SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 26 September 2023

doi: 10.2903/j.efsa.2023.8338

Safety of a change in specifications of the novel food oleoresin from *Haematococcus pluvialis* containing astaxanthin pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Margarita Aguilera-Gómez, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Miguel Prieto Maradona, Josef Rudolf Schlatter, Alexandros Siskos, Henk van Loveren, Wolfgang Gelbmann and Helle Katrine Knutsen

Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the safety of a change of specifications of the novel food (NF) oleoresin from *Haematococcus pluvialis* containing astaxanthin (ATX) pursuant to Regulation (EU) 2015/2283. The NF is already authorised as ingredient for the use in food supplements as defined in Directive 2002/46EC in accordance to Regulation (EU) 2017/2470. The NF concerns an oleoresin which contains ~ 10% ATX, obtained by supercritical CO_2 extraction of the homogenised and dried biomass of cultivated *H. pluvialis*. This NF has been assessed by the Panel in 2014. With the present dossier, the applicant proposed to lower the minimum specification limits for protein and ATX monoesters for the NF, and to increase the maximum specification limit for the relative amount of ATX diesters in total ATX. An increase of the maximum specification limit for the 9-cis isomer is also applied for. Although the data are limited regarding bioavailability and distribution in humans of these three naturally occurring ATX isomers, the available *in vitro* and *in vivo* data suggest that the 13-cis rather than the 9-cis ATX is selectively absorbed, i.e. has a higher bioavailability and/or possibly emerges from isomerisation of all-trans ATX. The Panel notes that the toxicity of the individual ATX isomers has not been studied individually. However, the ADI of 0.2 mg/kg, which was established for synthetic ATX and ATX from *H. pluvialis*, applies also for ATX in the oleoresin from *H. pluvialis* with the proposed changes of specifications. The Panel concludes that the NF, oleoresin from *H. pluvialis* containing ATX, is safe with the proposed specification limits.

Keywords: Astaxanthin, 9-cis astaxanthin, isomer, *Haematococcus pluvialis*, micro algae, specification change, food supplements

Requestor: European Commission

Question number: EFSA-Q-2022-00588

Correspondence: nif@efsa.europa.eu

Panel members: Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Aguilera-Gómez, M., Cubadda, F., Frenzel, T., … Knutsen, H. K. (2023). Safety of a change in specifications of the novel food oleoresin from *Haematococcus pluvialis* containing astaxanthin pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *21*(11), 1–15. https://doi.org/10.2903/j.efsa.2023.8338

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 20 June 2022, the company Astareal AB submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1], to authorise the change in the specifications of use of the novel food (NF) oleoresin from *Haematococcus (H.) pluvialis* containing astaxanthin (ATX).

The applicant requests modifications of the ranges in the specifications for ATX monoesters, ATX diesters, 9-cis ATX stereoisomer, and the modification of the range of the protein content.

The applicant has not requested data protection under Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority (EFSA) to provide a scientific opinion for a change in specifications of the novel food oleoresin from *H. pluvialis* containing ATX.

1.2. Additional information and previous assessments

The first authorisation for ATX in the EU was granted in Sweden in 1995, with a maximum intake of 4 mg/day for its use in food supplements (FS). This was before the cut-off date of 15 May 1997, when the first Novel Food Regulation (EC) No 258/97 came into effect. That application in 1995 concerned a 'dried powder of *H. pluvialis*' and was submitted by the company Astareal AB (formerly named 'Astacarotene AB'), which is also applicant of the application subject of this Opinion.

Subsequently, 11 notifications for oleoresins from *H. pluvialis* containing ATX were submitted from 10 different applicants pursuant to Article 5 of Regulation (EC) No 258/97 to EU Member States and the Commission, based on substantial equivalence to that powder obtained from the dried biomass of *H. pluvialis* authorised by the Swedish competent authority in 1995 (European Commission, 2018). One of these notifications was submitted by Astareal AB in 2006 for their oleoresin from *H. pluvialis* which contains ~ 10% ATX and for which the applicant informs that is produced from the same homogenised and dried biomass powder as the product authorised in 1995.

Commission Implementing Regulation (EU) 2017/2470[2] establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283, applies to all oleoresins from *H. pluvialis* containing ATX, which were authorised under Article 5 of Regulation (EC) No 258/97. They are allowed to be marketed for the use in FS at levels of up to 40–80 mg/day which should correspond to no more than 8 mg ATX per day. Commission implementing Regulation (EU) 2021/1377 and Commission Implementing Regulation (EU) 2023/1581 amended Commission Implementing Regulation (EU) 2017/2470 by imposing a labelling requirement for food supplements containing the NF should not be consumed by children younger than 3 years of age.

In 2014, the EFSA NDA Panel[3] assessed the safety of three formulations obtained from the cultivated microalgae *H. pluvialis* intended for the use in fermented liquid dairy products, non-fermented liquid dairy products, fermented soya products and fruit drinks: (1) a powder containing 5–5.6% w/w ATX (that concerned the product which was approved by the Swedish authority in 1995), (2) a red viscous oleoresin containing about 10% w/w ATX (called 'AstaReal L10' in that Opinion from 2014 and which concerned the product which has been authorised in 2006 under Article 5 of Regulation (EU) No 258/1997, 'substantial equivalence', for FS). Since this is an authorised NF, it must comply with the NF Union list (Commission Implementing Regulation (EU) 2017/2470) and (3) a water-dispersible formulation containing 2.5–2.7% w/w ATX under Regulation (EC) No 258/1997 made from (2).

The present opinion concerns the oleoresin from *H. pluvialis* produced by the applicant which contains about 10% ATX (i.e. formulation (2) which has been assessed by the Panel in 2014). The applicant seeks to lower the minimum specification limits as set in the EU Union list for the protein content in the NF from currently 0.3% to 0.0% and for ATX monoesters (as % of total ATX, i.e. esterified and free) from currently 79.8% to 66.7%. Furthermore, they propose to increase the maximum specification limits for ATX diesters (as % of total ATX, i.e. esterified and free) from currently 19.0% to 32.5% and for 9-cis-ATX from currently 17.3% to 30.0%.

