European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety of astaxanthin-rich ingredients (AstaREAL A1010 and AstaREAL L10) as novel food ingredients[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of astaxanthin-rich ingredients AstaREAL A1010 and AstaREAL L10 as novel food ingredients (NFIs) in the context of Regulation (EC) No 258/97. The NFIs are produced from astaxanthin-rich alga *Haematococcus pluvialis.* Astaxanthin content is 5.0–5.6 % in AstaREAL A1010 powder, 10.0–12.0 % in AstaREAL L10 oil and 2.5–2.7 % in AstaREAL L10 encapsulated oil. Sufficient information was provided regarding the composition, specification, manufacture and stability of the NFIs. The NFIs are intended to be used in fermented liquid dairy products, non-fermented liquid dairy products, fermented soya products and fruit drinks for healthy adults. The applicant recommends a maximum consumption of astaxanthin from the NFIs of 4 mg/day. Mean and high-level (95th percentile) daily intakes of 0.106 mg/kg bw and 0.256 mg/kg bw astaxanthin from the NFIs were estimated, based on European consumption data of the proposed food categories. The consumption of the NFIs is not considered to be nutritionally disadvantageous. There are no safety concerns regarding genotoxicity. There is no indication from the available toxicological data that the NFIs would be more toxic than astaxanthin. Therefore, the Panel bases the evaluation of the NFIs on the acceptable daily intake (ADI) of 0.034 mg/kg bw for astaxanthin derived by the FEEDAP Panel. The Panel notes that the maximum recommended intake of 4 mg astaxanthin per day (0.06 mg/kg bw) and the estimated mean intake based on the use levels in the proposed food categories (0.106 mg/kg bw per day) exceed the ADI by approximately two- and three-fold, respectively. The Panel therefore concludes that the safety of the NFIs at the proposed use and use levels has not been established.

KEY WORDS

astaxanthin, *Haematococcus pluvialis*, novel food, ingredient

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[1] On request the European Commission, Question No EFSA-Q-2011-00990, adopted on 25 June 2014.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of astaxanthin-rich ingredients AstaREAL A1010 and AstaREAL L10 as novel food ingredients in the context of Regulation (EC) No 258/97, taking into account the comments and objections of a scientific nature raised by Member States.

The novel food ingredients (NFIs) are produced from *Haematococcus pluvialis*, a microalga naturally rich in the carotenoid pigment astaxanthin. AstaREAL A1010 is a powder containing 5.0–5.6 % astaxanthin. AstaREAL L10 is available in the form of a red viscous oil, containing 10.0–12.0 % astaxanthin, or as a powder of modified starch encapsulating the red viscous oil, containing 2.5–2.7 % astaxanthin. The applicant provided sufficient information regarding the composition, specification, manufacture and stability of the NFIs.

The NFIs are intended to be used in fermented liquid dairy products, non-fermented liquid dairy products, fermented soya products and fruit drinks for healthy adults at a maximum incorporation level of 1.6 mg astaxanthin per 100 g or 100 mL. The applicant recommends a maximum consumption of astaxanthin from the NFIs of 4 mg/day. Based on data from the EFSA comprehensive European food consumption database, mean and high intakes of astaxanthin were estimated from the mean and 95th percentile consumption data of fermented liquid dairy products, non-fermented liquid dairy products and fruit drinks in European Union Member States. The highest daily intake estimates were of 0.106 mg/kg body weight (bw) for the mean consumption and 0.256 mg/kg bw astaxanthin for the high-level consumption. In the European diet, astaxanthin is primarily consumed through seafood, with wild and farmed salmonids as a major source. Considering the additional intake from salmon and trout consumption, total mean and high daily intakes of astaxanthin of 0.125 mg/kg bw and 0.286 mg/kg bw were calculated.

The Panel considers that the composition of the ingredients and results from available studies do not indicate that the consumption of the NFIs is nutritionally disadvantageous at the proposed daily intake.

Astaxanthin is absorbed in the human gastro-intestinal tract. Its bioavailability and distribution seem to depend on a variety of factors, including its form, its mode of consumption and the smoking habits of the consumer. Astaxanthin is also absorbed in rodents. The metabolic fate of astaxanthin involves the cleavage of the polyene chain at the C9, C9′ positions and stepwise reduction in both rats and humans. On the basis of similarities in absorption and metabolic fate, the Panel considers that rats are an acceptable species for toxicity testing of astaxanthin.

Based on the results of *in vitro* and *in vivo* genotoxicity studies on the biomass of *H. pluvialis* and other astaxanthin products, the Panel concludes that it has no safety concerns regarding genotoxicity of the NFIs.

In the human studies provided, which addressed safety endpoints, no clinically relevant changes or adverse effects were observed after consumption of the NFIs or other astaxanthin-rich ingredients from *H. pluvialis* at doses ranging from 2 to 40 mg/day astaxanthin for 10 days to 3 months. However, as these studies were of only short duration, the Panel considers that no conclusion can be drawn as regards long-term effects.

Supplemental intakes of β-carotene have been shown to increase the risk of lung cancer in smokers, and concerns were expressed that the NFIs may induce similar effects. There are neither human nor animal studies that have investigated astaxanthin and smoking with regard to the risk of lung cancer. There are differences in structure, metabolism and function between astaxanthin and β-carotene. In contrast to β-carotene, astaxanthin is more polar, is not a precursor of vitamin A and is considered as an antioxidant with no indication of pro-oxidative properties. The Panel concludes that the available

data do not indicate that the NFIs at the proposed level of use would increase the risk of lung cancer in smokers.

The Panel considers that the likelihood of adverse allergic reactions to the NFIs is low.

Biomass of *H. pluvialis* (algal meal containing 3 % astaxanthin) and astaxanthin-rich oil obtained by solvent extraction from *H. pluvialis* biomass (containing ca. 5 % astaxanthin) were tested for subchronic toxicity in rats. There is no indication from these studies that the NFIs would be more toxic than astaxanthin. Therefore, the Panel bases the evaluation of the NFIs on astaxanthin and considers the acceptable daily intake (ADI) of 0.034 mg/kg bw for astaxanthin derived by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP).

The Panel notes that the maximum intake of 4 mg astaxanthin per day (0.06 mg/kg bw per day for a 70 kg person) from the NFIs as proposed by the applicant and the estimated mean intake based on the use levels in the proposed food categories (0.106 mg/kg bw per day) exceed the ADI for astaxanthin of 0.034 mg/kg bw per day by approximately two- and three-fold, respectively. The Panel therefore concludes that the safety of the NFIs AstaREAL A1010 and AstaREAL L10 at the proposed use and use levels has not been established.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 5 June 2008, the company BioReal (Sweden) AB submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97[4] to place on the market 'Astaxanthin' as novel food ingredient.

On 29 December 2008, the competent authorities of Finland forwarded to the Commission their initial assessment report, which came to conclusion that the 'Astaxanthin' ingredients meet the criteria for acceptance as novel foods, with the restriction that they should be directed to healthy adults.

On 12 January 2009, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- The sums of the percentages of the constituents of AstaREAL A1010 and AstaREAL L10 do not amount to 100. The precise composition of the two products should be provided.
- Evidence of accreditation of the laboratories which carried out the analyses commissioned by the applicant should be provided.
- Levels of contaminants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), furans and dioxins, which are soluble in lipids, should be provided.
- Since the manufacturing processes involve high temperatures, composition in trans-fatty acids and newly-formed lipid products should be provided.
- Insufficient data are provided on the equivalence between astaxanthin from *Haematococcus pluvialis* and salmon astaxanthin, with respect to the proportions of geometric isomers, bioavailability and metabolism. The potential effect of the process on isomer conversion is not addressed.
- Presence of residues of the nutrients used in the cultivation of the alga should be provided. The quantities of additives used and their identification and quantification in the final products should be provided.
- Exposure to compounds derived from the alga *Haematococcus pluvialis* other than astaxanthin should be considered.
- Particles size of encapsulated AstaREAL L10 should be provided.
- High intake scenarios (95th percentile) indicate potential intakes significantly higher than 6 mg per day.
- The target population should be restricted to healthy adults and the product should not be consumed by children or pregnant women and during breastfeeding (no teratological studies provided) and for persons being treated with pharmaceuticals of various kinds or suffering from liver or metabolic conditions.
- *In vitro* studies indicate that astaxanthin may interact with enzymes CYP3A4 and CYP2B6 which are involved in the metabolism of medicines; the potential effect of astaxanthin on xenobiotic metabolism should be addressed.

[4] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

- The antioxidant vs. prooxidant effect of astaxanthin should be addressed.
- Potential effect of astaxanthin on the absorption and metabolism of liposoluble vitamins should be addressed.
- Full reports of *in vivo* toxicological studies should be provided in order to allow a full assessment.
- No histopathological examinations were carried out in the subacute toxicity study.
- The outcome and relevance of the rat studies should be further evaluated (elevated prothrombin and activated partial thrombin time in male rats at the highest dose of AstaREAL L10). Potential differences in astaxanthin metabolism between human and rat and the suitability of the rat model should be considered.
- Several studies show biological effects, sometimes dose-dependent (e.g. increase in blood cholesterol levels at the highest doses, reduced platelet count at the highest doses, increased prothrombin time, increased kidney weight, increased serum alkaline phosphatase).
- A no-observed-adverse-effect level (NOAEL) of 14 mg/kg per day was set for a purified synthetic astaxanthin on the basis of a carcinogenicity study in mice (EFSA, 2007a). The maximum acceptable daily intake of astaxanthin would be 9.8 mg/day for a 70 kg adult.
- Long-term exposure and potential accumulation of astaxanthin in tissues (e.g. eyes) need to be further examined.
- The same data requirements as for the risk assessment of food additives should be applied, i.e. including data from long-term and developmental studies.
- Clinical studies cannot be used in the safety evaluation due to their small size and narrow scope.
- The astaxanthin potential to increase risk of lung cancer (in heavy smokers), similar to β-carotene, should be examined.
- The information provided is not sufficient to establish an acceptable daily intake (ADI) for astaxanthin or ruled out an increased risk of lung cancer, thus the highest acceptable level of additional intake of astaxanthin should not exceed the current intake from foodstuffs.
- The allergic potential of AstaREAL A1010 should be further examined. In particular, whether reactions observed are due to soya lecithin rather than to algal proteins should be further investigated.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002,[5] the European Food Safety Authority is asked to carry out the additional assessment for astaxanthin-rich ingredients (AstaREAL A1010 and AstaREAL L10) as novel food ingredients in the context of Regulation (EC) No 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States.

[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. 1–24.

