Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request from the European Commission on the safety of use of colouring agents in animal nutrition

PART I. General Principles and Astaxanthin

(Question No. EFSA-2003-060)

Adopted on 30th November 2005

SUMMARY

Astaxanthin [(3S,3'S)-3,3'-dihydroxy-β,β-carotene-4,4'-dione) is a natural carotenoid with red pigmenting properties occurring in yeasts, algae, crustaceans, and predator fish like salmonids. It is approved at EU level as feed additive for salmon and trout at 100 mg kg^{-1} complete feed from 6 months of age onwards without time limit (and for ornamental fish). No specifications could be found at EU level, however the FEEDAP Panel recommends the inclusion of specifications. Products on the market are either synthetically or biotechnically produced. Astaxanthin is a vitamin A precursor for fish, and important for growth and survival, specific functions in reproduction and metabolism, and health in salmonids.

Astaxanthin is the major carotenoid in supplementing salmonid feed. The absorption capacity is limited, salmon would built a plateau at about 10 mg kg^{-1} flesh, trout at a higher level of about 10-25 mg kg^{-1} flesh. Absorption is determined by several factors, the occurrence of free or esterified astaxanthin, and dietary factors (mainly lipid level), excretion of undigested astaxanthin amounts highest to about 40%. Astaxanthin is metabolised in salmonids through reductive pathways, leading to idoxanthin, adonixanthin and zeaxanthin. No cleavage of the polyen chain is observed. Metabolites were mainly excreted via the bile. After astaxanthin application, the pigments deposited in flesh of trout and Chinook salmon are predominantly astaxanthin, in the arctic charr also idoxanthin.

Safety assessment was difficult due to the scarceness of fully published toxicity studies. The FEEDAP Panel therefore decided to consider also summaries and abstracts as additional information.

Astaxanthin is not mutagenic and not clastogenic. In three subchronic studies on rats performed with astaxanthin rich algae *Haematococcus pluvialis* or yeast *Phaffia rhodozyma*, no toxic effects were described at 2 mg, 11 mg kg^{-1}, and about 40 mg asthaxanthin kg^{-1} bodyweight. In a 37 week rat study a corresponding value of at least 25 mg asthaxanthin kg^{-1} bodyweight could be seen. A reproductive toxicity study, including developmental toxicity, with astaxanthin up to 400 mg kg^{-1} bodyweight did not show (statistically) significant adverse effects. Carcinogenicity studies could not be found, but several subchronic studies showed an anticarcinogenic effect of astaxanthin in experimental models with different carcinogens.

The FEEDAP Panel could not set a NOEL (no observed effect level) nor an ADI (acceptable daily intake) for different reasons (astaxanthin content of the product not clearly stated, no chronic study available, full toxicological data set not known).

Studies on healthy human volunteers showed that a daily intake of 5 and 12 mg astaxanthin for four weeks, and 6 mg for 8 weeks was tolerated without clinical signs. The data available on intake of trout and salmon flesh by the consumer are insufficient. Worst case calculations indicate that the mean astaxanthin uptake of the European consumer would not exceed 2 mg day^{-1}.

Supplementing salmonid feed with astaxanthin would not increase flesh astaxanthin of farmed fish essentially compared to wild catches. The FEEDAP Panel considers therefore the use of

astaxanthin as feed additive to salmonid feed at the maximum level approved safe for the human consumer.

No data is available for a qualified assessment of the environmental impact of asthaxanthin in salmonid feed. Astaxanthin added to the feed of farmed fish is a substitute of natural sources in the habitat of wild living salmon and trout. The FEEDAP Panel concludes mainly based on the oxydative suceptibility of astaxanthin and the large distribution volume in water of fish operations that the use of astaxanthin as feed additive to salmon and trout will not pose a significant risk to the environment.

Key words: Carotenoids, astaxanthin, capsanthin, citranaxanthin, cryptoxanthin, pigments, egg yolk colour, skin pigmentation, flesh colour, salmonids

BACKGROUND

In its opinion of April 2002 on the use of canthaxanthin in feedingstuffs, the SCAN suggested that the required levels of canthaxanthin should be reviewed in order that human exposure to canthaxanthin remains within the Acceptable Daily Intake established for that compound. The lowering of the levels of this pigment would lead to an increasing use of alternative colorants.

Other substances are indeed authorised for use in feedingstuffs as colouring agents, as described in the table hereafter. In its opinion on canthaxanthin, as well as in the report on an astaxanthin-rich product, the SCAN drew the attention of the Commission to the fact that no risk assessment has ever been carried out and that no ADI has ever been established for carotenoids other than canthaxanthin.