[1] Regulation (EU) 2015/2283 of the European Parliament and of the council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001; OJ L 327, 11.12.2015, p. 1–22.
[2] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32017R2470&from=EN
[3] EFSA Journal 2014; 12(7):3757. https://www.efsa.europa.eu/it/efsajournal/pub/3757

Already in the application assessed by the EFSA NDA Panel in 2014, the applicant had provided some compositional data on these four parameters, but they were not proposed in the specifications in the Opinion. In that assessment from 2014, the NDA Panel considered that the production process and the composition of the NF were sufficiently described and that also the specifications did not raise safety concerns. In the present application, the applicant informs that since the establishment of the NF Union list (which included specification limits for these four components), they have performed more compositional analyses of several batches from both of their production sites in the US and Sweden, including batches from earlier years. The results made the applicant realise that several of the batches did not comply with these four specification parameters set in the NF Union list. As a consequence, the applicant submitted this application with analytical data for a larger number of batches covering the period since 2013 to support their request to amend the applicable specifications limits in the NF Union list.

In their assessment, which addresses also consumer safety, the FEEDAP Panel assessed several times the safety and efficacy of synthetic ATX and ATX produced by microorganism, as feed additive for fish such as salmon and trout (EFSA, 2004, 2006, 2007a,b; EFSA FEEDAP Panel, 2010, 2014a,b, 2019, 2022).

In 2020, the EFSA NDA Panel considered that the updated ADI for ATX of 0.2 mg/kg body weight (bw) derived by the EFSA FEEDAP Panel in 2019, for synthetic ATX-DMDS (dimethyldisuccinate) also applies to ATX from *H. pluvialis* algae (EFSA NDA Panel, 2020).

2. Data and Methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[4].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2021). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

The applicant has submitted a confidential and a non-confidential version of a dossier following the 'EFSA guidelines on the preparation and presentation of a NF application' (EFSA NDA Panel, 2021) and the 'Administrative guidance for the preparation of applications on novel foods pursuant to Article 10 of Regulation (EU) 2015/2283 (EFSA, 2021)'.

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

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[2] EFSA carried out a public consultation on the non-confidential version of the technical dossier from 20 April to 11 May 2023 for which no comments were received.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2021 and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee.

[4] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

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

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

[7] The non-confidential version of the dossier has been published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/dossier/NF-2022-6010

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF which is the subject of the application concerns oleoresin from *H. pluvialis* containing ATX. The NF is already authorised (Commission Implementing Regulation (EU) 2017/2470[2] amended by Commission Implementing Regulation (EU) 2021/1377[8] and Commission Implementing Regulation (EU) 2023/1581[9]) for its use in FS as defined in Directive 2002/46/EC, excluding for children younger than 3 years. The authorised maximum daily intake is 2.3, 5.7 and 8 mg ATX for children 3 to less than 10 years of age, for adolescents 10 to less than 14 years of age and for the general population older than 14 years of age, respectively.

According to Regulation (EU) 2015/2283, the NF falls under the following categories:

i) 'food consisting of, isolated from or produced from microorganisms, fungi or algae'.

3.2. Identity of the NF

According to Commission Implementing Regulation (EU) 2017/2470[2] establishing the Union list of novel foods, the NF, 'Astaxanthin-rich oleoresin from *Haematococcus pluvialis algae*' concerns (citation): 'ATX is a carotenoid produced by *H. pluvialis* algae. Production methods for the growth of the algae are variable; using closed systems exposed to sunlight or strictly controlled illuminated light, alternatively open ponds may be used. The algal cells are harvested and dried; the oleoresin is extracted using either supercritical CO_2 or a solvent (ethyl acetate). The ATX is diluted and standardised to 2.5, 5.0, 7.0, 10, 15 or 20% using olive oil, safflower oil, sunflower oil or MCT (medium chain triglycerides)'.

Regarding the NF produced by the applicant, oleoresin is obtained with supercritical CO_2 extraction from the homogenised and dried biomass of cultivated *H. pluvialis*.

Astaxanthin, 3,3′-dihydroxy-β,β-carotene-4,4′-dione, has a molecular weight of 596.85 Da. Its Chemical Abstracts Service (CAS) number is 472-61-7. It occurs naturally in *H. pluvialis*.

3.3. Production process

According to the applicant, two minor changes have been made in the production as compared to the application subject of the EFSA NDA Panel (2014a,b) opinion, but otherwise the production process remained the same. The two changes concern the use of a vitamin E from a natural source (instead of a synthetic one) and the pasteurisation step, which is now applied right after the crushing and before the spray-drying (while previously it was employed after spray-drying). The applicant also noted that after the previous EFSA assessment from 2014, the applicant passed the audit of a Food Safety System Certification 22000.

3.4. Composition

The applicant limited the provided data on the composition to the specification parameters included in the NF Union list.

Information on proximate analysis for five batches including the certificates of analysis is provided for five batches in Table 1.

[8] https://eur-lex.europa.eu/eli/reg_impl/2021/1377/oj
[9] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32023R1581&qid=1694609675876

Table 1: Proximate analysis of five batches of the NF

Parameter	Specification limits[(a)]	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Method/Reference
Fat (g/100 g)	42.2–99	92.7	93.1	97.4	92.8	93.6	NMKL 160 mod.
Protein (g/100 g)	0.3–4.4	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	NMKL 6
Carbohydrates (g/100 g)	0–52.8	7.1	6.6	2.5	7.2	6.3	EU(No)1169/2011
Fibre (g/100 g)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	AOAC 991.43 mod.
Ash (g/100 g)	0.0–4.2	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	NMKL 173

AOAC: Association of Official Analytical Collaboration; NMKL: Nordic-Baltic Committee on Food Analysis.
(a): According to the Union list of novel foods, Commission Implementing Regulation (EU) 2017/2470.