ASSESSMENT

In accordance with Commission Recommendation 97/618/EC,[6] the astaxanthin-rich ingredients AstaREAL A1010 and AstaREAL L10 are allocated to Class 2.1, i.e. "a complex (non-GM derived) novel food ingredient. The source of the novel food has a history of food use in the Community". The assessment of the safety of these novel food ingredients (NFIs) is based on data supplied in the original application, the initial assessment by the competent authority of Finland, the concerns and objections of the other Member States and the responses of the applicant. The data are required to comply with the information required for the novel foods of Class 2.1, i.e. structured schemes I, II, III, IX, X, XI, XII and XIII of Commission Recommendation 97/618/EC. In the text, these structured schemes are addressed under headings 1 to 8. The applicant indicates that the novel ingredients are intended to be marketed as food ingredients for their antioxidant properties. This assessment concerns only risk that might be associated with consumption and is not an assessment of the efficacy of the astaxanthin-rich ingredients AstaREAL A1010 and AstaREAL L10 with regard to any claimed benefit.

1. Specification of the NFIs

AstaREAL A1010 and AstaREAL L10 (oil and encapsulated oil, respectively) are astaxanthin-rich ingredients manufactured by the company AstaReal AB (formerly BioReal (Sweden) AB). The NFIs are produced from *Haematococcus pluvialis*,[7] a microalga naturally rich in the carotenoid pigment astaxanthin. 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.

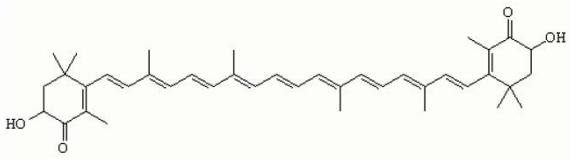

Figure 1: Chemical structure of astaxanthin

AstaREAL A1010 is a powder containing 5.0–5.6 % w/w astaxanthin. AstaREAL L10 is available in the form of a red viscous oil, containing 10.0–12.0 % w/w astaxanthin, or as a powder of modified starch encapsulating the red viscous oil, containing 2.5–2.7 % w/w astaxanthin.

According to the applicant, astaxanthin is mainly present in an esterified form in all three products (80 % monoester, 18 % diester and 2 % free astaxanthin). The fatty acid profiles of three different batches of AstaREAL A1010 and L10 have been analysed. Of the 27 fatty acids identified, 13 were quantified. The main fatty acids were palmitic acid (C16:0), oleic acid (cis-C18:1), linoleic acid (C18:2) and alpha-linolenic acid (C18:3). Upon the request from a Member State, the applicant determined the trans-fatty acid contents in one batch of AstaREAL L10 oil and AstaREAL A1010 to be 0.1 % and 0.2 % (area of total fatty acids; analysed by gas chromatography), respectively.

The main astaxanthin optical stereoisomer present in the NFIs is the 3S-3′S form, while its main geometric isomer is all-E-astaxanthin (all-trans-astaxanthin), representing ca. 80 % of total astaxanthin in AstaREAL A1010 and 77–79 % in AstaREAL L10. Isomerisation of all-E-astaxanthin to its Z- (cis) configuration is induced by heat, light and metal ions. The applicant notes that the proportion of Z-astaxanthin isomers increases during the process, from 13 % in the spray-dried biomass to ca. 14 % in AstaREAL A1010, ca. 20 % in AstaREAL L10 and ca. 21.5 % in AstaREAL L10 encapsulated oil (analysed by high-performance liquid chromatography (HPLC)).

[6] Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

[7] Taxonomic classification: phylum, Chlorophyta; class, Chlorophyceae; order, Volvocales; family, Haematococcaceae.

The typical contents of total carotenoids are 5.2–5.8 % w/w, 10.5–12.5 % w/w and 2.7–2.9 % w/w for AstaREAL A1010, AstaREAL L10 oil and AstaREAL L10 encapsulated oil, respectively. Astaxanthin represents ca. 95 % of the carotenoid content, whereas the remaining 5 % consists of small quantities of canthaxanthin, β-carotene, lutein and zeaxanthin, and other carotenoids. The applicant provided analytical reports of three batches of AstaREAL A1010 and L10 oil (Table 1).

Specifications for the three ingredients are found in Table 2.

Table 1: Carotenoid content of the novel food ingredients; analytical results from three batches

	AstaREAL A1010 (% w/w) Mean (min–max)	**AstaREAL L10 oil (% w/w)** Mean (min–max)
Total astaxanthin [(a)]	5.16 (5.04–5.37)	10.61 (10.32–11.07)
All-E-astaxanthin	4.16 (4.03–4.30)	8.30 (7.92–8.70)
9-Z-astaxanthin	0.63 (0.55–0.69)	1.86 (1.61–2.10)
13-Z-astaxanthin	0.37 (0.35–0.38)	0.46 (0.43–0.50)
Total carotenoids [(b)]	**5.36 (5.24–5.56)**	**11.22 (10.92–11.69)**
β-Carotene	0.02 (0.01–0.02)	0.03 (0.02–0.03)
Lutein	0.02 (0.02–0.02)	0.04 (0.03–0.05)
Canthaxanthin	0.02 (0.02–0.03)	0.06 (0.05–0.06)
Others	0.10 (0.10–0.10)	0.30 (0.30–0.30)

(a): High-performance liquid chromatography analysis of hydrolysed samples.
(b): Spectrophotometric analysis of hydrolysed samples.

Table 2: Specifications for AstaREAL A1010 (powder) and AstaREAL L10 (oil and encapsulated oil) as proposed by the applicant

	AstaREAL A1010	**AstaREAL L10 (oil)**	**AstaREAL L10 (encapsulated oil)**	**Method of analysis**
Physical specifications				
Appearance	Dark red powder	Dark red oil	Free-flowing powder	
Odour	Algal	Algal	Algal	
Particle size	> 97 % passes 60 mesh	–	> 96 % between 500 and 800 µm	
Chemical specifications				
Astaxanthin	5.0–5.6 %	10.0–12.0 %	2.5–2.7 %	HPLC
Moisture	< 4 %	< 0.5 %	< 3 %	NMKL 23
Heavy metals				
Pb	–	< 0.1 ppm	< 0.1 ppm	ICP MS
Total Pb, As, Cd, Hg	–	< 1 ppm	–	ICP MS
Microbiological specifications				
Total plate count	< 10 000 cfu/g	< 1 000 cfu/g	< 1 000 cfu/g	NMKL 86
Mould/yeast	< 100 cfu/g	< 100 cfu/g	< 100 cfu/g	NMKL 98
Enterococcus	< 100 cfu/g	–	–	NMKL 68
Enterobacteriaceae	< 100 cfu/g	–	–	NMKL 144
Escherichia coli	Absent/g	Absent/g	< 10 cfu/g	NMKL 44
Staphylococcus aureus	Absent/g	Absent/g	< 10 cfu/g	NMKL 66

	AstaREAL A1010	AstaREAL L10 (oil)	AstaREAL L10 (encapsulated oil)	Method of analysis
Bacillus cereus	< 100 cfu/g	–	–	NMKL 67
Clostridium perfringens	< 10 cfu/g	–	–	NMKL 95
Salmonella spp.	Absent/25 g	Absent/25 g	Absent/25 g	NMKL 71

cfu, colony-forming unit; HPLC, high performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectrometry; NMKL, Nordisk Metoddikomité for Naeringsmidler (Nordic Committee on Food Analysis); ppm, parts per million.

The typical nutrient composition of the NFIs is provided in Table 3. The applicant provided analyses of three batches of AstaREAL A1010 and of AstaREAL L10 oil.

Table 3: Nutrient composition of AstaREAL A1010 (powder) and AstaREAL L10 (oil and encapsulated oil)

	AstaREAL A1010	AstaREAL L10 (oil)	AstaREAL L10 (encapsulated oil)	Method of analysis
Fat [(a)]	45–50 %	95–102 %	26–30 %	SBR modified
Protein [(a)]	9–12 %	< 0.3 %	< 0.5 %	Dumas
Carbohydrates [(a)]	20–30 %	< 1 %	70–74 %	By difference
Dietary fibre [(a)]	10 %	≤ 1 %	≤ 1 %	AOAC 985.29
Ash [(a)]	1.5–2.5 %	< 0.1 %	0.8 %	NMKL 173

(a): Typical values. The values may vary slightly between samples.
AOAC, Association of Official Analytical Chemists; NMKL, Nordisk Metoddikomité for Naeringsmidler (Nordic Committee on Food Analysis); SBR, Schmid-Bondzynski-Ratzlof.

In response to a comment from a Member State, the applicant indicated that the particle size of encapsulated AstaREAL L10 ranges from 100 to 800 µm, and that over 96 % is between 500 and 800 µm.

The iodine content was shown to be < 1.0 ppm in one batch of the three NFIs.

1.1. Contaminants

The applicant provided certificates of analyses of three batches of AstaREAL A1010 and one batch of AstaREAL L10 oil, in which the concentrations of lead, arsenic, cadmium and mercury are below the maximum levels allowed in food supplements.[8] Aflatoxins (B1, B2, G1, G2) and pesticides (organophosphate, carbamate, organochlorine) were not detected in three batches of AstaREAL A1010. The Panel notes that the NFIs have to comply with existing European Union (EU) legislations.

The applicant examined the presence of algal toxins. In three batches of AstaREAL A1010, saxitoxin was not detected (enzyme-linked immunosorbent assay (ELISA); detection limit, 33 pg/g), while microcystins were detected in one out of three batches at a level of 0.3 µg/g (test material extracted with methylene chloride and methanol; analyses by ELISA; detection limit, 0.05 µg/g). The same batches gave negative results in another test: microcystin-LR (detection limit: 0.025 µg/g), nodularin (detection limit: 0.025 µg/g), anatoxin-a (detection limit: 0.012 µg/g) and cylindrospermopsin (detection limit: 0.012 µg/g) were not detected in three batches of AstaREAL A1010 (test material

[8] Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

extracted with methanol; analyses by HPLC tandem mass spectrometry (MS/MS)). The applicant indicates that the HPLC-MS/MS method for the determination of algal toxins in water is reported in the literature (Hedman et al., 2008). The applicant notes that the level of microcystins, which were detected once in one sample, is below the regulatory limit of 1 µg/g for blue-green algae-containing food supplements set by the US Oregon Department of Health (Gilroy et al., 2000). This limit is based on a no-observed-adverse-effect-level (NOAEL) of 40 µg/kg body weight (bw) per day for microcystin-LR determined for liver damage in mice (Fawell et al., 1999). Based on the same data, the World Health Organization (WHO) guideline value for total microcystin-LR (free plus cell-bound) is 1 µg/L in drinking water (WHO, 1998). Upon request from EFSA, the applicant clarified that growth of microorganisms is a critical control point in the Hazard Analysis Critical Control Points (HACCP) plan, including microscope examination for unwanted microorganisms of each culture at each stage of the production process. The applicant also indicated that water used for cultivation is sterile-filtered to avoid contamination. The risk of airborne contamination is minimised through the location of the cultivation units in rooms with over-pressure relative to the surroundings. Taking these measures into account, the Panel considers that the presence of algal toxins in the final product is unlikely, but suggests periodic controls.