TERMS OF REFERENCE

In the light of these opinions on some of the colouring agents, EFSA is asked to assess the safety of use of capsanthin (E160c), beta-apo-8'-carotenal (E160e), ethyl ester of beta-apo-8'carotenic acid (E160f), lutein (E161b), cryptoxanthin (E161c), zeaxanthin (E161h), citranaxanthin (E161i), astaxanthin (E161j) in feedingstuffs for laying hens, other poultry, salmon, trout, on the basis of currently available scientific literature.

In making its assessment, it is requested to prioritise the substances which may be used as alternatives to canthaxanthin.

Table 1. **ANNEX ENTRY**

EC No.	Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	End of period of authorisation
					mg kg^{-1} of complete feedingstuff			
Colourants including pigments								
1.Carotenoids and xanthophylls								
E 160c	Capsanthin	$C_{40}H_{56}O_3$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 160e	Beta-apo-8'-carotenal	$C_{30}H_{40}O$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 160f	Ethyl ester of beta-apo-8'-carotenoic acid	$C_{32}H_{44}O_2$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161b	Lutein	$C_{40}H_{56}O_2$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161c	Cryptoxanthin	$C_{40}H_{56}O$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161h	Zeaxanthin	$C_{40}H_{56}O_2$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161i	Citranaxanthin	$C_{33}H_{44}O$	Laying hens	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161j	Astaxanthin	$C_{40}H_{52}O_4$	Salmon, trout	-	-	100	Use only permitted from the age of 6 months onwards. The mixture of astaxanthin with canthaxanthin is allowed provided that the total concentration of the mixture does not exceed 100 mg kg^{-1} in the complete feedingstuff.	Without a time limit
			Ornamental fish	-	-	-	-	Without a time limit

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ASSESSMENT OF COLOURING AGENTS IN ANIMAL NUTRITION

1. Introduction Carotenoids

Carotenoids - a class of hydrocarbons and their oxygenated derivatives - are polyenic compounds characterised by a system of conjugated double bounds. Currently more than 700 carotenoids including their geometrical isomers are known. The basic structure is derived from eight isoprenoid chains arranged symmetrically around a central double bound to form a tetrapene (a terpene is a C_{10} molecule). The number of conjugated double bounds varies from 7 to 15. Figure 1 gives the structural formula of β-carotene, one of the most widespread natural carotenoids, as an example.

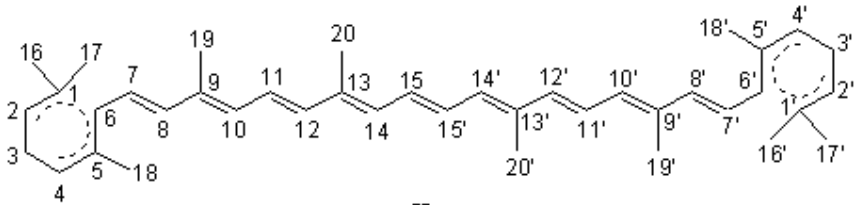

Figure 1. β-carotene, its structure includes two terminal β-ionone-rings

Due to the particular chemical structure of carotenoids, a large number of stereo (optical) and geometrical isomers occurs, which are interconvertible in solution. Stereoisomerism exerts a marked influence on the physical properties. Isomers differ not only in their melting points, solubility and stability, but also in UV characteristics. In nature all-E (formerly all-trans) isomers are the most abundant.

Carotenoids containing hydrogen and carbon only are classified as carotenes and do not have significant colouring abilities. Natural carotenoid pigments containing oxygen are called xanthophylls. (El Boushy and Raterink, 1992).

1.1. Function of carotenoids

Carotenoids are not synthesized in animals but they can be synthesized in plants and microorganisms. They are responsibles for the bright colours of various fruits and vegetables. The colour of carotenoids in green plant tissues is covered by chlorophyll and becomes evident only after degradation of the green pigment in the fall. Dietary carotenoids also serve as natural colorants in organisms that lack carotenoid synthesis and are responsible for the typical colour of e.g. salmon flesh, lobster shells or bird plumage.