The Panel notes that the protein content of the five batches analysed is below the minimum limit for the protein content in the NF Union list (which is 0.3%). The information is however identical to the value reported as 'typical protein content' for this NF assessed by the EFSA Panel in 2014.

With regards to the carotenoid composition, the applicant provided results from five batches produced in their production facilities in the US and in Sweden, presented in Table 2 and 3, respectively.

Table 2: Carotenoid content of the NF; analytical results from five batches produced at the US production site; CoA has been provided; analysed by Fuji Chemical (method similar to USP presented in Annex 7, astaxanthin esters: HPLC method as described by Holtin et al., 2009)

Carotenoid as w/w % of the NF	Specification limits[(a)]	A	B	C	D	E
Total ATX	2.9–11.1%	10.3	10.5	10.4	10.4	10.5
9-*cis*-ATX[(b)]	0.3–17.3%	13.0	13.5	12.7	10.6	9.3
13-*cis*-ATX[(b)]	0.2–7.0%	5.8	5.8	5.7	6.1	5.4
ATX monoesters[(c)]	79.8–91.5%	75.4	75.5	76.1	75.8	74.9
ATX diesters[(c)]	0.16–19.0%	23.7	23.6	23.1	23.3	24.4
ß-carotene	0.01–0.3%	0.27	0.24	0.26	0.27	0.31
Lutein	0–1.8%	0.4	0.4	0.43	0.38	0.50
Canthaxanthin	0–1.30%	0.70	0.73	0.69	0.71	0.78

CoA: certificate of analysis; HPLC: high performance liquid chromatography; USP: United States Pharmacopeia.
(a): According to the Union list of novel foods (Commission Implementing Regulation (EU) 2017/2470).
(b): % of total ATX (covering all ATX isomers; note: all-*trans* ATX, which is not among specification parameters, is the predominant isomer with more than 60% of total ATX. Thus, the sum of 9-*cis* ATX and 13-*cis* ATX is below 40%.)
(c): % of total ATX (covering all esterified and free ATX; only small quantities of free ATX are present in the NF).

According to the results presented in Table 2, the carotenoid composition of the five batches from the applicant's US production facility complies with the current specifications set in the NF Union list, including for 9-*cis*-ATX, except for the contents of ATX mono- and diesters, which are below the minimum and above the maximum specification limits, respectively.

Table 3: Carotenoid content of the NF; analytical results from five batches produced at the Swedish production site; CoAs have been provided; analysed with an in-house HPLC method, except for total carotenoids, where spectrophotometry is used

Carotenoid as w/w % of the NF	Specification limits[(a)]	F	G	H	I	J
Total ATX	2.9–11.1%	10.78	10.78	10.87	10.85	10.77
9-cis-ATX[(b)]	0.3–17.3%	22.90	24.86	25.81	26.73	25.05
13-cis-ATX[(b)]	0.2–7.0%	3.17	2.98	3.05	2.79	2.90
ATX monoesters[(c)]	79.8–91.5%	78.77	78.99	79.29	79.47	79.18
ATX diesters[(c)]	0.16–19.0%	20.62	20.41	20.09	19.88	19.78
ß-carotene	0.01–0.3%	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

Carotenoid as w/w % of the NF	Specification limits[(a)]	F	G	H	I	J
Lutein	0–1.8%	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Canthaxanthin	0–1.30%	0.02	0.02	0.02	0.02	0.02
Total carotenoids	–	11.1	11.1	11.1	11.1	11.0

CoA: certificate of analysis; HPLC: high performance liquid chromatography; USP: United States Pharmacopeia.
(a): According to the Union list of novel foods (Commission Implementing Regulation (EU) 2017/2470).
(b): % of total ATX (covering all ATX isomers; note: all-*trans* ATX, which is not among specification parameters, is the predominant isomer with more than 60% of total ATX. Thus, the sum of 9-*cis* ATX and 13-*cis* ATX is below 40%.).
(c): % of total ATX (covering all esterified and free ATX; only small quantities of free ATX are present in the NF).

With regards to the results for the five batches from the applicant's Swedish production facility as presented in Table 3, the carotenoid composition complies with the current specifications set in the NF Union list, except for the contents of 9-*cis* ATX, and ATX mono- and diesters. The values for 9-*cis* ATX and for the ATX diesters exceed the respective NF Union list's maximum specification limits. The differences between the results from the US and Swedish production are explained by the applicant to be the result of producing such complex NF at two different facilities and the use of different protocols and methods which were employed for analysing the batches produced in the US and Sweden. In response to a question asked by EFSA, on whether there are differences between the production process employed in the US and Sweden, which could explain the differences of the batches presented in Tables 2 and 3, the applicant responded that they could not identify the specific reason for the observed differences. The applicant also noted that it is difficult to produce such a food with a highly constant composition within narrow ranges, when the production involves cultivation and a series of downstream process steps in different production sites over many years. The applicant also noted that even small differences between several factors during cultivation can have an impact on the ratio of 9-*cis* ATX compared to the other ATX isomers (which are mainly all-*trans* ATX and smaller amounts of 13-*cis* ATX) and also on the ratio between ATX mono- and diesters. According to the applicant, supported by two publications (Gong et al., 2020; Viazau et al., 2021), the factors that affect the relative amount of *cis*-forms (and of the other isomers) are mainly the degree of maturity of the aplanospores, but also the growth media composition, light and temperature. Also factors during the processing of the biomass such as temperature, the solvent used to extract the oleoresin and the presence of copper ions have been reported in the literature to have an impact on the isomerisation from *trans* to the 9- and 13-*cis* isomers (Yuan and Chen, 1999; Zhao et al., 2005).

With analyses of 10 other batches produced in Sweden and in the years from 2013 to 2021, the applicant provided also historical data on the geometric ATX isomers contained in the NF (Table 4). According to this batch testing, and except for the batches produced in 2013 and 2015, the 9-*cis* ATX contents exceed the maximum specification limit for this compound as currently set by the NF Union list (i.e. 17.3% of total carotenoids), with the highest value being 29.4% in a batch produced in 2021.