The presence of pheophorbide a, a product of chlorophyll degradation, was investigated. According to the applicant, the chlorophyll content of *H. pluvialis* is low and thus the amount of pheophorbides in the NFIs is likely to be very low. Analyses show that the total chlorophyll content of AstaREAL A1010 is < 0.25 % w/w (results from three batches). The pheophorbide a content of AstaREAL A1010 has not been analysed, whereas pheophorbide a was detected at a level of 17 mg/100 g in one out of three batches of AstaREAL L10 analysed.

In response to a request from a Member State, the concentrations of 24 polycyclic aromatic hydrocarbons (PAHs) were analysed in one batch of AstaREAL A1010 and of AstaREAL L10 (oil). In AstaREAL A1010, the highest concentrations were found for phenanthrene (7.4 ± 1.1 µg/kg), fluoranthene (3.4 ± 0.5 µg/kg) and pyrene (2.9 ± 0.4 µg/kg). In AstaREAL L10, the highest amounts were found for pyrene (54.2 ± 8.1 µg/kg), fluoranthene (11.4 ± 1.7 µg/kg), benzo(ghi)perylene (4.8 ± 0.4 µg/kg) and cyclopenta(cd)pyrene (4.8 ± 0.7 µg/kg). PAH concentrations found in the NFIs are comparable to those present in conventional foods (SCF, 2002). Benzo(a)pyrene[9] concentrations were < 0.2 µg/kg in AstaREAL A1010 and 0.9 µg/kg in AstaREAL L10, which is below the maximum level of 2.0 µg/kg w/w allowed in oils and fats for human consumption.[10]

In response to a request from a Member State, total dioxin (sum of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) and dioxin-like polychlorinated biphenyls (PCBs) concentrations were analysed in one batch of AstaREAL A1010 and AstaREAL L10 (oil), respectively. The total dioxin concentration found in AstaREAL A1010 was 0.101 ng/kg (0.24 pg/g fat) and the concentration of total dioxins and dioxin-like PCBs was 0.122 ng/kg (0.29 pg/g fat) expressed as WHO toxic equivalents (TEQs). The total dioxins found in AstaREAL L10 were 0.257 ng/kg (0.35 pg/g fat) and total dioxins and dioxin-like PCBs were 0.275 ng/kg (0.38 pg/g fat) expressed as WHO TEQs, which is below the maximum level allowed in vegetable oils and fats[10] (0.75 pg/g fat and 1.50 pg/g fat, respectively). Concentrations of six WHO indicator congeners of PCBs (PCB 28, 52, 101, 118, 153, 138 and 180) were < 0.001 mg/kg in both NFIs (one batch).

The applicant indicates that quality control analyses are performed on each batch of NFIs, in order to check compliance with specifications.

The Panel considers that the information provided on the composition, specifications and data from batch testing do not raise safety concerns.

[9] Benzo(a)pyrene, for which maximum levels are regulated by Commission Regulation (EC) No 1881/2006, is used as a marker for the occurrence and effect of carcinogenic polycyclic aromatic hydrocarbons.

[10] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

1.2. Stability analysis

The applicant provides results from stability tests carried out with food products containing astaxanthin from AstaREAL A1010 or AstaREAL L10 encapsulated. The tests lasted six to nine weeks at the temperature of + 5 °C (e.g. cow milk yoghurt and drink, soya milk and yoghurt), and for ultra-high temperature (UHT)-processed and pasteurised products (e.g. UHT-processed milk drink, pasteurised fruit drink) also at + 23 °C. Astaxanthin content was measured by HPLC and a maximum decrease of 10 % was detected.

The Panel considers that these data provided sufficient information with respect to the stability of the NFIs.

2. Effect of the production process applied to the NFIs

The proprietary strain ACO32 of *H. pluvialis* is cultivated in a defined medium of sterile-filtered water and nutrients, harvested by centrifugation and homogenised. The cell slurry is then crushed and spray dried. Antioxidants (sunflower lecithin, ascorbyl palmitate and dl-α-tocopherol) and an anti-caking agent (colloidal silicon dioxide) are added during the process.

AstaREAL A1010 is a powder, obtained as described above, containing 5.0–5.6 % w/w astaxanthin, which is vacuum-/steam-treated (pasteurised) before the final packaging in vacuum-sealed aluminium bags.

AstaREAL L10 is a red viscous oil containing 10.0–12.0 % w/w astaxanthin. The oil is produced by supercritical CO_2 extraction of the homogenised and spray-dried biomass of the microalga *H. pluvialis*, cultivated and processed as for AstaREAL A1010. The extract is collected in a mixing tank and dispensed into 1.0 or 10.0 kg bottles. In this process, the fat-soluble components (i.e. fatty acids, triglycerides and carotenoids) of the dried algal meal are recovered, while carbohydrates, proteins, minerals, chlorophylls and water are removed.

Encapsulated AstaREAL L10 is a water-dispersible formulation, containing 2.5–2.7 % w/w astaxanthin, manufactured from AstaREAL L10. An aqueous emulsion consisting of AstaREAL L10 oil, ascorbyl palmitate, dl-α-tocopherol and 73.5 % modified maize starch is prepared by intensive stirring. The emulsion is then spray encapsulated on a fluidised bed granulation dryer equipped with a cyclone and a filter bag system. The free-flowing product is sieved to remove fine and big particles and packaged in laminated aluminium bags.

The detailed processes are described in the application dossier and are considered confidential by the applicant.

The production plant has been approved by the Swedish authorities and it fulfils the requirements set out in the EU and Swedish legislation. The production includes an HACCP system. The company complies with ISO 9001:2008 quality control standards. Food-grade raw materials are used for the production. Quality control analyses are performed on each batch according to specifications.

The production processes utilise well-known technologies, widely used in food industries. The Panel concludes that the production processes are sufficiently described and do not give rise to safety concerns.

3. History of the organism used as a source

A non-genetically modified (GM) strain of *H. pluvialis* (ACO32) is used in the production of the astaxanthin-rich ingredients. *H. pluvialis* (division Chlorophyta, family Haematococcaceae) is a unicellular green microalga which grows in fresh and brackish waters. Under environmental stress conditions, such as high light intensity or nutrient shortage, the cells accumulate fats and astaxanthin and turn into dark-red cysts or aplanospores (Steinbrenner and Linden, 2001).

H. pluvialis occurs naturally in the food chain. Microalgae are consumed by zooplankton and crustaceans, which in turn are consumed by salmon, trout and other aquatic animals. The characteristic pink colour of salmonid fish typically comes from astaxanthin, which is primarily obtained from crustaceans, which form a significant part of their diet.

4. Anticipated intake/extent of the use of the NFIs

The NFIs are intended to be used in fermented liquid dairy products, non-fermented liquid dairy products, fermented soya products and fruit drinks. According to the applicant, the foods are manufactured by conventional methods. The astaxanthin ingredient is added to the food either during or after the fermentation process. In non-fermented products, the ingredient is added before the food (drink) is pasteurised.

In response to several comments from Member States, the applicant decreased the recommended maximum daily consumption from 6 to 4 mg astaxanthin from the NFIs, in one or two portions of foods containing the NFIs. The intended use levels in the selected food categories would range from 0.8 to 1.6 mg astaxanthin per 100 g or 100 mL.

The applicant proposes to label food products with the recommended maximum daily consumption of 4 mg. The applicant indicates that foods containing NFIs are intended for healthy adults and are not recommended for children, pregnant or breast-feeding women or people with chronic diseases, and would be labelled as such.

Intake scenarios were calculated by the applicant by using mean and high intake values of fermented and non-fermented liquid dairy products and fruit drinks, or equivalent products, from the national dietary surveys of Sweden (Riksmaten 1997–1998; (Becker and Pearson, 2002)), Finland (National Findiet 2002; (Männistö et al., 2003)) and United Kingdom (National Diet and Nutrition Survey 2004; (Hoare et al., 2004)). Summary statistics from these datasets were used as the basis for the calculations. Although no data were available for fermented soya products, the applicant considered that they are likely to be consumed as alternatives to milk-based yoghurts.

If all conventional yoghurts, sour whole milks, milks, juices and nectars were enriched with the NFIs, the mean daily intake of astaxanthin would amount to 3.5–6.9 mg in Swedish adults (Table 4) and 1.8–14.4 mg in Finnish and British adults (Table 5). The worst-case scenario, considering high-level consumers of yoghurt, sour whole milks, milks, juices and nectars, results in a total daily intake of 8.8–17.7 mg astaxanthin in Swedish adults and 18.6–37.2 mg in Finnish adults.

The Panel notes that this type of intake assessment methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates, assuming that all food items within a food category contain the ingredient at the maximum specified level of use and that an individual might be a high-level consumer of all food categories.

Table 4: Estimates of daily intakes of astaxanthin from the NFIs in adults from Swedish data

	Mean food intake [(a)] (g/day)	Mean astaxanthin intake [(b)] (mg/day)	High food intake [(c)] (g/day)	High astaxanthin intake [(d)] (mg/day)
Milk, sour whole milk and yoghurt	344	2.8–5.5	765	6.1–12.2
Juice and nectar	87	0.7–1.4	343	2.7–5.5
Total		**3.5–6.9**		**8.8–17.7**

(a): Average intake among Swedish adults (mean of men and women average intakes).
(b): Assuming that all conventional foods are replaced by foods containing the NFIs (0.8–1.6 mg astaxanthin per 100 g or 100 mL), using average consumption intakes among Swedish adults.
(c): 95th percentile intake among Swedish adults is considered as high intake (mean of men and women 95th percentile intakes).
(d): Assuming that all conventional foods are replaced by foods containing the NFIs (0.8–1.6 mg astaxanthin per 100 g or 100 mL), using high consumption intakes among Swedish adults.

Table 5: Estimates of daily intakes of astaxanthin from the novel food ingredients in adults from Finnish and British data

	Mean food intake [(a)] (g/day)	Mean astaxanthin intake [(b)] (mg/day)	High food intake [(c)] (g/day)	High astaxanthin intake [(d)] (mg/day)
Yoghurt	57–165	0.5–2.6	365	2.9–5.8
Milk	66–401	0.5–6.4	1 049	8.4–16.8
Fruit juices	100–332	0.8–5.3	914	7.3–14.6
Total		**1.8–14.4**		**18.6–37.2**

(a): Range of average intakes among Finnish and British consumers only (the lowest and highest values among Finnish and British men and women are reported).
(b): Assuming that all conventional foods are replaced by foods containing the NFIs (0.8–1.6 mg astaxanthin per 100 g or 100 mL), using average consumption intakes among consumers only.
(c): By adding two standard deviations to the mean intake of conventional foods, using food intake data of Finnish men consumers only.
(d): Assuming that all conventional foods are replaced by foods containing the NFIs (0.8–1.6 mg astaxanthin per 100 g or 100 mL), using high consumption intakes among consumers only.

The Panel considered intake estimates derived from the EFSA comprehensive European food consumption database (EFSA, 2011), which are more accurate than those of the applicant, because they are based on individual data rather than summary statistics.