In addition to their colouring properties, some carotenoids posses pro-vitamin A activitiy. Only 50 out of the approximately 700 naturally occurring carotenoids are converted to vitamin A. ß-Carotene is the major pro-vitamin A carotenoid. Among the components in the terms of reference, citranaxanthin, ß-cryptoxanthin and β-apo-8'-carotenal have pro-vitamin A property (Schiedt *et al*., 1985), and astaxanthin only for fish (Christiansen *et al.,* 1994). Apart from their function as provitamin A, carotenoids are reported to be of importance as antioxidants, immune response enhancers, and photoprotection agents. The functions also induce gap junction communication or carcinogen-metabolizing enzymes (Stahl *et al*., 1997; Gerster, 1993).

1.1.1. Molecular effects of carotenoids

Carotenoids produce changes in the expression of many proteins participating in these processes. Changes in the expression of proteins suggest that the initial effect of carotenoids involves modulation of transcription. Evidence is established that transcription systems such as the retinoid receptors, activator protein-1, peroxisome proliferator activated receptors (PPAR),

xenobiotic receptors and antioxidant responsive element (ARE) are involved. Effects are exerted by carotenoids but also their metabolites (Sharoni *et al*., 2004; Rühl *et al*., 2004). Sharoni *et al*. (2004) showed that asthaxanthin and canthaxanthin modulate the induction of phase I, II and antioxidant enzymes in some *in vitro* studies.

1.2. Human exposure

Humans are exposed to carotenoids through the diet. This exposure results from carotenoids present in vegetables and fruits as well as from animal products rich in carotenoids. The later products might be additionally enriched in these components by specific feed additives. Furthermore, exposure results from carotenoids used as food additives and from dietary supplements. For some specific carotenoids (α-carotene, β-carotene, lutein, lycopene, and β-cryptoxanthin) data concerning the typical intakes from the diets of healthy adults are available (O'Neill *et al.,* 2001; Pelz *et al.,* 1998). Efficacy of gastrointestinal absorption of the carotenoids mainly depends on the carotenoid and on the composition of the diet (Schweigert, 1998; van Het Hof *et al.,* 2000).

1.2.1. Transfer of carotenoids to the off-spring

In addition to the direct exposure through diet and/or supplements, in humans the transfer of carotenoids to the off-spring has to be considered. Carotenoids can be transferred via the placenta to the embryo in utero or via the milk to the suckling new-born. Indicators of the efficacy of a transfer to the embryo can be obtained from plasma or tissue levels from the newborn or by the determination of umbilical blood (Kiely *et al.,* 1999; Yeum *et al.,* 1998). Studies indicate that the transfer is quantitatively limited. As a consequence levels of carotenoids in newborn are low. Milk carotenoids correspond qualitatively but not quantitatively to carotenoids in plasma. (Canfield *et al.,* 2003; Schweigert *et al.,* 2003). Carotenoid concentration in formulated milk products is generally lower than in maternal milk (Sommerburg *et al.,* 2000).

1.3. Biomarkers of carotenoid exposure

Human exposure can be assessed through the determination of absorbed carotenoids in blood plasma and milk or through the evaluation of the diet. Valid biomarkers of carotenoid consumption are carotenoid levels in plasma because the concentration is readily effected by dietary intake (Granado *et al.,* 1996; Oshima *et al.,* 1997). Additionally carotenoid levels in human milk and adipose tissue might be used as biomarkers (El-Sohemy *et al.,* 2002). The major determinants of carotenoid plasma levels might include many factors such as the dietary carotenoid concentration, the degradation of carotenoids in the gastrointestinal tract, the efficiency of absorption and metabolism as well as the rate of tissue uptake. In general, fat increases the availability.

1.3.1. Carotenoids in human plasma and milk

Carotenoids have been determined in human plasma at varying concentrations (Etoh *et al.,* 2000; Irwig *et al.,* 2002; Mercke Odeberg *et al.,* 2003; Oshima *et al.,* 1997; Osterlie *et al.,* 2000). Among the 18 - 20 carotenoids identified in plasma of humans, the most important include β-carotene, α-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin, indicating that in humans both carotenes and xanthophylls are absorbed (Gatautis and Pearson, 1987; Khachik *et al.,* 1992).

Plasma concentrations vary greatly per country. Differences were found comparing populations in Spain and Sweden (van Kappel *et al.,* 2001) or between five European countries comparing healthy subjects (Olmedilla *et al.,* 2001). In a cross-sectional study plasma levels of six

carotenoids in nine European countries were measured on 3,043 test persons (Al-Delaimy *et al.*, 2004). In multivariate regression analyses, region was the most important predictor of total plasma carotenoid level (partial R^2 27.3 %), followed by BMI (partial R^2 5.2 %), gender (partial R^2 2.7 %) and smoking status (partial R^2 2.8 %).

