, poultry for fattening, poultry for laying

Requestor: European Commission

Question number: EFSA-Q-2010-01525

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from CARAC EEIG (Carotenoids Authorisation Consortium European Economic Interest Grouping)[2] for authorisation/re-evaluation of the product 'capsanthin', when used as a feed additive for poultry, cats and dogs, ornamental fish and birds (category: sensory additives; functional group: colourants) in feed and in water for drinking. During the assessment it was clarified with the applicant that the additive under assessment is the saponified paprika (*Capsicum annuum*) extract containing capsanthin as the main carotenoid source. During the assessment, the applicant requested a change in the species limiting the application to poultry for fattening (chickens for fattening and minor poultry for fattening) and poultry for laying (laying hens and minor laying poultry (ducks, partridges, and quails)).[3] The request for use in water was withdrawn.[4]

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 5 March 2012.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product 'capsanthin', when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

'Capsanthin' (E 160c) is included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. It is authorised without a time limit in application of Article 9t (b) of Council Directive 70/524/EEC[5] concerning additives in feedingstuffs (2004/C 50/01) for its use in poultry (with the exception of turkeys) as colourant ('carotenoids and xanthophylls') with a maximum concentration of 80 mg/kg of complete feedingstuffs (alone or with the other carotenoids and xanthophylls).

The common use of the terms 'carotenoids' and 'xanthophylls' are often misleading and needs therefore clarification. The term 'carotenoids' is the generic name for a class of hydrocarbons formally derived from the acyclic $C_{40}H_{56}$ structure, comprising 'carotenes' (non-oxygenated hydrocarbon forms) and 'xanthophylls' (oxygenated hydrocarbon forms, i.e. hydroxy and epoxy functions). They consist typically of a chain of eight isoprenoid units joined in such a manner that their arrangement is reversed at the centre (carbons 9 and 10) of the chain. Xantophylls are yellow compounds, more polar than carotenes.[6] Although the polyene chain double bonds present in lutein and zeaxanthin exist in a *cis* (*Z*) or *trans* (*E*) conformation, giving rise to a large number of isomers, the vast majority of carotenoids is in the all-*trans* configurations.

EFSA published an opinion in 2006 on the safety use of colouring agents in animal nutrition (including carotenoids from natural sources e.g. capsanthin from paprika) (EFSA, 2006).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] On 19/12/2012, the rights of CARAC/EEIG were transferred to FEFANA asbl, Rue de Trèves 45, 1040 Brussels, Belgium. Companies: NOVUS Europe, S.A., Spain; Industrial Tecnica Pecuaria S.A. (ITPSA), Spain; Sinotrade Technology UK Ltd. United Kingdom.
[3] Technical dossier/Supplementary information July 2015 and Supplementary information July 2017.
[4] Withdrawal received on 08/01/15.
[5] OJ C 50, 25.2.2004, p.1.
[6] https://www.qmul.ac.uk/sbcs/iupac/carot/car1t7.html

Paprika extract (E 160c), a natural dye with capsanthin and capsorubin being the principle colouring compounds, is authorised as a food colourant in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 (E 161c).[7] The specific purity criteria concerning the use of this food additive (E 161c) in foodstuffs are included in Regulation (EC) No 231/2012.[8] It is permitted *quantum satis* in food except for meat preparations and processed meat, in which it is allowed up to 10 mg/kg product, and foodstuffs in which the use of colours is specifically prohibited. The re-evaluation of this food additive was performed by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) in 2015 (EFSA ANS Panel, 2015).

Paprika extracts have been also evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA); the latest evaluation was performed in 2014 (JECFA, 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[9] in support of the authorisation request for the use of 'capsanthin' as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[10] and the applicable EFSA guidance documents. During the assessment, it was clarified with the applicant that the additive under assessment is the saponified paprika (*C. annuum*) extract containing capsanthin as the main carotenoid source.

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 to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[11]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of 'capsanthin' is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008a), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008b).

3. Assessment

The additive under assessment is the saponified extract of paprika (*C. annuum*) containing capsanthin as the main carotenoid source. The present opinion deals with the safety and efficacy of this product when used as a sensory feed additive (functional group: colourants) for poultry for fattening (chickens for fattening and minor poultry for fattening) and poultry for laying (laying hens and minor laying poultry (ducks, partridges, and quails)). The applicant proposes a maximum content of 40 mg total carotenoids (TC)/kg of complete feedingstuffs. When the additive is used in combination with other carotenoid containing additives, the TC content shall not exceed 80 mg/kg feed.

[7] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 345, 31.12.2008, p.16.

[8] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p.1.

[9] FEED dossier reference: FAD-2010-0373.

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

[11] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0373.pdf.

Data were provided by a consortium of three companies. It should be recognised that these data cover only a fraction of existing additives containing capsanthin.

The active colouring principles in the extracts need protection against oxidation and are therefore marketed as formulated preparations only.

3.1. Characterisation

The additive under assessment is the saponified extract of paprika (*C. annuum*) containing various carotenoids at a concentration of 25–90 g/kg of which capsanthin being the major one with quantity specified as > 35% of total carotenoids (TC).[12]

3.1.1. Characterisation of the saponified paprika extract

3.1.1.1. Manufacturing

The saponified paprika extract is manufactured by a two-step procedure involving first the extraction of the dried fruits from *C. annuum L*. (sweet peppers or paprika) with hexane, and second the saponification using potassium or sodium hydroxide, [redacted] As a result of the saponification, the carotenoid esters, triglycerides and other lipid-soluble esters present in the paprika extract are hydrolysed into free carotenoids, fatty acids and glycerol. Antioxidants are added several times during manufacturing to protect carotenoids against oxidation.

3.1.1.2. Composition

Data were provided from three companies.[13] The analysis of a total of seven batches of saponified paprika extract showed mean values of saponification rate of 82.6% (Company A, two batches), 78.4% (Company C, two batches) and 77.5% (Company E, three batches).[12] The concentration of TC was determined in four batches of each company and ranged between 25.0 and 55.5 g/kg of which 36.9–57.8% was capsanthin. Additional three batches from company A were analysed and showed higher values of TC (75.2–76.8 g/kg; capsanthin 41.8–44.7% of TC). These higher values are due to the variability of the raw material that is affected by growth conditions of the plant (*C. annuum*) including yearly changes due to natural variation in precipitation and soil conditions or occasionally changes in cultivation practices. The analysed batches were all within the proposed specifications (TC: 25–90 g/kg; capsanthin: > 35% of TC).[12]

The carotenoid profile was analysed in one batch of two companies (Table 1).[12] The major carotenoids in these two saponified extracts, further to capsanthin, were zeaxanthin, β-carotene and cucurbitaxanthin A. Capsorubin was present in small quantities.

Table 1: Carotenoid composition of the saponified paprika extract (% of total carotenoids)

	Company A(1)	Company C(2)
Capsanthin	54.6	36.9
(13/13′Z)-capsanthin	1.4	9.7
(9Z or 9′Z)-capsanthin	0.7	1.9
(9′Z or 9Z)-capsanthin	1.1	3.9
capsanthin 5,6-epoxide	0.4	2.2
capsorubin	2.0	1.8
zeaxanthin	14.9	8.0
β-cryptoxanthin	4.1	4.5
cucurbitaxanthin A	1.7	8.7
5,6-diepikarpoxanthin	4.8	3.1
(9Z)-neoxanthin	–	2.1
β-carotene	10.4	9.8
Unidentified	3.9	7.5

(1): Total carotenoid content of the extract: 52.2 g/kg.
(2): Total carotenoid content of the extract: 25.4 g/kg.

[12] Technical dossier/Supplementary information January 2018/Updated Section II.
[13] The three companies that provided data are designated as Company A, C and E.

The applicant provided results of analysis of zeaxanthin, lutein and β-carotene in further batches of the saponified extract (Table 2).

Table 2: Content of total carotenoids, capsanthin, zeaxanthin, β-carotene and lutein in the saponified paprika extract (one batch per company)

	Company A	Company C	Company E
Total carotenoids (g/kg)	55.5	25.3	41.5
Capsanthin (% TC)	57.8	42.7	45.3
Zeaxanthin (% TC)	14.8	9.72	5.98
Lutein (% TC)	1.08	10.2	nd
β-carotene (% TC)	nd	nd	4.75

nd: not determined.

Information on the content of protein, ash, water (Table 3) and fatty acids (Table 4) were provided in four batches per company.[14]

Table 3: Results of the analysis for protein, water and ash (%) in the saponified paprika extract (four batches per company)

	Company A				Company C				Company E			
Protein	0.5	nd	nd	0.2	0.5	nd	nd	0.2	0.5	nd	nd	0.2
Water	2.1	15.6	14.2	18.0	2.1	15.6	14.2	18.0	2.1	15.6	14.2	18.0
Ash	18.4	16.8	15.4	16.2	18.4	16.8	15.4	16.2	18.4	16.8	15.4	16.2

nd: not determined.