The mean and high intakes of astaxanthin were derived from the mean and 95th percentile consumption data of fermented liquid dairy products, non-fermented liquid dairy products and fruit drinks in EU Member States (Table 6). At the highest incorporation level of 1.6 mg astaxanthin per 100 g or 100 mL, the estimated mean astaxanthin intakes ranged from 0.025 mg/kg bw per day in Latvia to 0.106 mg/kg bw per day in Finland, while the estimated high astaxanthin intakes ranged from 0.082 mg/kg bw per day in Italy to 0.256 mg/kg bw per day in Finland for adults. For a 70 kg adult, this would correspond to estimated mean astaxanthin intakes from 1.8 mg/day to 7.4 mg/day and high astaxanthin intakes from 5.8 mg/day to 17.9 mg/day.

Table 6: Estimates of daily intakes of astaxanthin from the novel food ingredients, based on the EFSA comprehensive European food consumption database considering the highest incorporation level of 1.6 mg astaxanthin per 100 g or 100 mL

	Mean intake (mg/kg bw/day)		High intake (95th) (mg/kg bw/day)	
	Minimum [a] (country, sample size [b])	Maximum [a] (country, sample size [b])	Minimum [a] (country, sample size [b])	Maximum [a] (country, sample size [b])
Adults [c] (18–64 years)	0.025 (Latvia, n = 1 306)	0.106 (Finland, n = 1 575)	0.082 (Italy, n = 2 313)	0.256 (Finland, n = 1 575)
Elderly [d] (65–74 years)	0.023 (Belgium, n = 518)	0.101 (Finland, n = 463)	0.080 (Belgium, n = 518)	0.239 (Finland, n = 463)
Very elderly [e] (> 75 years)	0.025 (Belgium, n = 712)	0.091 (Denmark, n = 20)	0.090 (Belgium, n = 712)	0.167 (Germany, n = 490)

(a): Minimum and maximum intake estimates across countries.
(b): Total number of subjects in the age group for the dietary survey.
(c): For adults, the EFSA comprehensive food consumption database include data for 13 countries.
(d): For elderly, the EFSA comprehensive food consumption database include data for seven countries.
(e): For very elderly, the EFSA comprehensive food consumption database include data for six countries.

Astaxanthin is primarily consumed through seafood. In the European diet, major sources of astaxanthin are wild and farmed salmonids. There are large variations in astaxanthin content of salmonids (0–38 mg/kg flesh) (EFSA, 2005). In a recent assessment of astaxanthin as a feed ingredient, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) reported an estimation of the 95th percentile fish consumption of 125 g per day among consumers only, based on the EFSA comprehensive European food consumption database (EFSA FEEDAP Panel, 2014a, 2014b). If a proportion of salmon to trout in the daily food basket of 2:1 is applied, it results in 83 g salmon flesh and 42 g trout flesh. The corresponding exposure to astaxanthin would be 1.9 mg/day, considering mean astaxanthin concentration in salmon and trout flesh of 10 mg/kg and 25 mg/kg, respectively.

Astaxanthin anticipated intake estimates, including exposure through salmon and trout consumption, were also calculated based on individual consumption data from the EFSA comprehensive European food consumption database. The estimated mean astaxanthin intakes ranged from 0.035 mg/kg bw per day in Latvia to 0.125 mg/kg bw per day in Finland, while the estimated high astaxanthin intakes ranged from 0.101 mg/kg bw per day in Italy to 0.286 mg/kg bw per day in Finland for adults. For a 70 kg adult in Finland, this would correspond to a total mean intake of 8.8 mg astaxanthin and a high intake of 20.2 mg astaxanthin.

5. Information from previous exposure to the NFIs or their source

Astaxanthin is primarily consumed through seafood, with wild and farmed salmonids being the major sources of astaxanthin in the European diet (see section 4).

In response to a comment from a Member State, the applicant indicates that all-E-astaxanthin is the major geometric isomer in the NFIs as well as in wild and farmed salmon flesh, although distributed selectively in fish tissues (Nie et al., 2011). According to the applicant, there are no data on the distribution of E-/Z-astaxanthin isomers in human plasma after the ingestion of salmon.

As additional evidence of the history of safe intake of astaxanthin, the applicant reported results of observational studies on high dietary intake of fish in relation to diverse health outcomes in specific populations (Greenland Eskimos, Alaskan natives and Japanese fishermen). The Panel notes that the astaxanthin intakes related to fish consumption were not assessed in these studies.

The dried form or extract of *H. pluvialis* has a history of use as a dietary supplement since at least 1995 in Europe and at least 1999 in the USA. Several brands are available on the EU market, with a typical recommended daily intake of astaxanthin of 4 mg (maximum of 16 mg/day in one brand). Since 1995, BioReal (Sweden) AB has marketed the astaxanthin food supplement Astaxin®, which is similar to AstaREAL A1010. The applicant provided sales data of Astaxin®. In 2006, a substantial equivalence[11] approval was obtained by BioReal (Sweden) AB from the Swedish National Food Administration, under Article 5 of Novel Food Regulation (EC) 258/97, for the use of AstaREAL L10 in food supplements. Similarly, the UK Advisory Committee on Novel Foods and Processes (ACNFP) published positive opinions for the use of three astaxanthin-rich ingredients derived from *H. pluvialis* in food supplements, based on substantial equivalence to Astaxin® (UK ACNFP, 2004, 2007, 2008).

Several drinks containing astaxanthin at doses ranging from 0.5 to 15 mg/portion are marketed in Japan.

In the EU, astaxanthin is registered as a feed additive for salmon and trout consumption,[12] as synthetic astaxanthin (EFSA, 2005), astaxanthin-rich *Pfaffia rhodozyma* extract (EFSA, 2006), astaxanthin-rich *Paracoccus carotinifaciens* extract (EFSA, 2007b) and astaxanthin dimethyldisuccinate (EFSA, 2007a). The applicant also reports that astaxanthin from *H. pluvialis* has been approved as a feed colour additive for salmon feeds in the USA, Japan and Canada and is classified as feed raw material in Sweden. *H. pluvialis* is also used in poultry feed in Sweden.

6. Nutritional information on the NFIs

Astaxanthin is a xanthophyll carotenoid without provitamin A activity in humans (Olson, 1989) (see section 8.3.1).

A Member State raised concerns about the effect of prolonged oral administration of astaxanthin on the absorption and metabolism of fat-soluble vitamins.

There is evidence for a competition between simultaneously ingested carotenoids for intestinal absorption (Zaripheh and Erdman, 2002). Interactions between carotenoids during absorption and during post-absorptive metabolism have been observed in animal and human studies and were investigated in *in vitro* studies. Mutual and variable interactions between the hydrocarbon carotenoid β-carotene and xanthophylls such as lutein and canthaxanthin have been shown to occur (van den Berg, 1999), but less so for astaxanthin (Ershov Iu et al., 1993). Studies in humans showed a reduction of canthaxanthin absorption when consumed concurrently with β-carotene in doses of 25 mg each (White et al., 1994; Paetau et al., 1997). On the other hand, de Pee et al. (1995) reported low β-carotene absorption in women from green vegetables, which are generally rich in lutein and have a relatively high lutein:β-carotene ratio. In 10 healthy volunteers, the long-term ingestion of 10 mg per day of the xanthophyll zeaxanthin had no effect on plasma carotenoids, retinol or α-tocopherol concentrations (Hartmann et al., 2004).

The applicant supplied the abstract of a randomised double-blind study in which healthy men received 8 mg/day astaxanthin (AstaREAL A1010 capsules) or a placebo for eight weeks prior to a 90 km ski race (BioReal (Sweden) AB, 2011, unpublished). Analysis of carotenoids in plasma samples taken after the race showed that astaxanthin concentration was significantly higher in the supplemented group than in the control group (63 ± 20 µg/L vs. 7.4 ± 6.4 µg/L, $p < 0.001$) and no statistically significant differences in plasma α-/β-carotene, lutein/zeaxanthin, β-cryptoxanthin, lycopene and total carotenoid were observed between the control group ($n = 34$) and the astaxanthin group ($n = 33$). The Panel notes that limited information was available in this abstract, which limits the conclusions that can be drawn from this study.

[11] That is the novel food is considered substantially equivalent to an existing food in terms of nutritional value, metabolism, intended use and level of undesirable substances.

[12] 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. 29–43.

In a randomised double-blind clinical trial, healthy men received 8 mg/day astaxanthin (Astaxin® (AstaREAL A1010) capsules) or a placebo for three months (Karppi et al., 2007). At the end of the intervention, astaxanthin plasma concentration was significantly higher in the supplemented group than in the control group (0.032 ± 0.012 µmol/L vs. not detected, $p < 0.001$) and no significant differences in the plasma ascorbic acid, alpha-tocopherol, retinol, β-carotene and other carotenoid (lycopene, zeaxanthin + lutein, canthaxanthin, β-cryptoxanthin) concentrations between the placebo group (n = 19) and the astaxanthin group (n = 20) were observed.

The Panel notes that studies on the impact of astaxanthin on the absorption and metabolism of fat-soluble vitamins are very limited. Based on available studies with astaxanthin and other xanthophylls, at the proposed intake level of astaxanthin within normal diets, interactions are unlikely to have a relevant impact on the systemic bioavailability of β-carotene or individual fat-soluble vitamins.

The Panel considers that the composition of the ingredients and results from available studies do not indicate that, at the proposed daily intake, the consumption of the NFIs is nutritionally disadvantageous.

7. Microbiological information on the NFIs

Microbiological specifications of the NFIs are indicated in Table 2. Certificates of analyses were provided for three batches of AstaREAL A1010 and AstaREAL L10 oil each, and microbiological contents conformed with the specifications.

8. Toxicological information on the NFIs

8.1. *In vitro* and animal studies

8.1.1. Absorption, distribution, metabolism, excretion

Choi et al. (2011) reported on the pharmacokinetics and metabolism of astaxanthin in rats. Astaxanthin was unstable for up to four hours of incubation in the gastric juices of four rats and for up to 24 hours incubation in various buffer solutions having a pH of 1–13. Rats were given astaxanthin intravenously (5, 10 and 20 mg/kg bw) and orally (100 and 200 mg/kg bw; by gavage). Upon intravenous (i.v.) administration, the authors concluded that there was a saturation of metabolism in the rat (presumed to be in the liver); plasma half-life was in the range of eight to nine hours. Upon oral administration, astaxanthin reached peak plasma concentrations at around six hours after administration and no saturation of metabolism was apparent. At 8 and 24 hours, oral administration of astaxanthin resulted in tissue/plasma ratios > 1 in all tissues, suggesting high affinity of organs and tissues for astaxanthin. This is also supported by high volumes of distribution found after i.v. administration. At a dose level of 20 mg/kg bw, the hepatic and gastro-intestinal first-pass extraction ratios of astaxanthin were approximately 0.490 and 0.901, respectively. Regarding metabolism of astaxanthin, upon i.v. or oral administration, only a very low amount of unmetabolised astaxanthin was recovered from 24-hour urine samples, suggesting metabolic clearance of astaxanthin. Moreover, upon treating rats with an inducer of cytochrome P450 (CYP) 1A1/2 (3-methylcholanthrene), plasma area under the curve (AUC) was lower, also indicating metabolism of astaxanthin by this route. The Panel notes that this study indicates that astaxanthin is absorbed in the rat gastro-intestinal tract and metabolised upon absorption.