In human milk only information on the levels of β-cryptoxanthin is available. Concentrations in mature human milk vary greatly per country (Canfield *et al.,* 2003; Macias and Schweigert, 2001). The most important carotenoids in milk are β-carotene, α-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin.

1.4. Analytical methods of carotenoids

Open-column and thin-layer chromatography methods have been originally used for the analysis of carotenoids. At present, the separation and quantification of carotenoids are performed by high-performance liquid chromatography (HPLC) and detection by UV light absorption.

Other methods of detection include electrochemical array detection, thermal lens spectrometry, mass spectrometry, nuclear magnetic resonance and resonance raman excitation spectroscopy (Su *et al*., 2002).

1.4.1. Measuring colour in animal products

Yolk colour is usually measured with special colour fans covering the different levels of colouration (from pale yellow to orange and red) and reflecting the different combinations of yellow and red carotenoids in the diet. The most used colour fan is the Roche Yolk Colour Fan (RYCF) nowdays called DSM-YCF, which displays a scale from 1 (pale yellow) to 15 (reddish orange). A similar system has been developed for fish flesh and is called Roche Colour Card (RCC).

Reflectance colourimetry is a most accurate and repeatable method for determination of yolk colour used since 1990 (Nys, 2000). By this system colour is measured in a three dimensional colour space according to the eyesight of humans. Colour quantification is standardized by the CIE (Commission International de l'Eclairage) using specific lighting-viewing conditions. The main colour components are described by the variables L* (lightness, black to white reflectance, 1-100), a* (red = positive, green = negative) and b* (yellow, blue). Obviously, for DSM-YCF values between 6 and 12 a linear relation exists to a* and b* values. In fish the a* value (redness) generally exhibits the best correlation to increasing carotenoid levels.

1.5. Stability of carotenoids

Due to the long chain of conjugated double bounds, carotenoids are sensitive to oxygen, light and temperature (Su *et al*., 2002). Therefore carotenoid stability in the feed chain deserves critical attention. Carotenoids destined as feed additives are usually specifically treated to protect them during storage and later incorporation to the compound feed.

1.6. Colouring effects

1.6.1. Uses of carotenoids in poultry

In layer feeding it is common practice to provide a basic dietary yellow colour (by xanthophylls as lutein and zeaxanthin) by including yellow corn, plata corn, alfalfa meal or marigold in diets and to adjust the yolk colour desired by supplementing synthetic yellow (β-apo-8'-carotinal or ethyl ester of β-apo-8'-carotenoic acid) and/or red (e.g. canthaxanthin, citranaxanthin) pigments. However, the pigmenting efficiency of carotenoids depends on their absorption, transport in blood, excretion, rate of deposition in target tissues and conversion (Huyghebaert,

1993a; Seemann, 1997; Grashorn *et al*., 2000; Nys, 2000). The red xantophylls show a distinctly higher colouring efficiency than the yellow pigments. Therefore, they may only be used in diets in combination with yellow pigments to avoid off-colours (Marusich and Bauernfeind, 1981; Belyavin and Marangos, 1987; Huyghebaert, 1993b) and to improve homogeneity of yolk colour (Hernandez *et al*., 1999). Gurbuz *et al*. (2003) recommend to supplement red and yellow pigments in a ratio of 3:1 to result in an optimal yolk colour. The interactions between yellow and red pigments result in a decrease of colouring efficiency with increasing dietary levels of both pigments (Huyghebaert, 1993b; Hernandez *et al*., 1999). On the other hand, supplementing red pigments to diets with high levels of yellow pigments improves the efficiency of red pigments.

In general, deposition of carotenoids in the egg yolk may be depressed by the dietary content of non starch polysaccharides (NSP), oxidation, fatty acid profile, antibiotics, vitamin A (only for very high levels), yeast toxins, source and treatment of xanthophylls, trace elements (Ni), breed and health status of layers (El Boushy and Raterink, 1992; Fletcher, 1992; Hencken, 1992; Oscar *et al.,* 1995; Sidibé, 2001; Baker and Günther, 2004; Waldenstedt *et al.,* 2003). Also unhydrolyzed vegetable sucrose polyester (UVSP) from bakery byproducts reduces the deposition of yellow carotenoids (Damron *et al*., 2001).