Fatty acid distribution was determined in two batches of company A and C and one batch of company E. Major fatty acids in the extracts are linoleic acid (C18:2), palmitic acid (C16:0), oleic acid (C18:1), linolenic acid (C18:3), myristic acid (C14:0) and stearic acid (C18:0); they account for at least 92% of total fatty acids (Table 4). The fat content of the extract was not provided.

Table 4: Fatty acid profile (%) of the saponified paprika extract(1)

Component	Company A	Company C	Company E
Dodecanoic acid (C12:0)	1.9	0.9	2.24
Tetradecanoic acid (C14:0)	5.7	3.6	7.53
Hexadecanoic acid (C16:0)	19.2	20.3	19.6
Hexadecenoic acid (C16:1)	1.0	0.7	1.49
Hexadecadienoic acid (C16:2)	< 0.1	< 0.1	< 0.1
Octodecanoic acid (C18:0)	3.5	3.7	4.57
Octadecenoic acid (C18:1)	19.4	12.0	20.2
Octadecadienoic acid (C18:2)	40.2	45.4	37.7
Octadecatrienoic acid (C18:3)	6.1	3.7	3.21
Eicosanoic acid (C20:0)	0.7	0.6	0.815
Eicosenoic acid (C20:1)	0.1	0.2	0.205
Docosanoic acid (C22:0)	0.6	0.5	0.595
Erucic acid (C22:1)	< 0.1	< 0.1	< 0.1
Tetracosanoic acid (C24:0)	0.5	0.4	0.385
Tetracosenoic acid (C24:1)	< 0.1	< 0.1	< 0.1
Hexacosanoic acid (C26:0)	< 0.1	< 0.1	< 0.1
Fatty acid (total identified)	100.0	99.5	98.8

(1): Mean values for company A and C (two batches each), one batch from company E.

[14] Technical dossier/Supplementary information January 2018/Annex 6, 7, 8 and 12.

[redacted]

The mineral fraction was characterised by high amounts of potassium (up to 93.7 g/kg) due to the use of potassium hydroxide in the saponification.[17]

3.1.1.3. Purity

During the manufacturing process, hexane is used in the extraction. The content of hexane was analysed in at least three batches per company. Results were below the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline limit (290 mg/kg, Class 2 solvent). The highest value was 130 mg/kg in one batch; other values were < 25 mg/kg.[17]

Data on residues of other solvents were submitted. Considering that the extract will be marketed in more diluted forms, the data did not raise any concern. This was not the case for one batch from one company in which the concentration of benzene was 64 mg/kg which exceeds the VICH limit for this Class 1 solvent (2 mg/kg) and according to VICH guidelines, substances of Class 1 should be avoided in active substances, excipients and preparations. The company involved in the production confirmed that benzene is not used in any step of the manufacturing and HACCP procedure is in place to control solvent levels. In addition, analytical data for additional batches were submitted that showed values of benzene < 1 mg/kg.[18]

Capsaicin and closely related compounds summarised as capsaicinoids, the pungent principles in *C. annuum*, were present at levels < 300 mg/kg in the saponified paprika extracts.[19] The applicant provided analysis of three batches of a feed additive preparation (Company A) to show that capsaicin were either not detected (in two batches; limit of detection (LOD): 0.1 mg/kg) or was present at low concentration (13.5 mg/kg in one batch).[20] The characteristics of capsaicin were described in the EFSA opinion on the use of colouring agents in animal nutrition (EFSA, 2006) and in EFSA opinion on the re-evaluation of paprika extract as food additive (EFSA ANS Panel, 2015). The FEEDAP Panel notes that according to Regulation (EC) No 231/2012, concentration of capsaicin is limited to 'not more than 250 mg/kg' in the food additive (paprika extract, E 160c). The same specification is recommended for the feed additive. Considering that the extract is formulated (and diluted) to obtain the final additive, the values reported by the applicant are of no concern.

Heavy metals and arsenic were measured in five different batches from three companies. Three batches showed values of Pb < 1.0 mg/kg, Cd < 0.1 mg/kg, Hg < 0.01 mg/kg and As < 1.0 mg/kg. Values of two other batches were Pb < 0.05 mg/kg, Cd < 0.01 mg/kg, Hg < 0.005 mg/kg and As < 0.1 mg/kg.[17]

Aflatoxins (B1, B2, G1 and G2) were measured in one batch of each company. All values were < 0.1 μg/kg in two batches. In the third batch, aflatoxins were not detected (LODs were < 7.5, 2, 2 and 5 μg/kg).[17]

The applicant stated that dioxins (polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)), dioxin-like polychlorinated biphenyls (PCBs) and non dioxin-like PCBs are regularly monitored in the starting material (unsaponified oleoresin).[21] Data on dioxins in saponified oleoresin were provided from two companies: sum of dioxins were 2.27 ng (Comp A) and 8.09 ng WHO-PCDD/F-TEQ/kg (Comp E).[22]

Pesticides (> 300 substances) were analysed in one batch of saponified paprika extract from one company and in the non-saponified extract from another company.[23] Traces of buprofezin, etofenprox, diafenthiuron, diphenylamine or biphenyl were detected. However, considering that the additive is not marketed as such but in form of diluted, standardised and stabilised feed additive preparations, such amounts found in the saponified extract are not expected to pose a risk to target animal or consumer health. No pesticide residues were detected in a commercial powder preparation of the same company.

[redacted]

[17] Technical dossier/Supplementary information January 2018/Annex 6, 7 and 8.
[18] Technical dossier/Supplementary information January 2018/Annex 11.
[19] Total capsaicinoids amounted to 278, 300 and 65 mg/kg (Company A), 80, 85 and 69 mg/kg (Company C) and 28, 10 and 22 mg/kg (Company E).
[20] Technical dossier/Supplementary information January 2018/Annex 8.
[21] Technical dossier/Supplementary information January 2018/Annex 7 and 8.
[22] Technical dossier/Supplementary information January 2018/Annex 6 and 8.
[23] Technical dossier/Supplementary information January 2018/Annex 14 and 15.

3.1.1.4. Physicochemical properties of capsanthin

Capsanthin ((3*R*,3′*S*,5′*R*)-3,3′-dihydroxybeta,kappa-caroten-6′-one, CAS number 465-42-9) has the molecular formula $C_{40}H_{56}O_3$ and the molecular weight of 584.87 g/mol. Its structural formula is given in Figure 1.

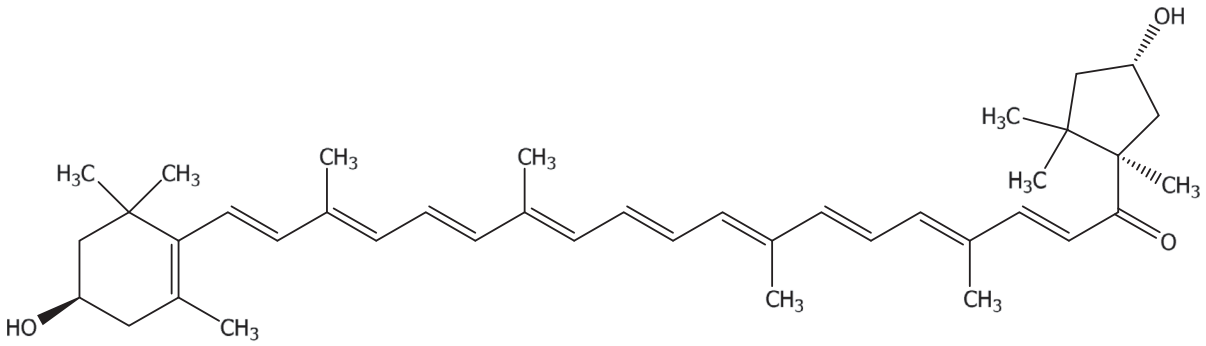

Figure 1: Structural formula of capsanthin

Physicochemical data of capsanthin were provided by the applicant and was also described in the EFSA opinion on the use of colouring agents in animal nutrition (EFSA, 2006).

3.1.2. Characterisation of feed additive preparations

3.1.2.1. Typical composition

The saponified extract is not used directly as a feed additive. It is diluted, standardised and stabilised before placing in the market. The applicant reported that such products typically contain 0.5–5.0% TC and 0.2–2.0% capsanthin. The preparations may be solid or liquid and may contain carriers (e.g. calcium carbonate, *Tagetes* meal bagasse, rice hulls, sesame meal, wheat bran, corncob meal, coconut shells, water), anticaking agents (e.g. silicic acid, colloidal silica and kaolinitic clay) and antioxidants.

3.1.2.2. Physical state

Particle size data obtained by sieve analysis were submitted for five preparations from the three companies. For company A, data showed that the amount of particles < 50 μm ranged between 45 and 55% (two preparations a total of six batches), no particles < 10 μm were found. Company C (three batches) reported that 5–11% of the particles were smaller than 63 μm and 3–6% were bigger than 495 μm. The third company reported that 98% of the particles were < 850 μm.

The dusting potential was determined in four different preparations (at least one per company) using the Stauber–Heubach method.[24] Results were < 0.1 (Company C), 0.3 (Company E), 20.9 (Company A) and 21.4/58.2 g/m^3 (Company A, two batches).