Clark et al. (1998) studied the absorption of lycopene and canthaxanthin in a rat model. The two carotenoids were administered directly into the duodenum at dose levels up to 20 µmol/L of infusion. The mesenteric lymph node was cannulated and both carotenoids were recovered from the lymph at a level between 6 and 16 % of the dose administered. In a sequel experiment in the same model, the absorption of astaxanthin (between 5 and 20 µmol/L of infusion) was found to be 13–20 % (Clark et al., 2000). The Panel notes that this study indicates that astaxanthin is absorbed in the rat gastro-intestinal tract.

Chew et al. (1999) measured the plasma concentrations of astaxanthin, β-carotene or canthaxanthin in female mice after dietary incorporation (0, 0.1 or 0.4 % in the diet) for 66 days, in a study to test their anticarcinogenic potential. Astaxanthin concentrations in plasma were of 20 µmol/L and 28 µmol/L with 0.1 or 0.4 % astaxanthin in the diet, respectively, and were significantly higher than plasma concentrations of β-carotene (0.1 µmol/L and 0.2 µmol/L) and canthaxanthin (3 µmol/L and 6 µmol/L). The Panel notes that this study indicates that astaxanthin is absorbed from the mouse gastro-intestinal tract.

Showalter et al. (2004) studied the absorption of an astaxanthin analogue, disodium disuccinate diester of astaxanthin, in mice. Upon oral dosing, they measured t_{max} of (non-esterified) astaxanthin (at approximately six hours) and plasma $t_{½}$ (at approximately four hours). The study also shows absorption of astaxanthin in the gut. The Panel notes that this study is initially on a compound other than the NFIs, and that only data after conversion into astaxanthin can be useful. The fact that astaxanthin is measured in plasma after oral administration of an astaxanthin *analogue* further supports that astaxanthin is absorbed in the gastro-intestinal tract in mice.

The organ distribution of astaxanthin was studied in female rats (Petri and Lundebye, 2007). Four groups of 10 Sprague–Dawley rats received a diet containing astaxanthin at levels of 0 (control), 0.3, 1 or 3 % of the feed for two weeks. Astaxanthin concentrations were measured at days 7 and 14 in the liver, spleen, kidneys, adrenals, heart, lungs and eyes and in tail skin and abdominal skin in five animals/group. Highest concentrations were found in the spleen, kidneys and adrenals. The authors reported that, from a visual examination, it appeared that the viscera of animals from the highest dose group were discoloured to varying extents and that the main site of astaxanthin accumulation was the hairless skin of the tail, which was associated with red coloration.

Data on astaxanthin metabolic fate in rats has also been reviewed by the FEEDAP Panel (EFSA, 2005; EFSA FEEDAP Panel, 2014a, 2014b). Biotransformation studies performed with [6,6′,7,7′-^{14}C]-astaxanthin in rat hepatocyte primary cultures showed the cleavage of the polyene chain at the C9, C9′ positions, leading to the formation of glucuronoconjugates of (rac)-3-hydroxy-4-oxo-β-ionone, and of its reduced form (rac)-3-hydroxy-4-oxo-7,8-dihydro-β-ionone (Wolz et al., 1999). A similar study performed with human hepatocytes (Kistler et al., 2002) concluded that the same metabolic pathway was followed (see section 8.2.1). Consistent with the results obtained *in vitro*, seven main metabolites were isolated in the urine of rats which had received radiolabelled astaxanthin, identified as products of the cleavage at C9, C9′ of the polyene chain, undergoing further stepwise reduction. In these studies, the radioactivity was mainly excreted in the faeces (53 % compared with 34 % in the urine after 48 hours), and, amongst the tissues, the highest concentration was found in the liver, followed by the kidney. The FEEDAP Panel concluded that a major metabolic pathway involving cleavage at the C9, C9′ positions and stepwise reduction appears to be common to salmonids, rats and humans.

Although one Member State noted that, based on studies with β-carotene, rodents are usually considered to be poor models of carotenoid absorption in humans, the Panel notes that the studies in rats and mice (Clark et al., 1998; Chew et al., 1999; Clark et al., 2000; Petri and Lundebye, 2007; Choi et al., 2011) suggest that astaxanthin is taken up in the gastro-intestinal tract of rodents upon oral exposure. Moreover, the absorption of astaxanthin appears higher than that of the other carotenoids studied. The low levels of the parent compound in the urine and the reported plasma half-lives suggest a metabolic clearance of astaxanthin in rodents (Choi et al., 2011).

Overall, the Panel considers that there are sufficient data to support the notion that astaxanthin is absorbed in rodents and humans and that the metabolic pathway is similar (see section 8.2.1). Hence, the Panel considers that rats are an acceptable species for toxicity testing of astaxanthin.

8.1.2. Genotoxicity studies

Tests on gene mutations in bacteria were conducted on biomass of *H. pluvialis* ("AstaCarox", 3 % astaxanthin) in accordance with Organisation for Economic Co-operation and Development (OECD)

Test Guideline 471 (OECD, 1997c) and under Good Laboratory Practice (GLP), using four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one strain of *Escherichia coli* (WP2 uvrApKM101) in the treat-and-plate variant. Biomass of *H. pluvialis*, dissolved in water, was not mutagenic with or without metabolic activation (S9), up to the highest tested concentration of 5 mg/plate (Edwards, 1998, unpublished-c).

Biomass of *H. pluvialis* ("AstaCarox", 3 % astaxanthin) was tested for genotoxicity using L5178Y TK+/– mouse lymphoma cells with and without metabolic activation (S9) (Edwards, 1998, unpublished-a). The tests were conducted in accordance with OECD Test Guideline 476 (OECD, 1997b) and under GLP. Biomass of *H. pluvialis* was prepared in cell culture medium and tested at levels up to 5 mg/mL of the test material, with and without S9. No increase of the mutation frequency was observed.

The genotoxicity of biomass of *H. pluvialis* ("AstaCarox", 3 % astaxanthin) was further investigated in an *in vivo* bone marrow micronucleus test using mice (Edwards, 1998, unpublished-b), in accordance with OECD Test Guideline 474 (OECD, 1997a) and under GLP. Five male and five female mice were administered 2 000 mg/kg bw of biomass of *H. pluvialis* in distilled water by gavage. Bone marrow samples were collected 24 and 48 hours after administration. No statistically significant reduction in the frequency of polychromatic erythrocytes was observed, indicating a lack of bone marrow toxicity by the test material. No increase in the number of micronucleated erythrocytes was found after treatment, indicating that the biomass of *H. pluvialis* was not genotoxic in this test.

In addition, tests on gene mutations in bacteria were conducted on an astaxanthin-rich oil from *H. pluvialis* ("AstaReal Oil 50F", ca. 5 % astaxanthin), extracted using acetone and standardised with medium-chain triglycerides, in accordance with OECD Test Guideline 471 (OECD, 1997c), but not under GLP, using four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one strain of *Escherichia coli* (WP2 uvrA), in the plate incorporation variant. The test substance, dissolved in acetone, was not mutagenic with or without metabolic activation (S9), up to the highest tested concentration of 5 mg/plate (Sarwar et al., 2002, unpublished; Takahashi et al., 2004). The Panel notes that the test material contains 5 % astaxanthin, which is half the content of AstaREAL L10 oil.

The genotoxicity tests indicate that the biomass of *H. pluvialis* is not genotoxic.

The Panel also notes that, where other astaxanthin products have been tested, there is no evidence for genotoxicity (EFSA, 2005, 2007a; EFSA FEEDAP Panel, 2014a, 2014b).

The Panel concludes that there are no concerns related to genotoxicity.

8.1.3. Acute oral toxicity studies

One acute oral toxicity study was conducted with biomass of *H. pluvialis*, in compliance with GLP. A dose of 12 000 mg/kg bw was administered by gavage to seven- to nine-week-old Sprague–Dawley rats (five animals/sex), as a suspension in 20 % Intralipid® solution (Gillio Tos et al., 1995, unpublished; Stewart et al., 2008). No rats died and no clinical signs or behavioural alterations were noted during the 14-day post-treatment observation period. Body weight gain was unaffected and no relevant macroscopic findings were noted at necropsy at the end of the study. The author concluded that the median lethal dose (LD_{50}) of the test substance was > 12 000 mg/kg bw.

One non-GLP complying acute oral toxicity study was conducted with an astaxanthin-rich oil derived from *H. pluvialis* biomass by solvent extraction. A single dose of 2 000 mg/kg bw of "AstaReal oil 50F" (ca. 5 % astaxanthin) was administered by gavage to six-week-old Sprague–Dawley rats (four animals/sex) (Sakurai et al., 2002, unpublished; Takahashi et al., 2004). The rats were observed for 14 days, body weights were recorded and gross necropsy was carried out at the end of period. Compound-coloured faeces were observed in all animals on day 2. Body weight development was normal and no clinical effect or abnormal findings at necropsy were observed. The LD_{50} of the test material was > 2 000 mg/kg bw.

8.1.4. Subacute oral toxicity study

A subacute oral toxicity study was conducted with biomass of *H. pluvialis* (3 % astaxanthin) (Yu, 1996, unpublished; Stewart et al., 2008). The study was performed in compliance with GLP principles but not in accordance with OECD Test Guideline 407 (OECD, 2008). Only one dose of the test material was administered and no justification for the shorter treatment period of 14 days was provided. Furthermore, at the end of the treatment period, the weights of only a limited number of organs were determined and no histological examinations of organs and tissues were carried out.

In this study, Sprague–Dawley rats (six animals/sex per group) received by gavage biomass of *H. pluvialis* as a suspension in 20 % Intralipid® solution at a dose of 6 000 mg/kg bw per day (test group) or vehicle alone (control group). Mortality, clinical signs, food and water consumption and body weight were recorded during the intervention. At the end of the 14-day period, haematology, clinical chemistry and urine analyses were carried out and macroscopic examinations and organ weight determinations were performed at necropsy. Two animals of the test group (a male and a female) died on day 11 and 15, respectively, owing to erroneous gavage of the test material into the trachea. Regarding the surviving animals, no clinical abnormalities were observed. Cumulative body weight gain in female animals of the test group was approximately 14 % lower than the control group, accompanied by a lower feed intake in females of the test group (approximately 10 %). Feed intake in male animals of the test group was only slightly lower and no difference in body weight development in relation to the control group was observed. No toxicologically relevant effects were identified in blood and urine analyses, organ weight determinations and macroscopic examinations at necropsy.