Kuchta *et al*. (2001) found that NSP hydrolyzing enzymes, used as feed additives improve deposition of citranaxanthin and yolk colour. Also the supplementation of organic acids (propionic acid and formic acid 1:1) may increase yolk colour scores (Steinberg *et al.,* 2001).

Free carotenoids (e.g. from corn or alfalfa) are better absorbed than esterified ones (from fruits) (Hamilton *et al*., 1990; Hencken, 1992; Seemann, 1997; Breithaupt *et al*., 2003), although in some experiments no clear difference was observed (Lai *et al*., 1996).

1.6.1.1. Egg yolk colour

Yolk colour, which directly reflects the concentrations of pigments in the diet of laying hens is one of the most dominant quality aspects of chicken eggs sold as table eggs (Hernandez *et al*., 2000; Hernandez and Blanch, 2000a 2000b). In addition to colour as such, homogeneity of yolk colour is important and associated with good quality.

Perception of yolk colour varies across Europe. Northern countries, with the exception of Germany, prefer weakly coloured yolks (range between 7-11 DSM-YCF score), whereas countries of the South and South West of Europe prefer more intensively coloured yolks (range between 11-14 DSM-YCF score). But, preferences for yolk colour of consumers vary also between regions within countries (Hernandez *et al*., 2000).

Yolk colouration is caused by deposition of dietary yellow and red pigments in the yolk. Depending on the intended use of eggs, pigments are added at various extents and ratios of yellow and red pigments to diets. Due to the contribution of the different carotenoids brought by feed components and feed additives and due to individual variations in the physiological transfer process in the hen, a given DSM-YCF score may correspond to a wide range of concentrations of individual carotenoids and their combinations. In order to reach the desired yolk colour, the supplementation of yellow and red pigments to the diet has to consider the original content of natural xanthophylls. Heat treatment of eggs may result in a decrease of the visual score, requiring an about 20% higher supplementation level to achieve a desired DSM-YCF value of 13 (Huyghebaert, 1993a; Grashorn *et al*., 2001), especially in case of low supplementation levels (Blanch *et al*., 2002).

1.6.1.2. Skin colour

Skin colour results from carotenoids deposited in the fat layer of the skin. Feeding diets with supplemental yellow and red pigments will result in a marked colouring of the skin, both of the body and the shanks. In some countries as in Austria, France and Spain consumers are

interested in poultry with coloured skin. In these countries pigmentation of skin is achieved by the use of feedingstuffs rich in natural xanthophylls. The additional use of red carotenoids is not very common, however canthaxanthin, capsanthin and β-cryptoxanthin are approved colouring feed additives. At present no reference scale to compare skin colouration hues is available.

1.6.2. Use of carotenoids in fish diets and flesh color

The characteristic red/pink colour of salmon flesh is perceived by the consumer as one of the most important quality criteria (Baker and Günther, 2004). Salmonids seem not to deposit other red carotenoids than astaxanthin and canthaxanthin in the flesh to significant amount. Corn gluten, containing lutein, reduces the deposition of astaxanthin or canthaxanthin. It is well documented that astaxanthin is more efficiently deposited in the flesh of rainbow trout compared to canthaxanthin (Torrissen, 1986; Storebakken and Choubert, 1991). A similar difference seems not to be evident in Atlantic salmon (Olsen, personal communication).

Astaxanthin and/or canthaxanthin are added to aqua feeds for salmonids (*Salmo, Oncorhynchus or Salvelinus* spp) to obtain an astaxanthin level and flesh colour comparable to those of their wild counterparts. The astaxanthin is normally supplemented to diets throughout the whole grow-out phase (100 g to harvest) and at levels between 30 to 100 mg kg^{-1} complete feed in order to obtain the desired pigment level and depending on species, fish size, dietary lipid level, farming environment and initial astaxanthin level. The muscle astaxanthin retention may reach levels of 6-7 % for Atlantic salmon and 20-25 % for rainbow trout.

Red pigments are also approved at the EU level for skin pigmentation of ornamental fish.

Following the Commission request to conduct a safety assessment and prioritise the carotenoids, which may be used as alternatives of canthaxanthin, the FEEDAP Panel focused in a first step in the red-colouring carotenoids Asthaxanthin, Capsanthin, Citranaxanthin, and Cryptoxanthin. The following is a first of a series of documents dealing with the assessment of carotenoids that has been prepared by the FEEDAP Panel and deals with Astaxanthin.