3.1.2.3. Stability and homogeneity

The sensitivity of carotenoids to temperature, light and oxygen requires the inclusion of antioxidants at the different steps of manufacturing of the active substance and the preparations of the additive to assure the stability of capsanthin.

Shelf-life

Data were submitted for five preparations from the three companies.

Three preparations of company A (0.5, 1 and 2% TC content)[25], one preparation of company C (2% TC content)[26] and one preparation of company E (0.5% TC content)[27] were kept at 25°C and

[24] Technical dossier/Supplementary information January 2018/Annex 16.
[25] Technical dossier/Supplementary information January 2018/Annex_26.
[26] Technical dossier/Supplementary information January 2018/Annex_27.
[27] Technical dossier/Supplementary information January 2018/Annex_28.

60% of relative humidity (RH)) in commercial packaging. Recoveries after 24 months showed no degradation of total carotenoids or capsanthin.

The same preparations of company C and E and one preparation of company A (1% TC content) were also tested during storage at 40°C and 75% RH. Recoveries after 3 months were 32% capsanthin and 29% TC (Company A), 93% both capsanthin and TC (Company C) and 86% capsanthin and 80% TC (Company E).

Stability in premixture and feedingstuffs

The stability of a capsanthin preparation (Company C) containing 2% TC in a standard vitamin mineral premixture for chickens for fattening was assessed at an intended inclusion level of 2.5 g TC/kg premix.[28] The composition of the premixture was not reported. Analysis of capsanthin showed no degradation over 6 months. Results reported for total carotenoids showed a slight decrease over time reaching 75% after 6 months.

The stability of a capsanthin preparation (Company C) containing 3% TC was assessed in a standard chicken layer feed at an intended inclusion level of 50 mg TC/kg complete feed.[29] The composition of the feed was not reported. Analysis of capsanthin showed no degradation over 3 months. Results reported for total carotenoids showed a slight decrease over time reaching 77% after 3 months.

The stability of a capsanthin preparation (Company C) containing 2% TC was assessed after pelleting at 75°C in a grower diet based on wheat, barley and soybean meal at an intended inclusion level of 15 mg TC/kg feed.[30] Analysis of capsanthin and TC showed a minimal decrease (about 5%) due to pelleting.

Homogeneity

The homogeneous distribution of two capsanthin preparations (Companies A and C, 2% TC content) in poultry feed was studied by the analysis of capsanthin and TC in 10 and 15 samples, respectively. The coefficients of variations (CV) were 5.3% for TC and 5.7% for capsanthin in feed containing product of Company A[31] and 8.3% for TC and 7.8% for capsanthin in feed containing product of Company C.[30]

3.1.3. Conditions of use

The applicant proposes the use of the saponified paprika extract (TC content 25–90 g/kg, capsanthin > 35% of TC) in feed for poultry for fattening (chickens for fattening and minor poultry for fattening) and poultry for laying (laying hens and minor laying poultry (ducks, partridges and quails)) to a maximum content of 40 mg TCs/kg complete feed. When used in combination with other carotenoids, the TC content shall not exceed 80 mg/kg feed.

3.2. Safety

3.2.1. Absorption, distribution, metabolism, excretion and residues

Metabolic studies

The metabolism of capsanthin was assessed by the FEEDAP Panel in 2006 (EFSA, 2006). No new data have been supplied by the applicant for the current assessment and, to the knowledge of the FEEDAP Panel, no specific data on capsanthin have been published since 2006. A summary of the information available on the metabolism of capsanthin in the target species, laboratory animals and humans is given as follows: (i) no data are available concerning the metabolic fate of capsanthin in poultry or laboratory animals; only evidence is given that the bioavailability of capsanthin from esterified capsanthin and from saponified (free) capsanthin from paprika extract in the chicken, measured through the plasma levels of free capsanthin, are similar, implying ester hydrolysis prior to absorption, (ii) capsanthin is eliminated in its free form in the egg yolk; the metabolic fate in laying hen of synthetic (3S,5R,3'S,5'R)-capsorubin, a chemically close compound harbouring two cyclopentanyl end rings instead of one in capsanthin, identified 14 metabolites with almost identical chromophoric values but diverse

[28] Technical dossier/Supplementary information January 2018/Annex_29.
[29] Technical dossier/Supplementary information January 2018/Annex_30.
[30] Technical dossier/Supplementary information January 2018/Annex_31.
[31] Technical dossier/Supplementary information January 2015/Annex_32.

polarities ranging from monosulfate to fatty acid monoesters; it can be assumed that a similar metabolic behaviour of the common structural part of capsanthin would occur, (iii) in humans, the bioavailability of capsanthin from paprika extract was shown to be very low; after administration of capsanthin from paprika juice for one week, capsanthin was distributed in plasma lipoproteins and its concentration plateaued between day 2 and day 7 to become undetectable after day 16. Humans may have the potential metabolic activity for the oxidation of secondary hydroxyl groups in various xanthophylls, i.e. the formation of capsanthon from capsanthin by the oxidation of the 3′-hydroxyl group to the 3′-keto group (Etoh et al., 2000).

In addition, it is noted that absorption and bioavailability of carotenoids will strongly depend on the matrix in which they are administered and thus may differ between unsaponified and saponified preparations and will be influenced by the presence of solubilisers in the additive.

3.2.1.1. Deposition of capsanthin in chicken tissues

Limited data reported in the former EFSA assessment (2006) on the deposition of capsanthin in chicken tissues refer to a study of Erkek et al. (1993) where chickens administered a diet supplemented with 20 mg xantophylls/kg exhibited capsanthin residues in the skin amounting to 0.4 mg/kg.

New residue data (total carotenoid and capsanthin residues) were measured in tissues of chickens for fattening from the tolerance study (see Section 3.2.3.1).[32] Four groups of 1-day-old chickens (20 birds/replicate, 6 replicates/treatment) were administered for 36 days a feed supplemented with 0, 40, 80 and 240 mg total carotenoids/kg in the form of a saponified paprika extract. The analysed values (given as mean of starter and grower diets at start) were 2.7, 40.0, 77.2 and 250.0 mg TC/kg (0.06, 17.5, 35.0 and 104 mg capsanthin/kg). Tissues, except those from the highest dose group, were sampled at slaughter (day 36) and kept frozen until analysis. Total carotenoids and capsanthin were determined by high-performance liquid chromatography (HPLC), with an LOD of 0.5 mg carotenoid/kg wet tissue. The results are shown in Table 5.

Table 5: Concentration of total carotenoid (TC) and capsanthin residues (mg/kg) in tissues of chickens for fattening administered feed supplemented with increasing amounts of TCs in the form of a saponified paprika extract

Feed(1)		Liver(2)		Kidney(2)		Muscle(2)		Skin/fat(2)	
TC	Caps.	TC	Caps.	TC	Caps.	TC	Caps.	TC	Caps.
2.7	0.06	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
40	17.5	3.31 ± 0.97	1.73 ± 0.35	0.85 ± 0.17	0.85 ± 0.17	0.39 ± 0.15	0.39 ± 0.15	0.29 ± 0.19	0.29 ± 0.19
77.2	35.0	7.96 ± 2.00	3.79 ± 0.89	2.29 ± 0.57	1.94 ± 0.35	1.26 ± 0.18	1.26 ± 0.18	1.35 ± 0.30	1.07 ± 0.13

(1): Concentrations measured.
(2): Mean ± standard deviation from 6 replicates with 2 animals per replicate.

3.2.1.2. Deposition of capsanthin in the egg yolk

The applicant submitted three studies assessing the deposition of dietary carotenoids from paprika extracts in the egg (Hamilton et al., 1990; Lai et al., 1996; González et al., 1999). These papers were already assessed by the FEEDAP Panel (EFSA, 2006) and considered insufficient to be used for the assessment of consumer exposure because of different analytical methods used, different background contents of diets, lack of additive identity and weaknesses in reporting.

New residue data (total carotenoid and capsanthin residues) were measured in whole egg samples from the tolerance study in laying hens (see Section 3.2.3.2).[33] Four groups (seven hens per replicate, eight replicates) of 24-week-old laying hens were administered for 56 days a feed supplemented with 0, 15, 80 and 240 mg total carotenoids/kg in the form of a saponified paprika extract. The analysed values were 4.8, 20.5, 83.4 and 265 mg TC/kg (< 1, 8.2, 40.4 and 128 mg capsanthin/kg). All eggs, except those from the highest dose group, laid on day 55 were collected per replicate. Liquid eggs were pooled per replicate and stored frozen until analysis. Total carotenoids and capsanthin were

[32] Technical dossier/Supplementary information July 2019/Annex 03.
[33] Technical dossier/Supplementary information July 2019/Annex 02.

determined by HPLC, with an LOD of 0.5 mg carotenoid/kg whole egg. The results are shown in Table 6.