8.1.5. Subchronic oral toxicity studies

One subchronic toxicity study was conducted with an astaxanthin-rich oil derived from *H. pluvialis* biomass by solvent extraction, "AstaReal Oil 50F" (ca. 5 % astaxanthin) (Takahashi et al., 2004; Yoshihiko et al., 2004, unpublished). Information on GLP compliance was not provided but, according to the report, the study was in accordance with the standard to be applied in drug safety testing in Japan. The study did not comply with the OECD Test Guideline 408 (OECD, 1998), as ophthalmological examinations were not performed and weight determinations as well as histological examinations were carried out on a limited number of organs and tissues.

Four groups of six-week-old Sprague–Dawley rats (10 animals/sex per group) were administered the astaxanthin-rich oil by gavage at daily doses of 0 (vehicle control: maize oil), 37.0, 185.2 and 925.9 mg/kg bw, equivalent to 0, 2, 10 and 50 mg/kg bw astaxanthin, respectively, for 90 days. The high-dose group received the undiluted form of the test material, and for the low- and mid-dose groups the test material was mixed with maize oil to produce concentrations of 20 % and 4 % (w/v), respectively. During the study, general conditions of the rats, body weight and feed intake were determined regularly. At the end of the study, haematology, coagulation, clinical chemistry and urine analyses were carried out. At necropsy, the weights of selected organs were determined and macroscopic as well as microscopic examinations were performed.

All animals survived the treatment period and there were no clinically relevant findings. Orange-coloured stools were recorded in all animals of the high-dose group and frequently in the mid-dose group. Body weights and feed consumption were comparable in all groups. Analysis of coagulation parameters showed a statistically significant increase in prothrombin time (PT) and activated partial thromboplastin time (APTT) in male rats in the highest dose group. The PT values were 12.6 ± 1.6 seconds in the control vs. 16.1 ± 2.4 seconds in the high-dose groups; the APTT values were 28.4 ± 3.2 seconds vs. 35.1 ± 3.2 seconds, respectively. There were no statistically significant differences in haematology parameters. Two animals of the high-dose group showed relatively high values for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities. One of these animals showed focal necrosis in the liver. Additionally, one animal in the mid-dose group showed relatively high ALT, AST and LDH activities and focal necrosis in the liver. There were no statistically significant differences in clinical chemistry analyses. No relevant findings were noted in urinalysis carried out on five animals per group.

In the macroscopic examinations, all animals of the mid- and high-dose groups showed an orange coloration of the mucosal surface of the forestomach as well as of the caecum content. No statistically significant differences were noted in the weights of the heart, lungs, spleen, liver and kidneys in comparison with the control group. Histological examinations of these organs, and of the stomach and duodenum, revealed no toxicologically relevant findings.

Taking into account the prolonged PT and APTT observed in male animals of the high-dose group, the Panel considers that the mid-dose of 185.2 mg AstaReal Oil 50F/kg bw per day, corresponding to approximately 10 mg astaxanthin/kg bw per day, should be regarded as the NOAEL in this study.

One subchronic toxicity study was conducted with biomass of *H. pluvialis* ("algal meal HPP", 3 % astaxanthin), in accordance with OECD Test Guideline 408. Four groups of Wistar strain rats (10 animals/sex per group) received diets containing biomass of *H. pluvialis* at levels of 0 (control), 10 000, 50 000 or 200 000 ppm for 13 weeks (Stewart et al., 2001, unpublished; Stewart et al., 2008). The highest average dose level of *H. pluvialis* biomass was determined to be 14 161 and 17 076 mg/kg bw per day for males and females, respectively, corresponding to an astaxanthin dose of 465 and 557 mg/kg bw per day, respectively. The Panel notes that the highest dose administered is relatively high and the diets were not nutritionally balanced. Mortality, clinical signs, body weight and food consumption were recorded during the intervention. Ophthalmoscopy was performed before the start of the treatments and in week 12 in control and high-dose animals. Urine samples were collected in week 12. Haematology and clinical chemistry analyses and organ weight determinations were performed at week 13. Histological examinations were performed on a restricted selection of tissues from all animals and a wider tissue selection from control and high-dose animals.

Plasma concentrations of *trans*-astaxanthin of animals receiving diets containing 200 000 ppm of the biomass were measured on days 2, 8, 31 and 91 and individual values ranged from 39.5 to 162.5 µg/L for males and from 54.8 to 190.2 µg/L for females. No relationship was noted between the mean plasma concentrations and length of exposure.

All animals survived the treatment period. Orange fur staining as well as faeces staining were noted in all animals receiving the test material. Body weights, body weight gains, feed consumption and feed efficiency were comparable in all groups. High-dose females consumed marginally less food than their controls. Absolute neutrophil counts were statistically significantly lower in males of the high-dose group. Mean platelet counts were lower in high-dose animals than in the control group, attaining statistical significance in females (791 (standard deviation (SD) = 109) vs. 956 (SD = 120)). In male rats, mean platelet counts showed a significant dose–response effect. A slight statistically significant increase occurred in PT in males of the high-dose group (21.1 (SD = 1.2) vs. 20.2 (SD = 1.0)), and there was also a trend regarding APTT. Males of the high-dose group showed a statistically significant lower potassium concentration as well as a dose-related (not significant) elevation in plasma alkaline phosphatase activity. An increase in plasma cholesterol concentration noted in high- and mid-dose males and females was considered to be associated with the high fat content of the test material. Urinalysis showed a statistically reduced urine volume as well as a lower pH value (not significant) for high-dose males. According to the report, there were a number of minor changes in the urinary composition, but the data were not provided.

In the macroscopic examinations at necropsy, orange/red coloration of the mucosal surface of the stomach was noted in several animals of the mid- and high-dose groups. Individual animals also showed coloration of the duodenum and caecum. No significant mucosal changes were noted microscopically in these organs. Minor renal pigmentation was noted in the microscopic examinations in 5 of the 10 females of the high-dose group. Males and females of this group also showed a significant increase in mean kidney weights relative to body weight compared with the control group (1.189 g vs. 1.063 g, $p < 0.01$ in females; 1.736 g vs. 1.611 g, $p < 0.05$ in males).

Taking into account the renal pigmentation reflecting the presence of xenobiotic material in 50 % of female animals and the increased relative kidney weights in both sexes in the high-dose group, the

Panel considers that the mid-dose level, i.e. 3 724 and 4 402 mg *H. pluvialis* biomass/kg bw per day, for males and females, respectively, should be regarded as the NOAEL in this study. This corresponds to 122 and 144 mg astaxanthin/kg bw per day for males and females, respectively.

8.1.6. Chronic toxicity/carcinogenicity studies and reproduction and developmental toxicity studies

No chronic toxicity studies, carcinogenicity studies or reproduction and developmental toxicity studies have been carried out with the NFIs.

The applicant makes reference to such studies conducted with rats, dogs and rabbits with synthetic astaxanthin, which have been evaluated in the context of feed additive applications by the FEEDAP Panel (EFSA, 2005, 2007a). These studies have been re-evaluated by the FEEDAP Panel in its most recent opinion on synthetic astaxanthin (EFSA FEEDAP Panel, 2014a, 2014b).

The FEEDAP Panel established an acceptable daily intake (ADI) of 0.034 mg astaxanthin/kg bw, based on a benchmark dose (BMD) lower confidence limit for a 10 % extra risk ($BMDL_{10}$) calculated for the liver hypertrophy observed in female rats in a chronic toxicity/carcinogenicity study and by applying an uncertainty factor of 100 to the $BMDL_{10}$ of 3.4 mg/kg bw (EFSA FEEDAP Panel, 2014a, 2014b).

8.2. Human studies

8.2.1. Absorption, distribution, metabolism, excretion

The bioavailability and distribution of astaxanthin has been studied in humans using single doses of 40 mg up to 100 mg with maximum concentrations in the blood observed between 6.7 ± 1.21 hours (Osterlie et al., 2000) and 21.3 ± 6.53 hours (Okada et al., 2009) and ranging from 55.2 ± 15.0 µg/L to 1.3 ± 0.1 mg/L (Osterlie et al., 2000; Mercke Odeberg et al., 2003; Okada et al., 2009). Reported apparent half lives ranged from 15.9 ± 5.3 hours (Mercke Odeberg et al., 2003) to about 55 hours (Coral-Hinostroza et al., 2004). Bioavailability and distribution in plasma seem to depend on a variety of factors including preparation (e.g. fractions of free and esterified astaxanthin, proportion of astaxanthin isomers), formulation (e.g. co-administration of fat or surfactants) and application (e.g. with or without meals) or smoking habits. In the relevant studies provided by the applicant, various preparations of astaxanthin from different producers were used, but not the NFIs per se. Although similar results may be expected with the NFIs, specific human data on its fractional absorption rates, its determinants of distribution in tissues and its metabolism, the latter in particular as regards to continued consumption, are not available.

Distribution of astaxanthin E/Z isomers in plasma were studied in three male volunteers (37–43 years) after ingestion of a dose of 100 mg astaxanthin (consisting of 74 % all-E-, 9 % 9Z-, 17 % 13Z-astaxanthin). The results indicate that a selective process increases the relative proportion of astaxanthin Z-isomers compared with the all-E-astaxanthin during blood uptake and that astaxanthin E/Z isomers have similar pharmacokinetics (Osterlie et al., 2000).

Astaxanthin metabolites 3-hydroxy-4-oxo-β-ionol and 3-hydroxy-4-oxo-β-ionone and their reduction products, 3-hydroxy-4-oxo-7,8-dihydro-β-ionol and 3-hydroxy-4-oxo-7,8-dihydro-β-ionone, were detected in the plasma of two human volunteers 24 hours after oral administration of 100 mg astaxanthin (8 % gelatine formulated beadlets) (Kistler et al., 2002). The C9,C9′ cleavage of the astaxanthin molecule and reduction of the polyenic 7,8-double bond has been shown to be common to humans and rats (see section 8.1.1).

One Member State raised a concern on the potential accumulation of astaxanthin in eyes and other tissues, based on the study by Petri and Lundebye (2007) in rats (see section 8.1.1). The study indicated that astaxanthin accumulated in rat eyes in a dose-dependent manner during a two-week exposure. The highest amount (80 ng/g) was detected in the eyes of rats exposed for two weeks to the

highest dose of astaxanthin (30 g astaxanthin/kg feed; > 2 000 mg astaxanthin/kg bw per day), which is the same order of magnitude as has been demonstrated for canthaxanthin in the eyes of albino rats (130 ng/g) and pigmented rats (20 ng/g) after administration of a 20-fold lower dose of 100 mg/kg bw per day for five weeks (JECFA, 1996). The Panel notes that none of the human studies addressed the potential accumulation of astaxanthin in tissues including the human eye lens. With regard to the study of Petri and Lundebye (2007), the lowest dose group received an amount of astaxanthin (215 mg/kg bw per day) which was one hundred times higher than the proposed use levels and anticipated intakes of the NFIs.