The Panel notes that the range for the 9-*cis* ATX isomer content of three batches (1.61–2.10% in the NF with a total ATX content in the NF ranging between 10.32 and 11.01%), reported by EFSA in 2014 indicated an exceedance of the applicable maximum specification limit for 9-*cis* ATX (i.e. 17.3% of total carotenoids), established later in 2017 by the NF Union list. Since the dossier assessed in 2014 is still held by EFSA, details on these batches could be retrieved from the archive. According to these archived data, one batch of the NF, produced in 2005 in Sweden, had a relative total ATX and 9-*cis* ATX content of 10.45% and 2.1%, respectively. Expressed as relative 9-*cis* ATX content per total ATX, this results to 20.1% (exceeding the maximum specification limit in the NF Union list).

Table 4: The relative distribution (%) of all-*trans*-, 9-*cis*- and 13-*cis* ATX of total ATX in batches of AstaReal produced in Sweden in the period 2013–2021; analysed with an in-house HPLC method

Production date	Batch	All-trans-ATX	9-cis ATX	13-cis ATX
Sep-13	K	86.0	9.7	4.5
Aug-14	L	76.8	18.5	4.6
Aug-15	M	78.3	17.0	4.7
Sep-16	N	75.9	20.0	4.1
Jun-17	O	74.4	22.3	3.3

Production date	Batch	All-trans-ATX	9-cis ATX	13-cis ATX
Aug-18	P	76.6	19.5	3.9
Okt-19	Q	70.5	26.2	3.3
Jul-20	R	68.7	28.1	3.2
Sep-21	S	67.5	29.4	3.0
Mar-21	T	73.0	23.8	3.2

The applicant noted the upward trend for the relative 9-*cis* ATX content over this period covered by this analysis of batches produced in Sweden from 2013 to 2021 (presented in Table 4).

Following a request by EFSA to specify whether changes of the growth conditions and maturation have been made during the cultivation process over the years, the applicant responded that the higher relative amount of 9-*cis* (% of total ATX) could indeed be a consequence of an extended maturation phase at the end of the cultivation of the microalgae from about 8 days in earlier years to now 11 days and not a result of a change of the production process after harvesting. The reason why no such trend was noted for batches produced in the US could not be explained by the applicant.

Regarding the ATX ester (mono- vs. diester ratio) the applicant provided more batch testing. The average ratio of 77 of batches produced in period of years 2014–2021 was 74.0% for monoesters and 25.0% for diesters. The maximum ratio was 81.1% for monoesters and 32.5% for diesters, respectively. The minimum values were 66.7% for monoesters and 13.6% diesters. The minimum value of monoesters was lower than found in analyses of US and Swedish batches presented in Tables 2 and 3, respectively. Correspondingly, the highest value for diesters, 32.5% was higher than any of those analysed in US and Swedish batches in Tables 2 and 3, respectively. These results describe the variability of the ester ratios. In the dossier assessed by the EFSA NDA Panel in 2014, no information from batch testing was provided on the ATX mono – vs. diester ratio, but 'typical values' (80% monoester, 18% diester and 2% free ATX) were reported. The applicant notes that when the dossier assessed by EFSA in 2014 was prepared, they were not aware of the higher variability of the ATX mono- vs. diester ratio. This was observed only later. For the present application, the applicant provided published articles reporting up to 35% of the ATX as diesters (Grewe and Griehl, 2008; Grung et al., 1992).

Considering the values reported for 9-*cis* ATX (Tables 3, 4 and 5) and that the average ratio between ATX mono- (74%) and ATX diesters (25%) derived from large number of batches, are already below and above the current specification limits of the NF Union list, i.e. 79.8% and 19.0%, respectively, the Panel considers that the relative contents of 9-*cis* ATX and of ATX diesters of many, if not most of the produced batches of this NF exceeded the respective specification limits in the NF Union list established in 2017.

3.5. Specifications

As presented in Table 5, the applicant seeks to lower the minimum specification limits as set in the EU Union list for the protein content in the NF from currently 0.3% to 0.0% and for ATX monoesters (as % of total ATX, esterified and free) from currently 79.8% to 66.7%. Furthermore, they propose to increase the maximum specification limits for 9-*cis*-ATX from currently 17.3% to 30.0% and for ATX diesters (as % of total ATX, esterified and free) from currently 19.0% to 32.5%.

Table 5: Current specifications for 'ATX-rich oleoresin of *H. pluvialis* algae'[(a)] as set by the NF Union list and changes as applied by the applicant

Parameter	Specification limit[(a)]	Applicant's proposal to change
	(g/100 g)	(g/100 g)
Fat	42.2–99	–[(d)]
Protein	0.3–4.4	≤ 4.4
Carbohydrates	0–52.8	–
Fibre	< 1.0	–
Ash	0.0–4.2	–
Specification of Carotenoids	(%)	(%)

Parameter	Specification limit[a] (g/100 g)	Applicant's proposal to change (g/100 g)
Total Astaxanthin	2.9–11.1	–
9-*cis*[b]	0.3–17.3	0.3–30.0
13-*cis*[b]	0.2–7.0	–
ATX Monoesters[c]	79.8–91.5	66.7–91.5
ATX Diesters[c]	0.16–19.0	0.16–32.5
β-carotene	0.01–0.3	–
Lutein	0–1.8	–
Canthaxanthin	0–1.30	–
Microbiological Criteria		
Total aerob. bact. (plate count)	< 3,000 CFU/g	–
Yeast	< 100 CFU/g	–
Moulds	< 100 CFU/g	–
Coliforms	< 10 CFU/g	–
E. coli	Negative	–
Salmonella	Negative	–
Staphylococcus	Negative	–

CFU: colony forming units.
(a): According to the Union list of novel foods (Commission Implementing Regulation (EU) 2017/2470).
(b): % of total ATX (covering all ATX isomers; note: all-*trans* ATX, which is not among specification parameters, is the predominant isomer with more than 60% of total ATX. Thus, the sum of 9-*cis* and 13-*cis* is below 40%)
(c): % of total ATX (covering all esterified and free ATX, only small quantities of free ATX are present in the NF).
(d): '–'means not to be changed.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the NF

As noted in Section 1.2 (Additional information) several oleoresins from the microalgae *H. pluvialis* produced by different manufacturers have been authorised within the EU based Article 5 of Regulation (EU) 258/1997, i.e. based on 'substantial equivalence' to the ATX containing 'dried powder of *H. pluvialis'* authorised in Sweden in 1995 and which is produced by the same applicant who now seeks to change the specification for 'astaxanthin-rich oleoresin of *H. pluvialis* algae' as currently set by Commission Implementing Regulation (EU) 2017/2470. Furthermore, this implementing Regulation notes that 'production methods for the growth of the algae are variable; using closed systems exposed to sunlight or strictly controlled illuminated light, alternatively open ponds may be used'.