Table 6: Concentration of total carotenoid (TC) and capsanthin residues (mg/kg) in whole eggs of laying hens administered feed supplemented with increasing amounts of TCs in the form of a saponified paprika extract

Feed(1)		Whole egg(2)	
TC	Capsanthin	TC	Capsanthin
4.8	< 1	0.59 ± 0.06	< 0.5
20.5	8.2	2.78 ± 0.25	1.24 ± 0.14
83.4	40.4	10.9 ± 1.17	5.48 ± 0.58

(1): Concentrations measured.
(2): Mean ± standard deviation from eggs collected from 8 pooled samples.

3.2.2. Toxicological studies

The FEEDAP Panel noted that the additive under assessment (saponified paprika extract) is different from that evaluated for food use (paprika extract, E 160c) and assessed by EFSA (EFSA ANS Panel, 2015) and JECFA (2014). The saponification of the extracts results in the hydrolysis of the carotenoid esters.

For the current assessment, the applicant performed two *in vitro* genotoxicity studies (a bacterial reverse mutation assay[34] and a mammalian cell micronucleus test[35]) and a modified 90-day toxicity study in rat (with extended parameters from OECD 407).[36] The test item was a saponified paprika extract with TC content of 48.2 g/kg and capsanthin 22.8 g/kg (expressed as 47% of TC) and it is considered representative for the additive under assessment.

3.2.2.1. Genotoxicity studies including mutagenicity

Saponified paprika extract was tested in a bacterial reverse mutation assay on Salmonella Typhimurium strains TA1535, TA1537, TA100 and TA98 and on *Escherichia coli* strain WP2uvrA both in the absence and presence of S9-mix (rat liver S9-mix induced Aroclor 1254) in compliance with OECD test guideline 471.[34] The vehicle of the test item was ethanol and both the plate incorporation and the pre-incubation methods were applied. Precipitation was reported at 5000 μg/plate and sporadically at 1600 μg/plate. In the S. Typhimurium tester strains, the maximum analysable concentration was in the range 17–52 μg/plate or mL and was limited by cytotoxicity. In the *Escherichia coli* tester strain WP2uvrA, no toxicity was observed up to and including the dose level of 512 μg/mL. No increase in the number of revertant colonies was reported in any experimental condition while the positive controls performed as expected.

The possible clastogenicity and aneugenicity of saponified paprika extract was tested in an *in vitro* micronucleus assay in cultured peripheral human lymphocytes, in compliance with OECD test guideline 487.[35] Two independent experiments were performed with and without metabolic activation (phenobarbital and ß-naphthoflavone induced rat liver S9-mix). Ethanol was used as the vehicle. In the first cytogenetic assay, the test item was tested up to 300 μg/ml for a 3-hour exposure time with a 27-hour harvest time in the absence and presence of S9-fraction. At this concentration, 13 and 31% cytostasis was reported with and without metabolic activation, respectively, and the test substance formed a precipitate in the culture medium.

In the second cytogenetic assay, the test item was tested up to 300 μg/mL for a 24-hour exposure time with a 24-hour harvest time in the absence of S9-mix. The top concentration caused 56% cytostasis and the formation of precipitate in the culture medium. No statistically significant or biologically relevant increase in the number of mono- and binucleated cells with micronuclei was reported in the absence and presence of S9-mix, in either of the two experiments. The positive control chemicals produced a statistically significant increase in the number of binucleated cells with micronuclei.

[34] Technical dossier/Supplementary information January 2018/Annex_40.
[35] Technical dossier/Supplementary information January 2018/Annex_41.
[36] Technical dossier/Supplementary information January 2018/Annex_42.

3.2.2.2. Subchronic toxicity

Groups of 10 male and 10 female Wistar rats were fed diets containing 0 (control), 1,000 (low dose), 5,000 (mid dose) or 10 000 (top dose) mg test saponified paprika extract[37] /kg feed for 90 days in a toxicological study in compliance with OECD test guideline 408.[36] The groups of male rats received mean dosages of the test item of 0, 84, 428 and 858 mg/kg body weight (bw) per day over the course of the study; the corresponding dosages for females were 0, 85, 448 and 879 mg/kg bw per day. Clinical observations were made daily on all animals with more detailed 'arena observations' being made weekly. Body weights and food consumption were measured weekly. Functional tests for pupillary reflex, hearing ability, static righting reflex, grip strength and locomotor activity were performed on 5 animals/sex per group towards the end of the study. Ophthalmic observations were made on all animals at the beginning and end of the study period. Daily vaginal lavage samples from all females were taken towards the end of the study and were examined for cytology for oestrus cycle determination. Blood samples were taken from all rats on the day of necropsy and were used for haematological and blood biochemical examination. All animals were subjected to necropsy, organs were weighed and tissues preserved for histological examination. Tissues and organs from all animals of the control and high-dose groups were independently examined microscopically by two pathologists, with particularly detailed examination of the testes.

No mortality occurred. No treatment-related clinical signs were seen other than red colouration of the faeces of mid and top dose group rats. Food consumption and body weights were not affected by the treatments. The only effect on functional observations was a significantly lower forelimb grip strength in mid and top dose males as compared with male controls. However, there was no effect on grip strength of the forelimbs in females and no effect on hindlimb grip strength in either sex. There were no treatment-related effects on ophthalmology results or oestrus cycle length. Haematology revealed no effects in females. In males, there were small but significant differences from control values in some haematological parameters (white blood cell and lymphocyte count decreased at the top dose, and mean corpuscular haemoglobin concentration decreased at all doses) in some treated groups, but in the absence of a dose–response relationship these were not regarded as treatment-related. There were also some small but significant differences from control values in some blood biochemistry parameters (elevated sodium in males at all dose levels and in mid-dose females, decreased glucose in low-dose females and elevated cholesterol in mid- and high-dose females). The only blood biochemistry changes that showed a dose–response relationship were those of cholesterol in females. The absence of corroborative findings in the opposite sex and/or the absence of a dose–response relationship suggested that none of the statistically significant changes in blood biochemistry parameters were caused by the treatment. The only treatment-related effect seen at necropsy was a yellowish discolouration of the adipose tissue in some males in the mid-dose group and all males and females in the top dose groups. There were no treatment-related effects on organ weights or on histopathology.

The FEEDAP Panel concludes that no adverse effects were caused in rats given the saponified paprika extract at dietary concentrations of up to the highest dose tested of 10 000 mg/kg (up to 858 mg (41.4 mg TC)/kg bw per day in males up to 879 mg (42.4 mg TC)/kg bw per day in females).

3.2.2.3. Conclusions

The saponified paprika extract assessed is not genotoxic in a bacterial reverse mutation assay and in an *in vitro* mammalian cell micronucleus test. No adverse effects were caused in rats given the paprika saponified extract under assessment at dietary concentrations up to the highest dose tested of 10 000 mg/kg (up to 858 mg (41.4 mg TC)/kg bw per day in males up to 879 mg (42.4 mg TC)/kg bw per day in females). The NOEL from the study is 41.4 mg TC/kg bw per day for saponified paprika extract. The FEEDAP Panel is aware that the above results are obtained with one saponified paprika extract, however, they could be considered representative of other saponified paprika extracts with a TC content in the range of 25–90 g/kg and a capsanthin content of > 35% of TC. The FEEDAP Panel also notes that reproduction toxicity studies were not provided.

[37] Technical dossier/Supplementary information January 2018/Annex_39.

3.2.3. Safety for the target species

Two new tolerance studies, one in chickens for fattening and one in laying hens, were submitted following the FEEDAP Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a).

3.2.3.1. Tolerance study in chickens for fattening

A total of 480 1-day-old male chickens for fattening (Ross 308) was distributed in 24 pens of 20 birds and allocated to four treatment groups (representing six replicates per treatment).[32] The test item was a saponified paprika extract on a mineral carrier containing 21.35 g TC/kg and 8.5 g capsanthin/kg (expressed as 40% TC). The groups were fed diets supplemented with 0 (control), 40 (1× maximum recommended level), 80 (2×) or 240 (6×) mg TC/kg complete feed for 36 days. The pelleted corn–soybean-based diets (methionine hydroxy analogue supplemented) were given as starter (day 1–21; 18.9% crude protein (CP), 12.8 MJ metabolisable energy (ME)/kg, 0.94% methionine-cysteine (met-cys) and grower diet (day 22 until study completion; 18.0% CP, 13.2 MJ ME/kg, 0.86% met-cys). Total carotenoids and capsanthin were analysed in the feed before and at the end of the two feeding periods, the results are given in Table 7.

Table 7: Analysed dietary levels of total carotenoids (TC) and capsanthin from saponified paprika extract at start and end of the two feeding periods (nominal concentration: 0, 40, 80 and 240 mg TC/kg feed)

		Total carotenoids (mg/kg feed)				Capsanthin (mg/kg feed)			
Starter phase	Start	2.2	40.1	78.7	251	< 0.5	17.6	35.2	104
	End	2.7	37.3	73.9	222	0.09	13.8	28.7	87.1
Grower phase	Start	3.2	39.8	75.7	248	0.08	17.4	34.8	104
	End	2.7	34.3	66.6	204	0.06	13.1	24.6	83.5

Health status, including mortality, was monitored daily, body weight and feed intake (per replicates) was measured on day 22 and 36 and feed to gain ratio was calculated for the corresponding periods. Blood samples for haematology[38] and clinical chemistry[39] were taken at the end of the study from two randomly selected birds/pen (12 per treatment). The same birds were killed for necropsy, organ weight determination (liver, spleen, kidneys, heart) and histological examination of liver, kidney, muscle and skin/fat samples. Another two birds per replicate were killed for edible tissue sampling (liver, kidneys, breast muscle and skin+fat (from breast area)).