2. Astaxanthin (E 161j)

2.1. Specifications

Although asthaxanthin has an E-number, it is not allowed to use in the EU as a food additive, therefore it has no specification on the food additive-legislation.

Because of a lack of formal specification the following assessment for approval as a feed additive refers to asthaxanthin with a molecular formula $C_{40}H_{52}O_4$. The FEEDAP Panel is aware of two different origins of asthaxanthin products available in the EU market, one extracted from *Pfaffia rhodozyma* and the other a synthetic product.

2.2. General characteristics

Astaxanthin [(3S,3'S)-3,3'-dihydroxy-β,β-carotene-4,4'-dione) has many commonly used synonyms like astahxanthine, astahaxanthin all-trans, 3,3'dihydroxy-β-carotene-4,4'dione. The molecular formula of astaxanthin is $C_{40}H_{52}O_4$ and the molar mass is 596.847 g mol^{-1}, the molecular structure is given in Figure 2. The Chemical Abstract Services Registry Number (CAS) of astaxanthin is 472-61-7 and the corresponding number in the EC database is 207-451-4.

Melting point of astaxanthin is 182.5°C and log P (octanol/water partition) 13.27. Astaxanthin has a characteristic absorption maximum wavelenght at 503 nm in carbon disulphide, 472 nm in methanol, 466-467 nm in hexane and 485 nm in chloroform. Astaxanthin is hydrophobic, forms aggregates or adheres unspecifically to structural surfaces.

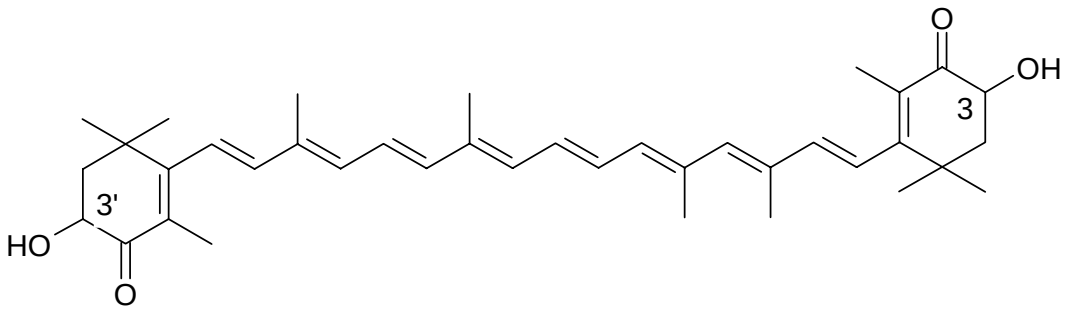

Figure 2. Molecular structure of astaxanthin.
The assymetric carbon atoms are shown using numbers 3 and 3', respectively.

Astaxanthin has two chiral atoms which are at the 3 and 3' positions of the rings. The two chiral centers can exist either in R or S form, and thus there is a total of three isomers; (3S,3'S), (3R,3'S) or (3R,3'S). The (3S,3'S) and (3R,3'R) are enantiomers of opposite optical activity. The (3R,3'S) form is an optically inactive meso form. Astaxanthin has several double bonds in the polyenic part of the molecule, each of which can potentially exist in the Z or E form. The thermodynamically most stable form of the molecule is *all-E* (all-trans) astaxanthin. In nature, Z isomers have been observed at positions 9, 13, and 15. Thus several geometric isomers are possible (Bernhard, 1990): all-E, (9Z), (13Z), (15Z), (9Z,13Z), (9Z,15Z), (13Z,15Z), and (9Z,13Z,15Z).

2.2.1. Analytical methods

Analysis of astaxanthin (free and esters) by HPLC/MS negative ionization technique was described recently (Breithaupt, 2003). The configurational isomers (all-E, 9Z and 13Z geometrical and (3R3'R), (3R,3'S) and (3S,3'S) optical isomers) of astaxanthin could also be determined in the liver, gut tissues, kidney, skin and blood plasma of rainbow trout (Osterlie *et al*., 1999).

2.3. Astaxanthin in fish

During the early development of salmonid aquaculture, by-products from the shrimp or crayfish industry and different astaxanthin extracts were used as pigment sources. The use of crustaceans or by-products of these was hampered by low astaxanthin level, low stability of the astaxanthin and limited availability. Marketing of chemically synthetised canthaxanthin was started in 1964 and became soon the dominant pigment source in dry pelleted diets for salmon and trout. Synthetic racemic astaxanthin became available in the market in 1984, and over the next 5 years nearly totally replaced canthaxanthin as a pigment source in the major salmon producing country, Norway. Due to lower price, canthaxanthin is still used to some extent in UK and Ireland (as in Chile, USA and Canada), either as the only pigment source or in combination with astaxanthin. Combination of astaxanthin and canthaxanthin in ratio of 75% astaxanthin and 25% canthaxanthin is reported to give an improved carotenoid deposition and retention compared to the deposition and retention upon the use of astaxanthin or canthaxanthin alone (Torrissen, 1989).