The NFIs contain a maximum of 0.06 % w/w of canthaxanthin in AstaREAL L10 oil. Assuming the highest intake of 17.9 mg/day astaxanthin from the NFIs estimated from the data of the EFSA comprehensive European food consumption database (see section 4), equivalent to ca. 160 mg AstaREAL L10 oil, this would correspond to a canthaxanthin intake of 0.1 mg/day. This corresponds to an intake of 1.4 μg/kg bw for a 70 kg person, which is below the ADI of 30 μg/kg bw for canthaxanthin (JECFA, 1996).

The Panel concludes that the proposed use levels of astaxanthin from the NFIs do not raise a safety concern with regard to the accumulation of astaxanthin or canthaxanthin from the NFIs in the human eye lens.

8.2.2. Clinical studies

8.2.2.1. Clinical studies with the NFIs

The applicant reported nine clinical studies which used astaxanthin-rich biomass from *H. pluvialis* produced by BioReal (Sweden) AB. In one acute study, 32 healthy adults received a single dose of 40 mg astaxanthin (Mercke Odeberg et al., 2003). Five studies involved 13 to 20 healthy adults who received daily doses of astaxanthin from 2 to 12 mg for two weeks to six months (Lignell, 2001, unpublished; Shimada, 2003, unpublished; Nagaki et al., 2005; Nagata et al., 2006; Karppi et al., 2007). Three studies involved 10 to 88 patients with dyspepsia or *Helicobacter pylori* infection who received 16 to 40 mg/day astaxanthin for three to four weeks (Lignell et al., 1999; Borody, 1999, unpublished; Kupcinskas et al., 2004). Five of these studies included some safety endpoints such as clinical chemistry parameters in blood, physical examination (body weight, blood pressure) and/or adverse effects reported by the subjects (Borody, 1999, unpublished; Shimada, 2003, unpublished; Nagaki et al., 2005; Karppi et al., 2007; Kupcinskas et al., 2008). None of the studies, which used astaxanthin doses ranging from to 2 to 40 mg/day for four weeks to three months, reported significant effects of the study treatment on these parameters.

8.2.2.2. Clinical studies with other astaxanthin-rich ingredients from *H. pluvialis*

The applicant reported six clinical human studies with other astaxanthin-rich extracts from *H. pluvialis.* The studies involved 8 to 73 healthy subjects who received 6 to 40 mg/day astaxanthin for 10 days to 8 weeks (Sawaki et al., 2002; Spiller and Dewell, 2003; Nitta et al., 2005; Shiratori et al., 2005; Miyawaki et al., 2008; Satoh et al., 2009; Kim et al., 2011). Safety concerns were considered in these studies by clinical chemistry parameters in the blood, physical examination (weight, blood pressure, temperature and pulse) and reported adverse events. If any changes in these parameters were observed, these were small and considered of no clinical importance. Similarly, no adverse event was considered to be caused by the test food.

The Panel concludes that no safety concerns are raised by the clinical studies at daily intakes of astaxanthin of 2 to 40 mg over periods of 10 days up to 3 months for healthy adults. However, as most of these studies were of short duration, no conclusion can be drawn as regards long-term effects.

The applicant indicates that no clinical studies were carried out with children and pregnant or breast-feeding women. As regards people with chronic diseases, the applicant reports that there are no data except from two studies in patients with dyspepsia or *Helicobacter pylori* infection which did not

report adverse events during the course of the treatment (Lignell et al., 1999; Kupcinskas et al., 2004; Kupcinskas et al., 2008).

8.3. Risk of lung cancer

Some Member States noted that β-carotene has been shown to increase the risk of lung cancer in smokers and expressed concerns that the NFIs may induce similar effects. An impaired metabolism of retinoids, caused by the induction of CYP enzyme CYP1A1 by β-carotene oxidative metabolites, has been discussed as a possible mechanism for the effect of β-carotene (Russell, 2004).

8.3.1. Pro-oxidant activity of astaxanthin

The applicant provided several *in vitro* studies indicating that astaxanthin lacks pro-oxidant activity (Martin et al., 1999; Beutner et al., 2001). Although, under certain experimental conditions, pro-oxidant effects were demonstrated for apolar carotenoids such as lycopene and β-carotene, this was not the case for astaxanthin (McNulty et al., 2007).

The Panel notes that there is no evidence of pro-oxidant activity of astaxanthin based on *in vitro* studies. No *in vivo* human data are available.

8.3.2. Interaction with cytochrome P450 enzymes

The applicant identified one human study which addressed the effect of astaxanthin on CYP enzymes. Kistler et al. (2002) investigated the CYP-inducer properties of astaxanthin in human hepatocytes. Liver samples were obtained from four patients who underwent a partial hepatectomy for the resection of liver metastases. Human hepatocytes were cultured with 0, 0.375 and 3.75 μM astaxanthin for 96 hours, after which CYP proteins, CYP mRNAs concentrations and monoxygenase activities were measured. No effects were observed at 0.375 μM astaxanthin. At 3.75 μM astaxanthin, CYP3A4 and CYP2B6 concentrations and their related mRNAs were increased. The oxidation of cyclosporin A and 7-ethoxy-4-thrifluoromethylcoumarin catalysed by CYP3A4 and CYP2B6, respectively, was increased. No effect was observed on CYP1A1 and CYP1A2.

The applicant is of the opinion that, at the intended use levels, astaxanthin is not expected to affect CYP enzymes or the metabolism of drugs in humans. The applicant argues that, in a clinical trial, where patients with non-ulcer dyspepsia received 40 mg astaxanthin/day for four weeks, the plasma level of astaxanthin did not exceed 125 μg/L (ca. 0.2 μM astaxanthin) (Kupcinskas et al., 2008). The Panel notes that blood samples were taken within 24–48 hours after ingestion of the last astaxanthin capsules and may not reflect maximum plasma concentrations. Furthermore, these plasma concentrations may not be indicative of tissue concentrations.

Gradelet et al. (1996b) studied the effect of various carotenoids on liver xenobiotic-metabolising enzymes in rats. The activities of several enzymes were assessed in liver microsomes as markers of CYP isoenzymes activity, including the activity of ethoxyresorufin O-deethylase (EROD) as marker of CYP1A1, methoxyresorufin O-demethylase (MROD) as marker of CYP1A2, pentoxyresorufin O-dealkylase (PROD) as marker of CYP1B2 and benzoxyresorufin O-dealkylase (BROD) as a marker of CYPs 1A, 2B and 3A. Immunoblots of CYP1A1 and CYP1A2 were also performed. In a first experiment, five groups of five rats (24–27 days old) received a diet containing 300 mg/kg diet lycopene, canthaxanthin, astaxanthin; 15 mg/kg ethoxyquin; or a placebo for 15 days. The Panel notes that this is equivalent to daily doses of ~ 35 mg/kg bw lycopene, canthaxanthin, astaxanthin, considering a conversion factor from test compound concentration in feed (mg/kg) into daily dose (mg/kg bw) of 0.12 for this 15-day study in young rats (EFSA SC, 2012). Significant induction of all four enzymes was observed in groups receiving astaxanthin and canthaxanthin. This was accompanied by an elevation of CYP1A1 and CYP1A2 levels, as assessed by immunoblot. No effects were observed in the other groups. In a second experiment, rats (24–27 days old) were administered doses of 10, 30, 100 or 300 mg/kg diet astaxanthin or canthaxanthin for 15 days (three animals/group). The Panel notes that this is equivalent to daily doses of ~ 1.2, 3.5, 12 and 35 mg/kg bw lycopene, canthaxanthin, astaxanthin. A significant increase in the activity of EROD, MROD and BROD was

observed with doses of astaxanthin of 100 mg/kg and 300 mg/kg. No effect was observed on total microsomal CYP content assessed at any dose. Significant induction of the enzymes was observed with doses of 30, 100 and 300 mg/kg diet canthaxanthin, accompanied with a significant increase in total microsomal CYP content.

Similar effects of astaxanthin on EROD and MROD were observed in a subsequent experiment using a dose of 300 mg/kg diet (equivalent to ca. 35 mg/kg bw) (Jewell and O'Brien, 1999), in the liver, lungs and kidneys. Induction of PROD and BROD were observed in the liver only.

Finally, Ohno et al. (2011) fed rats with an astaxanthin-containing oil (100 mg/kg bw per day for three days) and observed an increase in CYP1A mRNA expression, protein and its activity in the rat liver.

The Panel notes that orally administered astaxanthin has been shown to induce CYP1A1 and CYP1A2 enzymes in various tissues in rats, at doses above 3.5 mg/kg bw (Gradelet et al., 1996b; Jewell and O'Brien, 1999; Ohno et al., 2011). The implications of these findings on the putative cancer risk of astaxanthin have not been investigated. One study investigated metabolism of CYP-inducer properties of astaxanthin in primary human hepatocytes (Kistler et al., 2002). The Panel considers that this study is of limited relevance as it was carried out in a very small number of cancer patients who may not be representative of healthy subjects and investigation was limited to the liver and did not address other tissues, such as gut or lung tissues.

The Panel considers that the knowledge on the potential of astaxanthin to impact xenobiotic metabolism is not sufficient to draw a conclusion for humans. The relevance of the observed induction of CYP enzymes by astaxanthin in rats for the cancer risk in humans is unclear.

8.3.3. Discussion and conclusions

The Panel notes that a series of experiments conducted in ferrets have provided indications regarding the observed interactions between β-carotene, cigarette smoking and lung tumorigenesis. In ferrets, exposure to cigarette smoke and/or high doses of β-carotene (30 mg/day) was found to induce CYP enzymes in the lungs, to enhance retinoic acid catabolism, and to decrease levels of retinoic acid receptor β (RAR-β) (Wang et al., 1999; Liu et al., 2000; Liu et al., 2003).

It has been proposed that β-carotene, when administered at high doses, has pro-oxidant properties based on its interaction with free radicals in cigarette smoke, particularly in the oxidative environment of a smoker's lungs (Mayne et al., 1996; Arora et al., 2001). The increased formation of oxidative metabolites of β-carotene (β-apo-carotenals) might lower the levels of retinoic acid in lungs from ferrets exposed to cigarette smoke. It was found in *in vitro* studies that the eccentric cleavage product of β-carotene, β-apo-13-carotenone, functions as an antagonist of the retinoic X receptor and was effective at concentrations as low as 1 nM. It has also been shown that low levels of retinoic acid may interfere with retinoid signal transduction, resulting in an enhancement of cell proliferation and an increased potential for malignant transformation (Eroglu et al., 2010). In a study by Gradelet et al. (1996a), it was demonstrated that CYP enzymes induced by eccentric cleavage breakdown products of β-carotene are involved in the degradation of retinoic acid.