Statistical evaluation was made by analysis of variance (ANOVA) of data from a randomised complete block design (with blocks (four adjacent pens) as random effect). The experimental unit was the pen. Group means were compared with Tukey test (adjusted for continuous variables). Mortality data were assessed using categorical data analysis (Wilcoxon rank sum, Kruskal–Wallis test). A significance level $\alpha = 0.05$ was used.

Mortality was low (average 1.9%) and not treatment related. No differences between the groups were detected by statistical analysis for the final body weight (mean: 2,465 g), daily feed intake (mean: 98 g) and feed to gain ratio (mean 1.46).

No significant differences were detected among treatments in serum enzyme activities, protein and lipid related variables, or electrolytes except for bilirubin, creatinine and alanine aminotransferase (ALT). Bilirubin level was 0.115 mg/dL for the control group, whereas it was below the LOD of 0.05 mg/dL for all groups treated with paprika extract. Since no adverse consequences of lower bilirubin levels are known, this effect (if present) appears to be without biological relevance for the safety of target animals. Creatinine significantly decreased with the inclusion of saponified paprika extract (from 0.214 mg/dL to 0.180, 0.158 and 0.141 for the groups with 40 (1×), 80 (2×) and 240 (6×) mg TC/kg supplementation level of the test item); these changes are not considered to be of any safety relevance for poultry. ALT was only lower in the intermediate level group (1.7 U/L) compared to

[38] Haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, erythrocytes count, and white blood cell count and differentials, and prothrombin time. Fibrinogen was not analysed as preliminary tests from untreated birds offered results below level of detection on 10 out of 10 chickens.

[39] Sodium, potassium, chloride, calcium, phosphorus, magnesium, total protein, albumin, globulin, glucose, uric acid, cholesterol, creatinine, bilirubin, amylase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, gamma-glutamyltransferase, alkaline phosphatase and creatine kinase.

the control group (2.5 U/L). No significant differences were seen between the groups for haematological variables, cell counts or differential cell counts.

No significant differences were found in organs weights of birds expressed in relative weight.

The high-dose group produced yellowish to orange coloured fat, skin, subcutaneous tissue and liver, but this was not associated with any gross pathology or histopathology.

3.2.3.2. Tolerance study in laying hens

The tolerance study in laying hens was performed using a saponified paprika extract on a mineral carrier as a test item containing 20.8 mg TC/g and 7.7 mg capsanthin/g (expressed as 37% TC). A total of 224 laying hens (Hy-Line Brown, 24 weeks old, 1.83 kg bw) was allocated to four treatment groups fed diets supplemented with 0 (control), 15, 80 (2×) or 240 (6×) mg TC/kg complete feed for 56 days according to a randomised complete block design.[33] Group size was eight replicates (enriched laying hen cages) with seven hens each. Blocking factors were 2-wk previous laying rate and battery side, initial body weight was also considered when building groups.

The diet, given in mash form, consisted mainly of maize and soybean meal and was supplemented with MHA and tryptophan. The basal diet was calculated to provide 14.9% CP, 0.58% met+cys and 11.1 MJ ME/kg. The analysed TC levels in the diets were initially 4.8, 20.5, 83.4 and 265 mg TC/kg for the control diet and the diets with nominal 15, 80 and 240 mg TC/kg feed, the capsanthin contents were < 1, 8.2, 40.4 and 128 mg/kg, respectively. These concentrations were measured 4 months afterwards as follows: 4.7, 21, 82 and 197 mg TC/kg, and < 1, 7.2, 38.6 and 98.8 mg capsanthin/kg. An essential reduction of the test substance during the 2-month experimental period could therefore only be supposed for the high-dose group, its magnitude is probably less than 15%.

Health status including mortality was monitored daily. Animals were weighed by replicate at the beginning and end of the study. Feed consumption was calculated for both 28-day periods. All eggs laid were counted and weighed per replicate every second day on weekly days. Performance variables (laying rate, average egg weight and mass, average daily feed intake and average feed to egg mass ratio) were calculated. At the end of the study, two birds/replicate (16 per treatment) were selected for blood sampling (haematology[40] and clinical chemistry[39]) and killed thereafter for gross pathology examination, organ weight determination (heart, liver, spleen, kidneys) and histological examination of liver, kidney, muscle and skin/fat. Egg quality (yolk colour and Haugh Units, shell and yolk percentages, shell index and egg classification) was assessed at the end of each period. Whole egg samples were collected to measure total carotenoid and capsanthin residues.

Statistical evaluation (comparison of Tukey-adjusted means) was made by ANOVA of data from a randomised complete block design (with blocks as random effects). Statistical significance was declared at $p < 0.05$.

No mortality occurred during the study. No differences between the experimental groups were seen for the zootechnical endpoints: initial body weight (mean: 1,828 g), final body weight (mean: 1,936 g), average daily feed intake (mean: 122 g), laying rate (mean: 94.9%) and egg weight (mean: 58.8 g). Daily egg mass showed differences near to significance ($p = 0.052$) and significant differences for feed to egg mass ratio ($p = 0.028$); highest daily egg mass (56.9 g) and lowest (best) feed to egg mass ratio (2.12) were found in the control group, lowest daily egg mass (54.1 g) and highest feed to egg mass ratio (2.26) were found in the low TC group, for the intermediate and the high level groups both parameters were between the extremes and not significantly different from those. These differences are considered accidental.

No differences were detected for haematology and blood biochemistry with the exception of bilirubin. For bilirubin, the following blood concentrations were measured: 0.15 mg/dL in the control and 0.08 mg/dL[41] in the group with 15 mg TC/kg feed, respectively. All bilirubin values of the two groups with higher dietary TC concentrations were below LOD (0.05 mg/dL); this effect appears to be without biological relevance for the safety of target animals. Creatinine ($p = 0.054$), and mean corpuscular volume (MCV) ($p = 0.051$) showed nearly significant differences. Creatinine in the overdose group (0.280 mg/dL) was lowest and in tendency ($p < 0.10$) different from the highest value measured for the intermediate level group (0.297 mg/dL), the values for the control and the low-level group being in between. MCV was lowest in the control groups (115.5 fL) and highest (127.8 fL) in the low-level group, the other groups not being different from both ($p < 0.10$).

[40] Haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, erythrocytes count, and white blood cell count and differentials as well.

[41] With two values below LOD (0.05 mg/dL) taken with 0.025 mg/dL (half of LOD) for calculation.

There were no statistically significant differences detected in relative organs weights (means for heart: 0.521%, for liver: 2.040%, for spleen: 0.114% and for kidneys: 0.671%). Histopathological assessment of organs and tissues did not result in adverse findings.

3.2.3.3. Conclusions on safety for the target species

Saponified paprika extract was tolerated by chickens for fattening and laying hens at the highest level tested (240 mg TC/kg feed). The FEEDAP Panel concludes that maximum recommended use level of 40 mg TC from the saponified paprika extract/kg feed is safe for chickens for fattening and laying hens. The margin of safety is at least 6. This conclusion is extrapolated to minor poultry species for fattening and laying.

3.2.4. Assessment of consumer safety

3.2.4.1. Determination of a safe concentration

Based on the available data, the FEEDAP Panel concludes that no adverse effects were caused in rats given a saponified paprika extract at dietary concentrations of up to the highest dose tested of 10,000 mg/kg (up to 858 mg (41.4 mg TC)/kg bw per day in males up to 879 mg (42.4 mg TC)/kg bw per day in females) in a 90-day study. The NOEL from the study is 41.4 mg TC/kg bw per day.

3.2.4.2. Consumer exposure

The chronic exposure of consumers to residues of TC from the saponified extract of paprika in tissues of chickens end eggs of laying hens is calculated (Table 9) following the methodology described in the Guidance on the safety of feed additives for consumers (EFSA FEEDAP Panel, 2017b) and using the Feed Additives Consumer Exposure (FACE) calculator available on EFSA's website (for further details see Appendix A). The residue data originating from tolerance studies (see Sections 3.2.2 and 3.2.3) are summarised in Table 8.

Table 8: Residue data derived from the use of saponified paprika extract in chickens for fattening fed 40 mg TC/kg and in laying hens fed 80 mg TC/kg[(1)]

Source	TC concentration in tissue/product (mg/kg)	
	Mean ± SD	Mean + 2SD
Liver	3.31 ± 0.97	5.25
Kidney	0.85 ± 0.17	1.19
Muscle[(2)]	0.39 ± 0.15	0.69
Skin/fat	0.29 ± 0.19	0.67
Egg yolk	10.9 ± 1.17	13.24

SD: standard deviation.
(1): Since residue concentrations in eggs at the proposed use level (40 mg TC/kg feed) was not provided, the values measured at higher use level (80 mg/kg feed) were taken as a worst-case scenario.
(2): The residue concentration in muscle and skin/fat will be applied to the intake of meat at the following proportions: 90% muscle and 10% skin/fat (EFSA FEEDAP Panel, 2017b). This corresponds to 0.609 mg/kg.