2.3.1. Sources and use

The European Feed Manufacturers Federation (FEFAC) made available some non quantitative market data on the use of astaxanthin covering responses from nine EU Member States and Norway (FEFAC, 2004). According to these data astaxanthin is used by the majority of feed manufacturers in four Member States and Norway, by about half the feed manufacturers in

two Member States, by a small proportion in two Member States and not in one Member State. In five Member States, astaxanthin is used together with cantaxanthin (mainly depending on price).

SCAN (EC, 2002) suggested that the lowering of the levels of canthaxanthin would lead to an increasing use of astaxanthin for salmon pigmentation. This assumption might be valid only in EU countries where canthaxanthin was predominantly used but not to the same extent in Norway and the Faroe Islands. The lowering of the permitted inclusion level of canthaxanthin in Salmonid diets in Europe has not significantly changed the pigmentation strategies or use of astaxanthin.

Astaxanthin occurs in several different forms which can be classified according to stereoisomers, geometric isomers and free or esterified forms. All of these forms are found in various natural sources. For example, the predominant stereoisomer of astaxanthin found in the Antarctic krill *Euphausia superba* is (3R,3'R) (Bernhard, 1990) and the majority of this is esterified (Foss *et al*., 1987). In wild Atlantic salmon, the predominant stereoisomer is (3S,3'S) which occurs as the free carotenoid (Bernhard, 1990). The relative distribution of esters and optical isomers in some crustaceans is shown in Table 2.

Table 2. **Free and esterified astaxanthin and ratio of optical isomers (RR':RS':SS') in some crustaceans, the yeast *Phaffia rhodozyma and Haematococus pluvialis.***
Foss *et al*., 1987; Grung *et al*., 1992; Müller *et al*., 1980; Bowen *et al*., 2002

Astaxanthin	Free	Diester	Monoester	Ratio optical isomers RR' : RS' : SS'
Euphausia superba (Antarctic krill)	5	64	31	9 : 21 : 70
Thysanoessa inermis (Antarctic krill)	4	61	35	55 : 7 : 38
*Calanus finmarchicus (*Marine copepode*)*	11	46	43	83 : 3 : 14
Acanthephyra purpurea (Deap sea shrimp*)*	20	43	37	20 : 44 : 15
Cancer pagurus shell (Edible crab shell)	58	22	13	20 : 24 : 56
Phaffia rhodozyma (Red yeast)	100			98 : + : +
Haematococcus pluvialis (Alga)	5	59	22	4 : 8 : 88

The yeast *Phaffia rhodozyma* (Johnson *et al*., 1977; Whyte and Sherry, 2001) and the algae *Heamatococcus pluvialis* (Sommer *et al*., 1991) containing high levels of astaxanthin (≥ 2000 mg kg^{-1}) from biotechnological production is also available in some markets. In Europe, astaxanthin from a *Phaffia rhodozyma* product is approved for use in salmon diets. Astaxanthin from *Phaffia rhodozyma* is in the free form 3R, 3'R while astaxanthin from *Haematococcus pluvialis* is present mainly as di- or mono-fatty acid esters and the stereochemical form 3R,3'R dominates (Bernhard, 1990; Renstrom and Liaaen-Jensen, 1981). Synthetic astaxanthin contains free asthaxanthin in the ratio of 1:2:1 of the three stereoisomers: (3S,3'S), (3R,3'S) and (3R,3'R). The all-E isomer is the major geometric isomer in synthetic astaxanthin (Turujman *et al*., 1997).

2.3.2. Astaxanthin levels in wild and farmed salmonids

Large variations in the muscle astaxanthin level in the wild *Oncorhynchus* species was reported, ranging from 38 mg kg^{-1} in sockeye salmon down to 3 in chum. Today commercially farmed Atlantic salmon is targeted to 6 to 8 mg astaxanthin kg^{-1} flesh and it does not exceed 10 mg

astaxanthin kg^{-1}. Portion size trout has concentrations of asthaxanthin of 4 mg astaxanthin kg^{-1} and large trout 12 mg astaxanthin kg^{-1} in the European markets. The large trouts produced for the Japanese market contains up to 25 mg asthaxanthin kg^{-1}. Based on the available literature (Table 3) it can however be concluded that the astaxanthin level of farmed and wild salmonid species presents the same order of magnitude.