The Panel also notes that according to the information provided by the applicant in the present application, and consistent with their application assessed by the EFSA NDA Panel in 2014, the oleoresin is produced by supercritical CO_2 extraction of the homogenised and spray-dried biomass of the microalga *H. pluvialis*, which is cultivated and processed as for the ATX containing 'dried powder of *H. pluvialis'* authorised in Sweden in 1995 for the use in food supplements (FS).

The batch testing results provided by the applicant and a batch noted in the Opinion from 2014, suggest that there has been exposure to batches which had contents of 9-cis ATX and of ATX diesters higher than currently permitted by the NF Union List. Furthermore, and according to the applicant, the NF is produced from the same dried biomass of *H. pluvialis* which has been used in FS, authorised by Sweden in 1995. The Panel considers therefore that there is a history of use of FS which contain higher 9-*cis* ATX and ATX diester contents than the currently applicable maximum specification limits for these two parameters.

3.6.2. History of use of the source

As noted by the EFSA NDA Panel (2014), ATX from *H. pluvialis* occurs also naturally in the food chain. *H. pluvialis* and other microalgae are consumed by zooplankton and crustaceans, which in turn are consumed by salmon, trout and other aquatic animals.

3.7. Absorption, distribution, metabolism, excretion

The applicant performed a literature review on the bioavailability of the 9-*cis* ATX isomer. Articles were found on studies with human cell lines and performed *in vivo* in rodents and human.

Yang et al. (2017) showed that both, *trans*-*cis* isomerisation of highly purified all-*trans*-ATX, and *cis*-*trans* isomerisation of the purified 9-*cis*- and 13-*cis* ATX isomers occur during digestion in an *in vitro* digestion model and also during the cellular uptakes of ATX isomers by human intestinal Caco-2 cells. Higher absorption (measured by cellular uptake) was seen with 13-*cis* ATX than with 9-*cis* ATX- or all-*trans* ATX. The 9-*cis* ATX showed higher transport efficiency (measured as concentration in the basolateral compartment) than the other studied stereoisomers. The authors concluded that these findings might explain why *cis* isomers occur in higher concentrations in human plasma than the all-*trans*-isomer.

In a study with Balb/c mice, the animals received a single dose of either free ATX or highly purified ATX esters at 100 mg (as ATX equivalent) per kg bodyweight via gavage. Plasma and different tissues were studied for the presence of ATX isomers from 1 to 72 h (Zhou et al., 2019). The ATX esters consisted of 85% of monoesters and 15% diesters. In the small intestine wall, plasma and liver tissues, only free ATX forms were found. The relative ratios of 13-c*is*, all-*trans* and 9-*cis* ATX isomers were 7.0, 75.9 and 17.1% in the small intestine content, and 11.4, 71.4 and 17.1%, respectively, in the small intestinal wall. Thus, the 13-*cis* isomer had a slightly higher relative uptake by the small intestine. In the plasma, the relative ratios were 51.8, 41.6 and 6.6%, and in the liver 62.5, 30.6 and 6.9%, which shows selective accumulation of 13-*cis* ATX, but not of the 9-*cis* isomer over all-*trans* astaxanthin in the plasma and liver. The authors considered that this result is probably due to a combination of isomerisation (from all-*trans* to 13-*cis*) reactions after ingestion, isomer discrimination during absorption, translocation in enterocytes and subsequent update in the blood and/or discrimination during metabolism.

Honda et al. (2021) compared the bioavailability and tissue accumulation efficiency of all-*trans* and *cis* ATX isomers in rats. Male rats received a diet over 2 weeks containing ATX isomers with either a relative ratio of 99.5, 0.3 and 0.2% for all-*trans*, 9-*cis* and 13-*cis* ATX, respectively (i.e. the 'all-*trans* ATX diet') or a '*cis* ATX diet' with a relative ratio of 19.2 (all-*trans*), 17.7 (9-*cis*), 40.1 (13-*cis*) and 23% (other *cis* isomers), respectively. ATX levels in tissue samples (skin, liver, kidney, adrenal, lung, prostate, testis, eye and cerebral cortex) were analysed after the treatment period. The ATX concentrations in 6 h-plasma and 2-week plasma of rats fed with the *cis*-ATX diet were 36.8 and 67.4 times higher than those fed the all-*trans* diet. Although the all-*trans* ATX diet contained only 0.5% *cis*-ATX isomers, the relative concentration of *cis*-ATX isomers (of total ATX) in the rats' plasma receiving all-*trans* diet was 9 and 44.2% after 6 h and at 2-week, respectively. In the group fed with the *cis*-ATX diet, the relative proportion of *cis*-ATX was 76.1% to 79.4%, respectively. The 13-*cis* ATX isomer was the major ATX *cis* isomer detected in plasma samples of each group, regardless of the isomer profile of the diets. The 9-*cis* ATX concentration in the plasma at 2 weeks was less than 10% of the total ATX isomers (as compared to 17.7% in the '*cis*-ATX diet'). In skin, liver, kidney, adrenal, lung, prostate, testis and eye the concentration of ATX was significantly higher when fed with the *cis*-ATX diet. The relative amount of the 9-*cis* ATX isomer in the tissues was less than 10%, except in the cerebral cortex. The authors concluded that, in rats, *cis* isomers are more bioavailable than the all-*trans* isomer and that 13-*cis* ATX has a greater bioavailability than the 9-*cis* ATX isomer.