Table 9: Chronic dietary exposure of consumers to residues of TC from the saponified extract of paprika – Summary statistics across European dietary surveys

Population class	Number of surveys	Highest exposure estimate (mg/kg bw per day)
Infants	6	0.0472
Toddlers	10	0.0530
Other children	18	0.0562
Adolescents	17	0.0364
Adults	17	0.0200
Elderly	14	0.0173
Very elderly	12	0.0219

The results showed that the highest chronic exposure was for the age class 'other children' with 0.0562 mg/kg bw per day, the lowest for the age class 'elderly' with 0.0173 mg/kg bw per day (for detailed results per age class, country and survey see Appendix A, Table A.1).

3.2.4.3. Safety assessment

Based on the NOEL of the 90-day study and the exposure estimates, the Panel considered that there would be an adequate margin of exposure (MOE) (between 700 and 2,000) to conclude that the level of exposure to residues of the saponified paprika extract in animal tissues and products does not raise concern for the safety for consumers (EFSA Scientific Committee, 2019). The MOE is enough wide to account for the uncertainty in the inter- and intraspecies extrapolation and for the absence of reproduction toxicity studies.

3.2.4.4. Conclusions on safety for the consumer

The FEEDAP Panel concludes that the use of the saponified paprika extract (TC: 25–90 g/kg, capsanthin not less than 35% of TCs) for poultry for fattening and laying would not be of concern for the consumer.

3.2.5. Safety for the user

No specific studies were submitted by the applicant to assess the safety of the products under assessment for the user.

The saponified paprika extract is a viscous paste and as such users will not be exposed by inhalation. The applicant recognises that the extract may be irritant to skin and eyes. Due to the susceptibility of the active colouring principles to oxidation (Section 3.1.2.3), the additive will be placed in the market only in the form of preparations. The applicant provided data in the dossier on the physical properties of some of these preparations which showed dusting potential ranging from < 0.1 to 58.2 g/m^3,[24] indicating a likely exposure of user by inhalation.

The FEEDAP Panel recognises that once authorised, multiple formulations of the additive can be placed in the market, and consequently, not all preparations can be directly tested for user safety. No information has been provided on the inhalation toxicity or the irritation/sensitisation potential of any preparation. In the absence of these data, the FEEDAP Panel cannot conclude on the potential of the preparations to be toxic by inhalation or on their potential as skin/eye irritant or skin sensitiser.

3.2.6. Safety for the environment

The additive is extracted from a natural source (*C. annuum*) and contains compounds which occur in nature: free carotenoids, mainly capsanthin, mono- and diglycerides, fatty acids, glycerol, waxes, potassium/sodium and water. The use of paprika extracts in poultry feed as a source of capsanthin and other carotenoids would not alter the concentration or distribution of these compounds in the environment given the natural occurrence of these carotenoids and their oxidative susceptibility. Therefore, the FEEDAP Panel considers that use of extracts from paprika in poultry feed will not adversely affect the environment.

3.3. Efficacy

The additive is used to provide colour to the skin and egg yolk of poultry. In practice, the so-called red colouring pigments are used together in an appropriate proportion with yellow colouring pigments to achieve the desired golden yellow tone of egg yolk and skin. In layer feeding, it is common practice to provide a basic dietary yellow colour (e.g. by xanthophylls as lutein and zeaxanthin) and to adjust the yolk colour desired by supplementing red pigments (or other yellow pigments). The need for additional red or yellow carotenoids in any particular production system will also depend on the content of natural endogenous pigments in feed materials and on the relative deposition rates of the various carotenoids. Thus, there is no standard approach to egg pigmentation which can vary considerably between egg producers.

The applicant made reference to the previous FEEDAP opinion on red carotenoids (EFSA, 2006), to new papers from the literature and to the two tolerance studies submitted for the current assessment.

3.3.1. Skin pigmentation

The applicant submitted data on reflectance colorimetry with saponified paprika extract from the tolerance study in chickens for fattening (see Section 3.2.3.1). Five birds/replicate (6 replicates)

remained 6 days longer at farm (36 + 6 days treatment). They were slaughtered and colour measurements were performed the following day after cooling of carcasses. Measurements were performed on foot pad and breast skin (*pectoral pterilium*) (Table 10).

Table 10: Colour of breast skin and foot pads after 42 days of feeding different levels of saponified paprika extract to chickens for fattening

Supplemented TC (mg/kg feed)	0	40	80	240
Analysed TC (mg/kg feed)	2.2–3.2	34.3–40.1	66.6–78.7	204–251
Analysed capsanthin (mg/kg feed)	< 1	13.1–17.6	24.6–35.2	83.5–104
Breast skin colour(1)				
Lightness (L*)	65.76^{a}	64.08^{ab}	63.25^{bc}	61.03^{c}
Redness (a*)	6.66^{c}	7.90^{c}	10.17^{b}	15.13^{a}
Yellowness (b*)	10.18^{d}	16.76^{c}	20.86^{b}	26.87^{a}
Foot pad colour(1)				
Lightness (L*)	75.56^{a}	73.11^{b}	71.22^{b}	67.41^{c}
Redness (a*)	4.78^{d}	13.69^{c}	18.36^{b}	26.24^{a}
Yellowness (b*)	36.37^{c}	49.06^{b}	52.70^{b}	58.28^{a}

a, b, c, d: Values in the same row with different superscript letters are significantly different ($p < 0.05$).
(1): Colour by means of a Minolta CR 300 colorimeter in the CIELab scale.

The additive increased redness of both tissues, for breast skin significantly at 80 mg TC from saponified paprika extract/kg feed, for foot pads already at 40 mg TC/kg. In contrast to egg yolk colour, yellowness increased too, in both tissues significantly at 40 mg TC/kg feed.

Two other studies from literature could also be used to support efficacy of the additive.

The FEEDAP Panel made reference to a study of Erkek et al. (1993) with paprika meal in its previous opinion on red carotenoids (EFSA, 2006). The authors fed chicken for fattening (Erbro strain) depleted for 4 weeks of xanthophylls (60% wheat diet) for another 3 weeks diets supplemented with different carotenoid sources so that the TC level of the basal diet (10 mg/kg) was increased to 30 mg/kg. One of the carotenoid sources was paprika meal at a dietary level of 2.5%. Shank and skin colour were measured by means of the Roche Yolk Colour Fan and Hunter reflectance photometry. The results are summarised in Table 11.

Table 11: Hunter colour reflectance values of shanks and skin of chickens for fattening after 3 weeks feeding of a diet supplemented with 20 mg TC from paprika meal/kg

Diet	**Control**		**Paprika meal**	
Tissue	**Shank**	**Skin**	**Shank**	**Skin**
Lightness (L*)	76.15	66.67	74.37	68.75
Yellowness (b*)	30.80	14.55	30.97	12.48
Redness (a*)	−0.85	2.59	1.65	3.35

The addition of 20 mg TC from paprika meal/kg feed significantly ($p < 0.05$) increased redness of shanks over control values (background 10 mg TC/kg); it also increased redness of broiler skin, however not significantly. Supplementation with paprika meal significantly reduced yellowness of broiler skin, but not in shanks. Lightness of both tissues was not affected by paprika meal. The study supports the colouring properties of paprika meal in chickens for fattening.

Castañeda et al. (2005) compared the efficacy of synthetic and natural carotenoids on pigmenting broiler skin. As natural pigments, the authors used TCs from red pepper (*C. annuum*) for red pigmentation (2 and 3 mg/kg) together with a marigold extract for yellow pigmentation (25 and 45 mg/kg from day 21 to day 35 and 65 and 85 mg/kg until study end). The carotenoids were added to a pigment poor diet based mainly on milo and soybean meal and fed from day 21 to day 49. From the day 35 until day 49, individual bird weight, skin colour and blood pigment levels were measured weekly. Pigmentation was measured on 50 samples each by means of a Minolta CR-200 Chroma meter. There was a steady increase in b* values (indicating yellowness) of skin from 4.632 at day 28 until 11.959 at day 49 for the lower doses and from 6.503 until 15.572 for the higher doses. However, there was rather a decrease of a* values (indicating redness) from 3.517 to 1.708 and from 3.452 to

1.615 for the corresponding time points and dose groups. The authors suggest that the yellow pigments might have masked redness from the blood vessels and muscle, an effect that is observed as yellowness intensifies. There was an increase of red pigments in plasma (Table 12).