Contrary to the findings in animals, a study on archival lung tissue available from 52 men who received 20 mg/day β-carotene as a supplement or a placebo for several years in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study revealed no apparent effect of β-carotene supplementation on RAR-β expression (Wright et al., 2010). Similarly, in archival lung tissue samples (n = 40) from patients who were diagnosed with lung cancer in the Physicians' Health Study, no significant influence of 50 mg β-carotene supplementation on alternate days for 12 years was observed on RAR-β expression compared with a placebo (Liu et al., 2009).

The Panel notes that, while several potential mechanisms with regard to an increased risk of lung cancer in heavy smokers taking high-dose β-carotene supplements have been proposed from studies in animal models, the transferability of these findings in animals to humans has to be ascertained.

In an intervention study by (Kim et al., 2011), doses of astaxanthin from a preparation of *H. pluvialis* of 5, 20 or 40 mg (n = 13 each) were administered once daily for three weeks to 39 heavy smokers. At the end of the intervention, no adverse effect of the treatment was observed on markers of oxidative stress in any intervention group compared with baseline values. The Panel notes that this study was uncontrolled.

At present, there are neither human nor animal studies that have investigated the interaction of astaxanthin and smoking with regard to the risk of lung cancer. As outlined in section 8.3.2, the knowledge on the potential of astaxanthin to impact xenobiotic metabolism in humans is not sufficient to draw a conclusion.

The Panel notes that the effects of high-dose β-carotene observed in animal models of carotenoids research in lung cancer have been mainly attributed to the pro-oxidant properties of β-carotene.

There are differences in structure, metabolism and function between astaxanthin and β-carotene. In contrast to β-carotene, astaxanthin is more polar, is not a precursor of vitamin A and is considered as an antioxidant with no indication of pro-oxidative properties (see section 8.3.1).

The Panel concludes that available data do not indicate that additional astaxanthin at the proposed level of use would increase the risk of lung cancer in smokers.

8.4. Allergenicity

Soy lecithin was used in the original production process of AstaREAL A1010. However, the applicant has replaced it with sunflower lecithin. The Panel concludes that there is no concern for people allergic to soy regarding potential allergic reactions to the NFIs.

In response to questions concerning allergenicity potential of the NFIs owing to proteins of *H. pluvialis*, the applicant provided additional information. The immunoglobulin E (IgE) binding capacity of the crushed spray-dried biomass of *H. pluvialis* was tested with the ImmunoCap® test. Most of the detected IgE antibody concentrations for *H. pluvialis* biomass in serum from mould-, birch- and seafood-sensitised individuals were below cut-off levels considered to be negative in their specificity to the soluble proteins extracted from *H. pluvialis* biomass. Sera from multifood-sensitised individuals showed negative (3/10), very low (6/10) or low (1/10) responses to the algae protein. Although the level of IgE does not correlate very well with clinical allergy, these data do not allow allergic reactions to the NFIs to be excluded. No allergic reactions to *H. pluvialis* have been described in the literature.

The Panel considers that the likelihood of adverse allergic reactions to the NFIs is low.

DISCUSSION

The applicant has provided sufficient information regarding the composition and specification of the astaxanthin-rich novel ingredients AstaREAL A1010 and AstaREAL L10 and their manufacturing process. AstaREAL A1010 and AstaREAL L10 are produced from *H. pluvialis*, a microalga naturally rich in astaxanthin. AstaREAL A1010 is a powder containing 5.0–5.6 % w/w astaxanthin, while AstaREAL L10 is available in the form of a red viscous oil, containing 10.0–12.0 % w/w astaxanthin, or as a powder of modified starch encapsulating the viscous oil, containing 2.5–2.7 % w/w astaxanthin. Analyses of several batches confirmed that the products meet specification. There are no concerns with respect to the contents of the NFIs in heavy metals, pesticides, aflatoxins, PAHs, dioxin and dioxin-like PCBs. Taking the HACCP plan and manufacturing conditions into account, the Panel considers that the presence of algal toxins in the final product is unlikely, but suggests periodic

controls. The stability of the NFIs was analysed under relevant storage conditions and considered sufficient.

The NFIs are intended to be marketed as ingredients in fermented liquid dairy products, non-fermented liquid dairy products, fermented soya products and fruit drinks. The applicant proposes to advise consumers to limit their daily intake to a maximum of 4 mg astaxanthin by using product labelling. The applicant indicates that foods containing the NFIs are intended for healthy adults and are not recommended for children, pregnant or breast-feeding women or people with chronic diseases, and would be labelled as such.

Based on consumption data from EU Member States (EFSA comprehensive European food consumption database), the highest intake estimates from combined consumption of fermented liquid dairy products, non-fermented liquid dairy products and fruit drinks were calculated for Finnish adults. The estimated mean and high (95th percentile) astaxanthin intakes amount to 0.106 mg/kg bw per day and 0.256 mg/kg bw per day, respectively. These would be "worst-case" scenarios based on the assumption that all food categories consumed contain the maximum level of the novel ingredients. Considering the additional intake from salmon and trout consumption, this would correspond to total mean and high daily intakes of astaxanthin of 0.125 mg/kg bw and 0.286 mg/kg bw, respectively.

In the human studies provided which addressed safety endpoints, no clinically significant changes or adverse effects were observed after consumption of the NFIs or other astaxanthin-rich ingredients from *H. pluvialis*, at doses ranging from 2 to 40 mg astaxanthin per day for 10 days to 3 months. However, as these studies were of only short duration, no conclusion can be drawn as regards long-term effects.

Regarding the concern that astaxanthin intakes might cause an increased risk of lung cancer in smokers, as reported for β-carotene, the Panel notes that the mechanisms by which high-dose β-carotene supplements increase the risk of lung cancer in smokers still await further elucidation. At present, there are neither human nor animal studies that have investigated the interaction of astaxanthin and smoking with regard to the risk of lung cancer. The knowledge on the potential of astaxanthin to impact xenobiotic metabolism, including P450 CYP1A1, in humans is not sufficient to draw a conclusion. There are differences in structure, metabolism and function between astaxanthin and β-carotene. In contrast to β-carotene, astaxanthin is more polar, is not a precursor of vitamin A and is considered as an antioxidant with no indication of pro-oxidative properties. The Panel concludes that available data do not indicate that the NFIs at the proposed level of use would increase the risk of lung cancer in smokers.

The data available indicate that astaxanthin is absorbed in humans and rodents and follows similar metabolic pathways, consisting of its cleavage at the C9, C9′ positions and stepwise reduction. There is no concern with respect to the potential accumulation of astaxanthin or canthaxanthin in the human eye lens at the proposed use levels of the NFIs.

Based on the *in vitro* and *in vivo* studies available, the Panel considers that there are no concerns related to genotoxicity of the NFIs.

Biomass of *H. pluvialis* (algal meal containing 3 % astaxanthin) was tested for subchronic toxicity in rats and the NOAEL was 3.7 and 4.4 g/kg bw per day for males and females, corresponding to 122 and 144 mg astaxanthin/kg bw per day, respectively. An astaxanthin-rich oil obtained by solvent extraction from *H. pluvialis* biomass (containing ca. 5 % astaxanthin) was also tested for subchronic toxicity and the NOAEL was 185.2 mg/kg bw per day, corresponding to approximately 10 mg astaxanthin/kg bw per day.

There is no indication from the toxicity tests with the source of the NFIs and AstaREAL oil that the NFIs would be more toxic than astaxanthin. Therefore, the Panel bases the evaluation on astaxanthin.

An ADI of 0.034 mg/kg bw for astaxanthin has been derived by the FEEDAP Panel. Considering the maximum intake of 4 mg astaxanthin per day (0.06 mg/kg bw per day for a 70 kg person) from the NFIs as proposed by the applicant, the Panel notes that this intake exceeds the ADI by approximately two-fold. The estimated mean and high (95th percentile) astaxanthin intakes of 0.106 and 0.259 mg/kg bw per day from the combined consumption of food products containing the NFIs exceed the ADI by approximately three- and seven-fold, respectively.

CONCLUSIONS

The NDA Panel notes that the maximum intake of 4 mg astaxanthin per day (0.06 mg/kg bw per day) from the NFIs as proposed by the applicant and the estimated mean intake based on the use levels in the proposed food categories (0.106 mg/kg bw per day) exceed the ADI for astaxanthin of 0.034 mg/kg bw per day by approximately two- and three-fold, respectively. The Panel therefore concludes that the safety of the NFIs AstaREAL A1010 and AstaREAL L10 at the proposed use and use levels has not been established.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier "Application for the approval of the use of natural astaxanthin rich ingredients: AstaREAL A1010 and AstaREAL L10 in fermented and non-fermented fluid dairy products, fermented soya products and fruit drinks under Regulation (EC) No 258/97 of the European Parliament and the Council of 27 January 1997 concerning novel foods and novel food ingredients" received on 29 August 2011. Submitted by BioReal (Sweden) AB on 05 June 2008. Additional data were provided on 24 May 2012, 31 May 2012 and 09 December 2013.

2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of a "Astaxanthin". SANCO/E6/AK/bs ref. Ares(2011)894395, dated 22 August 2011.

3. Initial assessment report carried out by Finland: "AstaREAL A1010 and AstaREAL L10 manufactured from Haematococcus pluvialis green alga rich in astaxanthin as novel food ingredients, Initial assessment under Article 4 of Regulation (EC) No 258/97", the Novel Food Board, the Finnish Food Safety Authority Evira.

4. Member States' comments and objections.

5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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ABBREVIATIONS

ADI	acceptable daily intake
ALT	Alanine aminotransferase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under the curve
BMD	benchmark dose
BMD_{10}	benchmark dose associated with a 10 % response rate
BMDL	95^{th} percentile benchmark dose lower confidence limit
$BMDL_{10}$	95^{th} percentile benchmark dose lower confidence limit associated with a 10 % response rate
BROD	benzoxyresorufin O-dealkylase
bw	body weight
cfu	colony-forming unit
CYP	cytochrome P450
EC	European Commission
ELISA	enzyme-linked immunosorbent assay
EROD	ethoxyresorufin O-deethylase
EU	European Union
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
GLP	good laboratory practice
GM	genetically modified
HACCP	Hazard Analysis and Critical Control Points
HPLC	high-performance liquid chromatography
ICP -MS	inductively coupled plasma mass spectrometry
IgE	immunoglobulin E
i.v.	intravenous
LD_{50}	median lethal dose
LDH	lactate dehydrogenase

MROD	methoxyresorufin O-demethylase
MS/MS	tandem mass spectrometry
NDA	Panel on Dietetic Products, Nutrition and Allergies
NF(I)	novel food (ingredient)
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-para-dioxins
ppm	parts per million
PROD	pentoxyresorufin O-dealkylase
PT	prothrombin time
RAR-β	retinoic acid receptor β
SD	standard deviation
TEQ	toxic equivalent
UHT	ultra-high temperature
UK ACNFP	Advisory Committee on Novel Foods and Processes of the United Kingdom
WHO	World Health Organization
w/v	mass concentration
w/w	mass fraction