A review by Yu and Liu (2020), not provided by the applicant, concerned studies reporting on the presence of the main isomers (all-*trans*, 9-*cis*, 13-*cis* and 15-*cis*) in aquatic animals. The authors noted that selective distribution and isomerisation have been reported for various aquatic animals. With regards to the Atlantic salmon, however, and based on four studies, no obvious isomerisation and selectivity of ATX isomers have been found in the flesh of this species, irrespective of whether the animals got only one or a mixture of three ATX isomers (Schiedt et al., 1981; Storebakken et al., 1985; Schiedt et al., 1988; Whyte and Sherry, 2001).

The review of the applicant included two studies reporting human data. One study including three subjects regarded the bioavailability and distribution at a single dose of 100 mg ATX (consisting of 74% all-*trans*, 9% 9-*cis* ATX and 17% 13-*cis* ATX) (Osterlie et al., 2000). This study was also referred to by the Panel in 2014, who reported that all-*trans*- and *cis*-ATX isomers had similar kinetics, and also that a selective process increases the relative proportion of *cis*-ATX isomers compared with to all-*trans* ATX during uptake. However, the results also indicated that the higher ratio of *cis* ATX isomers in the plasma (as compared to the ratio in the diet) concerned only the 13-*cis* ATX and not the 9-*cis* isomer

(not mentioned in the Opinion from 2014). The plasma level of 9-*cis* ATX was proportionally (in relation to their exposure ratio) lower than the plasma level of all-*trans* ATX.

In the second human study provided by the applicant's literature review, only three subjects were also studied. They received a single dose of 10 mg of an ATX formulation composed of 95.2, 1.2 and 3.6% of the all-*trans*, 9-*cis* and the 13-*cis* ATX, respectively, followed by a single dose of 100 mg of the same formulation 4 weeks later (Coral-Hinostroza et al., 2004). A higher *cis*- versus all-*trans* ATX plasma level as compared to the *cis*/all-*trans*-ATX ratio in the diet was reported.

Despite the limited studies available, the Panel considers that the provided *in vitro* data and studies in rodents and humans consistently indicate that the 13-*cis* ATX is more bioavailable than the 9-*cis* ATX and the all-*trans* isomer, and that *trans*-to-*cis* isomerisation (demonstrated *in vitro* with human colon epithelial cells and reported for aquatic animals) may also occur in rodents and humans.

3.8. Toxicological information and human studies

The safety of ATX, both synthetic and of biological origin has been extensively assessed by EFSA (EFSA, 2004, 2006, 2007a,b; EFSA FEEDAP Panel, 2010, 2014a,b, 2019, 2022).

In 2020, the EFSA NDA Panel reviewed the safety of 8 mg ATX per day when used in FS. That assessment considered (i) previous assessments of ATX by EFSA Panels in 2014 (EFSA NDA Panel, 2014; EFSA FEEDAP Panel, 2014a,b), (ii) information received by the European Commission during the public consultation of the draft Union list (European Commission, 2017), (iii) an updated safety assessment by the EFSA FEEDAP Panel from 2019 on synthetic ATX dimethyldisuccinate (DMDS) (EFSA FEEDAP Panel, 2019), (iv) information received from a public call for data[10] launched by EFSA and (v) information retrieved by EFSA from an extensive literature search. The information received from the public consultation included also a submission concerning the safety assessment of ATX, which included an evaluation of the isomeric differences between ATX of natural origin and synthetic ATX, and a review of published clinical studies with ATX. The safety relevant aspects of these and other available studies in humans (in total 90) with ATX, both, synthetic and of biological origin, were briefly summarised in the Opinion by the FEEDAP Panel in 2019 when an acceptable daily intake (ADI) for ATX of 0.2 mg/kg bw per day was established. This ADI for ATX was confirmed by the NDA Panel (2020), and to be applicable also for ATX derived from *H. pluvialis*. For their assessments of the safety of ATX, the EFSA FEEDAP and NDA Panels did not consider the specification limits as set by the NF Union list, but the scientific evidence reported in both of their opinions, including a number of repeated dose toxicological studies addressing subchronic and chronic toxicity, and carcinogenicity. Two of the subchronic toxicity studies were performed with ATX from *H. pluvialis,* which were already assessed by the NDA Panel in 2014 (Takahashi et al., 2004; Stewart et al., 2001).

No additional toxicological or human study has been provided by the applicant.

4. Discussion

The NF is an already authorised oleoresin which contains ~ 10% ATX, obtained by supercritical CO_2 extraction of homogenised and dried biomass of cultivated *H. pluvialis*. This NF has been assessed by the Panel in 2014, when they considered that the production process and the composition were sufficiently described and that the specifications do not raise safety concerns.

In the present dossier, the applicant proposes to lower the minimum specification limits for protein and ATX monoesters for the NF. The Panel considers that this would not affect the NF's safety. Furthermore, an increase of the maximum specification limit has been requested for the relative amount of ATX diesters in total ATX. Since only free ATX from hydrolysed esters is expected to be absorbed, the Panel considers that this increase would also not affect the safety of the NF. The applicant also applied for increasing the maximum specification limit for the 9-*cis* isomer. According to the information provided by the applicant, the higher relative amount of 9-*cis* (% of total ATX) observed in batches produced in Sweden is probably a consequence of an extended maturation phase at the end of the cultivation of the microalgae from about 8 days in earlier years of their production to now rather 11 days.