Table 12: Concentration of red pigments in plasma (μg/mL) and skin yellowness (b*) and redness (a*) values of birds fed diets containing various levels of natural pigments

Day of study		28	49
Control	Plasma concentration	0.378	0.560
Low dose	Plasma concentration	1.684	2.209
	Yellowness (b*)	4.632	11.959
	Redness (a*)	3.517	1.708
High dose	Plasma concentration	2.056	3.692
	Yellowness (b*)	6.503	15.572
	Redness (a*)	3.452	1.615

In the view of the FEEDAP Panel, the supplementation levels of red pigments were too low, particularly compared to the yellow pigment concentration to result in an increased red pigmentation of broiler skin (and carcasses). However, the plasma values showed that the red pigment of *C. annuum* was absorbed, and since it is known that oxycarotenoids result also in skin coloration when present in the body, the study data are considered supportive for the efficacy of saponified paprika extract in colouring skin of chickens for fattening.

3.3.2. Egg yolk pigmentation

Several studies found a dose-dependent effect of paprika extract on egg yolk colour (Huyghebaert, 1993a,b; Lai et al., 1996; Galobart et al., 2004). Some other studies with a single dose confirmed this effect (Baiao et al., 1999; González et al., 1999; Santos-Bocanegra et al., 2004). The lowest effective dose was 4 mg TC/kg feed (Lai et al., 1996; Santos-Bocanegra et al., 2004).

The most recent data can be taken from the tolerance study in laying hens (see Section 3.2.3.2, Table 13).

Table 13: Effect of supplementing layer diets with saponified paprika extract on egg yolk colour after 56 days of feeding

Supplemented TC (mg/kg feed)	0	15	80	240
Analysed TC (mg/kg feed)	4.8	20.5	83.4	265
Analysed capsanthin (mg/kg feed)	< 1	8.2	40.4	128
Yolk colour characteristics(1)				
RCQO	8.6^{d}	13.4^{c}	17.2^{b}	20.1^{a}
Lightness (L*)	55.25^{a}	50.65^{b}	44.82^{c}	41.19^{d}
Redness (a*)	0.28^{d}	10.75^{c}	20.68^{b}	24.61^{a}
Yellowness (b*)	36.23^{a}	33.05^{b}	26.12^{c}	21.07^{d}

a, b, c, d: Values in the same row with different superscript letters are significantly different ($p < 0.05$).

(1): Yolk colour by means of a Minolta CR 300 colorimeter in the CIELab scale (Commission Internationale de l'Eclairage). RCQO: Roche Yolk Colour Fan value calculated with Contrôle Qualité des Oeufs V.1.0 software {Laboratoire de Développement et d'Analyses}.

Over the full dose range of TC from saponified paprika extract supplemented (15–240 mg TC/kg), all egg yolk parameters indicating higher intense red colour significantly increased with increasing levels of saponified paprika extract, whereas lightness and yellowness significantly decreased.

The data submitted allowed also to calculate the deposition rate for TC and capsanthin from saponified paprika extract. Independently from the supplemented levels (15 and 80 mg TC/kg feed), the deposition rate for TC and capsanthin was about 6% which confirms the values from literature (El Boushy and Raterink, 1992; Huyghebaert, 1993a,b; Steinberg et al., 2000) ranging from 6 to 29% (EFSA, 2006).

The Panel noted that a sufficient egg yolk pigmentation (calculated Roche Yolk Colour Fan value about 14, Table 13) was reached with 15 mg TC/kg feed while 80 and 240 mg TC/kg complete feed resulted in higher values, both above expectations of consumers (which vary from country to country)

for table eggs. The highest TC level tested (240 mg/kg) resulted in broilers in a reddish discoloration of fat, subcutaneous tissue and liver without other (adverse) tissue alterations.

3.3.3. Conclusions on efficacy

The FEEDAP Panel concludes that saponified paprika extract has the potential to pigment chicken skin and egg yolk. This conclusion is extrapolated to minor poultry species for fattening and laying.

4. Conclusions

The application is for the saponified paprika (*C. annuum*) extract containing various carotenoids at a concentration of 25–90 g/kg of which capsanthin being the major one with quantity specified as > 35% of total carotenoids (TC).

The highest TC level tested (240 mg TC from saponified paprika extract/kg feed), was tolerated by chickens for fattening and laying hens. The FEEDAP Panel concludes that the maximum recommended use level of 40 mg TC/kg feed is safe for chickens for fattening and laying hens. The margin of safety is at least 6. This conclusion is extrapolated to minor poultry species for fattening and laying.

The saponified paprika (*C. annuum*) extract is not genotoxic in a bacterial reverse mutation assay and a mammalian cell micronucleus test. Based on the NOEL of the 90-day study in rat and the exposure estimates, the Panel considered that there would be an adequate margin of exposure (between 700 and 2,000) to conclude that the level of exposure to residues of the saponified paprika (*C. annuum*) extract (capsanthin not less than 35% of TCs) in animal tissues and products does not raise concern for the safety for the consumer.

The saponified paprika extract is a viscous paste and as such users will not be exposed by inhalation. The applicant recognises that the extract may be irritant to skin and eyes. The FEEDAP Panel cannot conclude on the potential of any preparation to be toxic by inhalation, skin/eye irritant or skin sensitiser since no data were submitted.

The use of saponified paprika (*C. annuum*) extract in poultry feed as a source of capsanthin and other carotenoids would not alter the concentration or distribution of these carotenoids in the environment given their natural occurrence and oxidative susceptibility.

Saponified paprika extract has the potential to pigment broiler skin and egg yolk. This conclusion is extrapolated to minor poultry species for fattening and laying.

5. Recommendations

The name capsanthin does not provide sufficient clarity to the feed business operator since the additive contains a higher level of total carotenoids for which a maximum content is given by legislation. The FEEDAP Panel recommends the following definition of the additive to provide better clarity to the additive market: saponified extract of the dried fruits of *C. annuum* L., with a total carotenoid content of 25–90 g/kg of which capsanthin is not less than 35%.

The FEEDAP Panel recommends that the provision with regard the maximum content should be maintained but modified as follows: 'when used in combination with other carotenoids, the TC content shall not exceed 80 mg/kg feed'.

The active substances in the extracts need protection against oxidation; therefore, the extracts should be marketed as preparations only, containing antioxidants authorised for use in feed.

The FEEDAP Panel notes that according to Regulation (EC) No 231/2012, concentration of capsaicin is limited to 'not more than 250 mg/kg' in the food additive (paprika extract, E 160c). The same specification is recommended for the feed additive.

Documentation provided to EFSA/Chronology

Date	Event
8/11/2010	Dossier received by EFSA. Capsanthin for poultry and pets. Submitted by FEFANA asbl.
19/11/2010	Reception mandate from the European Commission
05/03/2012	Application validated by EFSA – Start of the scientific assessment
05/06/2012	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
05/06/2012	Comments received from Member States

Date	Event
11/07/2012	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation, conditions of use, safety for the consumer and efficacy*
8/1/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
27/2/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Characterisation*
21/4/2015	Complementary request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 *Issues: Safety for the consumer*
22/1/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
25/5/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Characterisation, safety for the target species and safety for the consumer*
1/7/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
29/1/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
BW	body weight
CAS	Chemical Abstracts Service
CV	coefficient of variation
EURL	European Union Reference Laboratory
FACE	Feed Additives Consumer Exposure
FAO	Food Agricultural Organization
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
HPLC	high-performance liquid chromatography
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
NOAEL	no observed adverse effect level
TC	total carotenoid
WHO	World Health Organization

Appendix A – Calculation of consumer exposure with FACE model

Methodology

As described in the Guidance on the safety of feed additives for consumers (EFSA FEEDAP Panel, 2017b), consumption data of edible tissues and products as derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) will be used to assess exposure to residues from the use of feed additives in different EU countries, age classes[42] and special population groups. For each EU country and age class, only the latest survey available in the Comprehensive Database will be used.

While the residue data reported for feed additives refer to organs and tissues (raw agricultural commodities. RAC), the Comprehensive Database includes consumption data for foods as consumed. In order to match those consumption data with the available residue data for feed additives, the consumption data reported in the Comprehensive Database have been converted into RAC equivalents. For assessing the exposure to coccidiostats from their use in (non-reproductive) poultry, the following list of commodities is considered: meat, fat, liver, other offals (including kidney).

Depending on the nature of the health-based guidance derived, either a chronic or acute exposure assessment may be required.

For chronic exposure assessments, the total relevant residues will be combined for each individual with the average daily consumptions of the corresponding food commodities, and the resulting exposures per food will be summed in order to obtain total chronic exposure at individual level (standardised by using the individual body weight). The mean and the higher percentile (usually the 95th percentile) of the individual exposures will be subsequently calculated for each dietary survey (country) and each age class separately.

As opposed to the chronic exposure assessments, acute exposure calculation will be carried out for each RAC value separately. The higher percentile (usually the 95th percentile) exposures based on the consuming days only will be calculated for each food commodity, dietary survey and age class separately.