The Panel notes that the maximum daily intakes of the NF and of ATX as currently permitted by the NF Union list for FS would not change with the proposed specification changes. The Panel furthermore notes that the relative increase of the maximum specification limit for 9-*cis* ATX (from 17.3% to 30% of total ATX), resulting in increased exposure to this isomer, would be accompanied by a corresponding decrease of the contents of and exposure to the 13-*cis* and in particularly the predominant all-*trans*

[10] https://www.efsa.europa.eu/en/consultations/call/180725

ATX isomer. Although the data are limited regarding bioavailability and distribution in humans of these three naturally occurring ATX isomers, the available *in vitro* and *in vivo* data suggest that the 13-*cis* rather than the 9-*cis* ATX is selectively absorbed, i.e. has a higher bioavailability and/or possibly emerges from isomerisation of all-*trans* ATX.

The Panel notes that the toxicity of the individual ATX isomers has not been studied individually. However, the ADI of 0.2 mg/kg which was established for synthetic ATX and ATX from *H. pluvialis*, applies also for ATX in the oleoresin from *H. pluvialis* with the proposed changes of specifications.

5. Conclusions

The Panel concludes that the NF, oleoresin from *H. pluvialis* containing ATX, is safe with the proposed specification limits.

6. Steps taken by EFSA

1. On 12/01/2023 EFSA received a letter from the European Commission with the request for a scientific opinion for a change of specifications of the novel food "oleoresin from *Haematococcus pluvialis* containing astaxanthin Ref.Ares(2023)268645.

2. During its plenary meeting on 26/09/2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of the specification changes of the novel food "oleoresin from *Haematococcus pluvialis* containing astaxanthin pursuant to Regulation (EU) 2015/2283.

References

Coral-Hinostroza GN, Ytrestøyl T, Ruyter B and Bjerkeng B, 2004. Plasma appearance of unesterified astaxanthin geometrical E/Z and optical R/S isomers in men given single doses of a mixture of optical 3 and 3'R/S isomers of astaxanthin fatty acyl diesters. Comparative Biochemistry, Physiology Toxicology and Pharmacology: CBP, 139, 99–110. https://doi.org/10.1016/j.cca.2004.09.011

EFSA (European Food Safety Authority), 2004. Scientific Opinion on environmental impact of Astaxanthin-rich Phaffia rhodozyma (Ecotone®) as feed additive in accordance with Council Directive 70/524/EEC. EFSA Journal 2004;2(4):43, 4 pp. https://doi.org/10.2903/j.efsa.2004.43

EFSA (European Food Safety Authority), 2006. Scientific Opinion on a request from the European Commission on the safety and efficacy of the product AQUASTA, an Astaxanthin-rich Phaffia rhodozyma ATCC SD-5340 for salmon and trout. EFSA Journal 2006;4(2):320, 19 pp. https://doi.org/10.2903/j.efsa.2006.320

EFSA (European Food Safety Authority), 2007a. Scientific Opinion on the safety and efficacy of CAROPHYLL® Stay-Pink (astaxanthin dimethylsuccinate) as feed additive for salmon and trout. EFSA Journal 2007;5(11):574, 25 pp. https://doi.org/10.2903/j.efsa.2007.574

EFSA (European Food Safety Authority), 2007b. Scientific Opinion on the safety and efficacy of Panaferd-AX (red carotenoid-rich bacterium Paracoccus carotinifaciens) as feed additive for salmon and trout. EFSA Journal 2007;5(10):546, 30 pp. https://doi.org/10.2903/j.efsa.2007.546

EFSA (European Food Safety Authority), 2021. Administrative guidance for the preparation of applications on novel foods pursuant to Article 10 of Regulation (EU) 2015/2283. EFSA supporting publication 2021;EN-6488, 33 pp. https://doi.org/10.2903/sp.efsa.2021.EN-6488

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010. Scientific Opinion on modification of the terms of authorisation of a red carotenoid-rich bacterium Paracoccus carotinifaciens (Panaferd-AX) as feed additive for salmon and trout. EFSA Journal 2010;8(1):1428, 55 pp. https://doi.org/10.2903/j.efsa.2010.1428

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014a. Scientific Opinion on the safety and efficacy of astaxanthin (CAROPHYLL® Pink 10% CWS) for salmonids and ornamental fish. EFSA Journal 2014;12(6):3725, 33 pp. https://doi.org/10.2903/j.efsa.2014.3725

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014b. ScientificOpinion on the safety and efficacy of synthetic astaxanthin as feed additive for salmon and trout, other fish, ornamental fish, crustaceans and ornamental birds. EFSA Journal 2014;12(6):3724, 35 pp. https://doi.org/10.2903/j.efsa.2014.3724

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2019. Scientific Opinion on the safety and efficacy of astaxanthin-dimethyldisuccinate (Carophyll Stay-Pink 10%CWS) for salmonids, crustaceans and other fish. EFSAJournal 2019;17(12):5920, 42 pp. https://doi.org/10.2903/j.efsa.2019.5920

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances usedin Animal Feed), 2022. Scientific opinion on thesafety and efficacy of a feed additive consisting of astaxanthin-richPhaffia rhodozymafor salmon andtrout (Igene Biotechnology, Inc.). EFSA Journal 2022;20(2):7161, 14 pp. https://doi.org/10.2903/j.efsa.2022.7161

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2014. Scientific opinion on the safety of ATX-rich ingredients (AstaREAL A1010 and AstaREAL L10) as novel food ingredients. European Food Safety Authority (EFSA) panel on dietetic products, nutrition and allergies (NDA). EFSA Journal 2014;12(7):3757, 35 pp. https://doi.org/10.2903/j.efsa.2014.3757

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2020. Scientific Opinion on the safety ofastaxanthin for its use as a novel food in food supplements. EFSA Journal 2020;18(2):5993, 9 pp. https://doi.org/10.2903/j.efsa.2020.5993

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2021. Guidance on the preparation and submission of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283 (Revision 1). EFSA Journal 2021;19(3):6555, 27 pp. https://doi.org/10.2903/j.efsa.2021.6555

European Commission, 2017. Available online: https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/1134-Implementing-act-on-the-Union-list_en

European Commission, 2018. Notifications pursuant to article 5 of Regulation (EC) No 258/97 of the European Parliament and of the council. Available online: https://food.ec.europa.eu/system/files/2018-06/novel-food_notifications_en.pdf