Detailed results on chronic exposure calculation

Table A.1: Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to residues of TC from the saponified extract of paprika

Population class	Survey's country	Number of subjects	HRP value(1)	HRP description
Infants	Bulgaria	523	0.0472242114	95th
Infants	Germany	142	0.0122860435	95th
Infants	Denmark	799	0.0111018501	95th
Infants	Finland	427	0.0021874941	95th
Infants	United Kingdom	1,251	0.0297510612	95th
Infants	Italy	9	0.0000000000	50th
Toddlers	Belgium	36	0.0263914819	90th
Toddlers	Bulgaria	428	0.0530473737	95th
Toddlers	Germany	348	0.0328757916	95th
Toddlers	Denmark	917	0.0230258485	95th
Toddlers	Spain	17	0.0416162353	75th
Toddlers	Finland	500	0.0180515680	95th
Toddlers	United Kingdom	1,314	0.0410998849	95th
Toddlers	United Kingdom	185	0.0358467133	95th
Toddlers	Italy	36	0.0295683045	90th
Toddlers	Netherlands	322	0.0376830487	95th
Other children	Austria	128	0.0329476039	95th
Other children	Belgium	625	0.0275098649	95th

[42] Infants: < 12 months old, toddlers: ≥ 12 months to < 36 months old, other children: ≥ 36 months to < 10 years old, adolescents: ≥ 10 years to < 18 years old, adults: ≥ 18 years to < 65 years old, elderly: ≥ 65 years to < 75 years old, and very elderly: ≥ 75 years old.

Population class	Survey's country	Number of subjects	HRP value(1)	HRP description
Other children	Bulgaria	433	0.0504273225	95th
Other children	Czech Republic	389	0.0343574046	95th
Other children	Germany	293	0.0335611472	95th
Other children	Germany	835	0.0344526486	95th
Other children	Denmark	298	0.0225807033	95th
Other children	Spain	399	0.0397374737	95th
Other children	Spain	156	0.0561527207	95th
Other children	Finland	750	0.0255768624	95th
Other children	France	482	0.0322294945	95th
Other children	United Kingdom	651	0.0274226902	95th
Other children	Greece	838	0.0486347064	95th
Other children	Italy	193	0.0399464844	95th
Other children	Latvia	187	0.0292490813	95th
Other children	Netherlands	957	0.0336485761	95th
Other children	Netherlands	447	0.0301027748	95th
Other children	Sweden	1,473	0.0235914522	95th
Adolescents	Austria	237	0.0202229801	95th
Adolescents	Belgium	576	0.0160154294	95th
Adolescents	Cyprus	303	0.0134156254	95th
Adolescents	Czech Republic	298	0.0208010768	95th
Adolescents	Germany	393	0.0274854433	95th
Adolescents	Germany	1,011	0.0142477670	95th
Adolescents	Denmark	377	0.0109679975	95th
Adolescents	Spain	651	0.0279384122	95th
Adolescents	Spain	209	0.0363794061	95th
Adolescents	Spain	86	0.0166484215	95th
Adolescents	Finland	306	0.0089090575	95th
Adolescents	France	973	0.0175339094	95th
Adolescents	United Kingdom	666	0.0159362299	95th
Adolescents	Italy	247	0.0225752525	95th
Adolescents	Latvia	453	0.0225148248	95th
Adolescents	Netherlands	1,142	0.0180388582	95th
Adolescents	Sweden	1,018	0.0150471027	95th
Adults	Austria	308	0.0121302366	95th
Adults	Belgium	1,292	0.0127660671	95th
Adults	Czech Republic	1,666	0.0144966506	95th
Adults	Germany	10,419	0.0134354017	95th
Adults	Denmark	1,739	0.0090750457	95th
Adults	Spain	981	0.0168874361	95th
Adults	Spain	410	0.0169334252	95th
Adults	Finland	1,295	0.0143854991	95th
Adults	France	2,276	0.0120440884	95th
Adults	United Kingdom	1,265	0.0128254530	95th
Adults	Hungary	1,074	0.0162294423	95th
Adults	Ireland	1,274	0.0122699949	95th
Adults	Italy	2,313	0.0153195623	95th
Adults	Latvia	1,271	0.0199828227	95th
Adults	Netherlands	2,055	0.0139532206	95th
Adults	Romania	1,254	0.0193581609	95th
Adults	Sweden	1,430	0.0186839144	95th

Population class	Survey's country	Number of subjects	HRP value(1)	HRP description
Elderly	Austria	67	0.0124864876	95th
Elderly	Belgium	511	0.0115120727	95th
Elderly	Germany	2,006	0.0121333166	95th
Elderly	Denmark	274	0.0103986656	95th
Elderly	Finland	413	0.0111664089	95th
Elderly	France	264	0.0104811996	95th
Elderly	United Kingdom	166	0.0112512771	95th
Elderly	Hungary	206	0.0135736657	95th
Elderly	Ireland	149	0.0135820816	95th
Elderly	Italy	289	0.0127290272	95th
Elderly	Netherlands	173	0.0118483657	95th
Elderly	Netherlands	289	0.0117546899	95th
Elderly	Romania	83	0.0172567744	95th
Elderly	Sweden	295	0.0171347358	95th
Very elderly	Austria	25	0.0088529668	75th
Very elderly	Belgium	704	0.0126900875	95th
Very elderly	Germany	490	0.0124001964	95th
Very elderly	Denmark	12	0.0073220136	75th
Very elderly	France	84	0.0104367093	95th
Very elderly	United Kingdom	139	0.0105551912	95th
Very elderly	Hungary	80	0.0141346112	95th
Very elderly	Ireland	77	0.0132681778	95th
Very elderly	Italy	228	0.0120915169	95th
Very elderly	Netherlands	450	0.0115013946	95th
Very elderly	Romania	45	0.0144971696	90th
Very elderly	Sweden	72	0.0218590701	95th

(1): HRP: highest reliable percentile, i.e. the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possibly raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are, respectively, required to derive 50th, 75th and 90th and 95th percentile estimates. Estimates with less than 5 observations were not included in this table.

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for capsanthin

In the current application, authorisation is sought under articles 4(1) and 10(2) for *capsanthin (E160c)* under the category/functional group 2(a) 'sensory additives'/'colourants', for the following subgroups: (i) substances that add or restore colour in *feedingstuffs*; (ii) substances which, when fed to animals, add colours to food of animal origin; and (iii) substances which favourably affect the colour of ornamental fish or birds, according to the classification system of Annex I of Regulation (EC) No 1831/2003.

The *feed additive* is a saponified paprika oleoresin, with a total carotenoids & xanthophylls content ranging from 35 to 130 g/kg, which corresponds to a capsanthin content ranging from 10 to 60 g/kg. The *feed additive* is to be marketed as solid or liquid formulations with a minimum total carotenoids & xanthophylls and capsanthin concentrations of 0.5% and 0.15%, respectively.

Specifically, authorisation is sought for the use of the *feed additive* for all poultry species, cats, dogs, ornamental fish and birds. The *feed additive* is intended to be incorporated in *premixtures, feedingstuffs* and *water*. While no maximum and minimum levels were proposed for cats, dogs, ornamental fish and birds, the Applicant proposed for all poultry species a maximum concentration of capsanthin or total carotenoids & xanthophylls in *feedingstuffs* and *water* of 80 mg/kg and 40 mg/L, respectively.

For the determination of capsanthin in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted a single laboratory validated and further verified method, based on normal phase High Performance Liquid Chromatography with visible wavelength detection (HPLC-Vis). The following performance characteristics were reported:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.6 to 2.3%;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1 to 8.5%;
- a recovery rate ranging from 80 to 105%; and
- a limit of quantification of 1 mg/kg *feedingstuffs*.

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified method, based on High-Performance Liquid Chromatography with visible wavelength detection (HPLC-Vis), submitted by the Applicant, to determine capsanthin in the *feed additive*, *premixtures* and *feedingstuffs*.

For the determination of total carotenoids & xanthophylls in the *feed additive* the Applicant proposed the internationally recognised FAO JECFA monograph for food additives, recommended by Commission Directive 2008/128/EC, where identification is based on several tests, including: solubility; colour reaction; high-performance liquid chromatography (HPLC), while the quantification of total carotenoids & xanthophylls is achieved by spectrophotometry at 462 nm. Even though no performance characteristics are provided, the EURL recommends for official control the methods recommended by Commission Directive 2008/128/EC and described in the above-mentioned JECFA monograph for the determination of total carotenoids & xanthophylls in the *feed additive*.

For the determination of total carotenoids & xanthophylls (including capsanthin) in *feedingstuffs*, the Applicant submitted the official method of Association of Analytical Communities (AOAC, 970.64) based on chromatographic separation (after saponification) of carotenes & xanthophylls and further detection of the different fractions by spectrophotometry at 436 and 474 nm. This method could be applied on *premixtures* samples diluted in blank feed. Even though no performance characteristics are provided, the EURL recommends for official control the above-mentioned AOAC official method for the determination of total carotenoids & xanthophylls in *premixtures* and *feedingstuffs*.

The Applicant did not provide any analytical method or experimental data for the determination of capsanthin and total carotenoids & xanthophylls in *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine capsanthin and total carotenoids & xanthophylls in *water*.

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