Gong F, Zhang C, Zhang L and Liu J, 2020. Changes of carotenoids contents and analysis of astaxanthin geometrical isomerization in haematococcus pluvialis under outdoor high light conditions. Aquaculture Research, 51, 770–778. https://doi.org/10.1111/are.14427

Grewe C and Griehl C, 2008. Time- and media-dependent secondary carotenoid accumulation in Haematococcus Pluvialis. Biotechnology Journal, 3, 1232–1244. https://doi.org/10.1002/biot.200800067

Grung M, D'Souza FML, Borowitzka M and Liaaen-Jensen S, 1992. Algal Carotenoids 51. Secondary carotenoids 2.Haematococcus pluvialis aplanospores as a source of (3S, 3′ S)- astaxanthin esters. Journal of Applied Phycology, 4, 165–171. https://doi.org/10.1007/BF02442465

Holtin K, Kuehnle M, Rehbein J, Schuler P, Nicholson G and Albert K, 2009. Determination of Astaxanthin and Astaxanthin Esters in the Microalgae Haematococcus Pluvialis by LC-(APCI)MS and Characterization of Predominant Carotenoid Isomers by NMR Oy Medfiles Ltd AstaReal – Draft – 12.4.2022 4 (6) Spectroscopy. Analytical and Bioanalytical Chemistry, 395, 1613–1622. https://doi.org/10.1007/s00216-009-2837-2

Honda M, Murakami K, Osawa Y, Kawashima Y, Hirasawa K and Kuroda I, 2021. Z-isomers of astaxanthin exhibit greater bioavailability and tissue accumulation efficiency than the all-E-isomer. Journal of Agricultural and Food Chemistry, 69, 3489–3495. https://doi.org/10.1021/acs.jafc.1c00087

Osterlie M, Bjerkeng B and Liaaen-Jensen S, 2000. Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin. Journal of Nutritional Biochemistry, 11, 482–490.

Schiedt K, Leuenberger FJ and Vecchi M, 1981. Natural Occurrence of Enantiomeric and *meso*-Astaxanthin. 5. Ex wild salmon (*Salmo salar* and *Oncorhynchus*). HCA, 64, 449–457. https://doi.org/10.1002/hlca.19810640209

Schiedt K, Mayer H, Vecchi M, Glinz E and Storebakken T, 1988. Metabolism of carotenoids in salmonids. Part 2. Distribution and absolute configuration of idoxanthin in various organs and tissues of one atlantic salmon (*Salmo salar*, L.) fed with astaxanthin. HCA, 71, 881–886. https://doi.org/10.1002/hlca.19880710425

Stewart J, Glaister J, Henderson P, Riches S and Everett D, 2001, unpublished. HPP: 13 Week oral (dietary administration) toxicity study in the rat. Covance Report 1840/002-D6154. Covance Laboratories Ltd, Harrogate, North Yorkshire, England, 281 pp.

Storebakken T, Foss P, Austreng E and Liaaen-Jensen S, 1985. Carotenoids in diets for salmonids: II. Epimerization studies with astaxanthin in Atlantic salmon. Aquaculture, 44, 259–269. https://doi.org/10.1016/0044-8486(85)90225-X

Takahashi J, Tsukahara H and Minato S, 2004. Toxicological studies of astaxanthin from Haematococcus pluvialis - Ames test, oral singl dose and 90-days subchronic toxicity studies in rats. Journal of Clinical Therapeutics and Medicines, 20, 867–888.

Viazau YV, Goncharik RG, Kulikova IS, Kulikov EA, Vasilov RG and Selishcheva AA, 2021. E/Z Isomerization of Astaxanthin and Its Monoesters in Vitro under the Exposure to Light or Heat and in Overilluminated Haematococcus Pluvialis Cells. Bioresources and Bioprocessing, 8, 55. https://doi.org/10.1186/s40643-021-00410-5

Whyte JNC and Sherry KL, 2001. Pigmentation and Composition of Flesh of Atlantic Salmon Fed Diets Supplemented with the Yeast Phaffia rhodozyma. North American Journal of Aquaculture, 63, 52–57. https://doi.org/10.1577/1548-8454(2001)063<0052:PACOFO>2.0.CO;2

Yang C, Zhang H, Liu R, Zhu H, Zhang L and Tsao R, 2017. Bioaccessibility, cellular uptake, and transport of astaxanthin isomers and their antioxidative effects in human intestinal epithelial caco-2 cells. Journal of Agricultural and Food Chemistry, 65, 10223–10232. https://doi.org/10.1021/acs.jafc.7b04254

Yuan JP and Chen F, 1999. Isomerization of trans-astaxanthin to cis-isomers in organic solvents. Journal of Agricultural Food Chemistry, 47, 3656–3660. https://doi.org/10.1021/jf981319u

Yu W and Liu J, 2020. Astaxanthin isomers: selective distribution and isomerization in aquatic animals. Aquaculture, 520, 734915.

Zhao L, Chen F, Zhao G, Wang Z, Liao X and Hu X, 2005. Isomerization of trans-astaxanthin induced by copper(II) ion in ethanol. Journal of Agricultural Food Chemistry, 53, 9620–9623. https://doi.org/10.1021/jf0517750

Zhou Q, Xu J, Yang L, Gu C and Xue C, 2019. Thermal stability and oral absorbability of astaxanthin esters from Haematococcus pluvialis in Balb/c mice. Journal of Science Food Agriculture, 99, 3662–3671. https://doi.org/10.1002/jsfa.9588

Abbreviations

ATX	Astaxanthin
bw	body weight
CAS	chemical abstracts service
CFU	colony forming units
CoA	certificate of analysis
DMDS	dimethyldisuccinate
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FS	food supplements
HPLC	high performance liquid chromatography
MCT	medium chain triglycerides
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	Novel food
NMKL	Nordic-Baltic Committee on Food Analysis
USP	United States Pharmacopeia
w/w	Weight per weight