Table 3. **Astaxanthin content of different salmonids**

Genus		Farmed	Wild	Astaxanthin mg kg^{-1} flesh	References
Oncorhynchus nerka	Sockeye salmon		X	26 - 38	1, 3, 9
Oncorhynchus kisutch	Coho salmon		X	10 – 21	1, 3, 9
Oncorhynchus keta	Chum salmon		X	3 – 5	1, 3
Oncorhynchus tschawyzscha	Chinook salmon		X	5.4	1
Oncorhynchus gorbuscha	Pink salmon		X	4 – 7	1, 9
Oncorhynchus Masou	Masu salmon		X	4.6	9
Salmo salar	Atlantic salmon		X	3 – 10	3, 4
Salmo salar	Atlantic salmon	X		1 – 9	4, 6
Oncorhynchus mykiss[a]	Rainbow trout	X		0 - 25	5
Salvelinus alpinus	Arctic charr	X		1-8	7, 8, 11
Salvelinus alpinus	Arctic charr		X	8.6	10

1) Kanemitsu and Aoe, 1958 2) Schiedt *et al.,*1986 3) Schiedt *et al.,*1981
4) Skrede and Storebakken, 1986 5) Rønsholdt and McLean, 2001 6) Torrissen *et al.,* 1995
7) Synowiecki and Shahidi, 1997 8) Aas *et al.*, 1997 9) Kitahara, 1984
10) Scalia *et al.*, 1989 11) Olsen and Mortensen, 1997

[a] including unpigmented trout

2.3.3. Effects of astaxanthin in fish

There is a general consensus in the literature that astaxanthin is beneficial for fish and shellfish. Reported functions of astaxanthin in fish range from a general enhancement of performance to specific functions in reproduction and metabolism. Among the reported actions of astaxanthin are improved reproduction and brood quality, reduced embryonic mortality, effects on photoresponse, behavior and respiration, improved health and immunstatus and functions as antioxidants and provitamin A (reviewed by Torrissen, 1989). Not all these actions are equally well documented, but skin colouration is important in communication (Fujii, 1969; Moyle and Cech, 1982).

The interactions of astaxanthin and vitamin A on growth and survival of Atlantic salmon, *Salmo salar* L., during the first-feeding period were examined using semi-purified diets by Christiansen *et al*., (1994). Astaxanthin was found to strongly influence growth, survival and vitamin A concentration in the fish. Poor growth and low survival rates were observed in groups fed diets without astaxanthin, including the group fed a diet with sufficient vitamin A. The results indicate both a provitamin A function of astaxanthin and its presumed essentiality as a micronutrient. In addition, astaxanthin was found to be essential to alevins during the first-feeding period. Astaxanthin deficiency was reported to strongly correlate to the Baltic M74 syndrome in salmon and trout and a threshold concentration level, 0.11 µg astaxanthin per egg, below which healthy egg batches are not found is implicated. Furthermore, all Baltic salmon family groups with an astaxanthin concentration >0.22 µg per egg were healthy (Pettersson and Lignell, 1999). Torrissen and Christiansen (1995) concluded that is recommended to supplement starter diets to salmonids as well as marine fish larvae with astaxanthin (Torrissen and Christiansen, 1995)

2.3.4. Deposition

The maximum astaxanthin level in the flesh of farmed salmonids seems species dependent. Rainbow trout, coho and chinook salmon have a significantly higher maximum astaxanthin level compared to Atlantic salmon and arctic charr (Table 3). This difference may be due to varying i) digestion and absorption, ii) muscle binding capacity for astaxanthin and iii) astaxanthin metabolising activity. It was concluded by March *et al.,* (1990) that poor flesh pigmentation results from rapid metabolism of absorbed pigment to colourless derivatives rather than failure of fish to absorb pigment.

The response to increased dietary dose is linear for low dietary doses, levels off for higher and finally reaches a plateau where no further increased flesh pigmentation is obtained by increases in dietary astaxanthin level (see Figure 3; Torrissen *et al.,* 1995; Olsen and Mortensen, 1997). It is shown that increased dietary lipid level improves deposition in the flesh (Torrissen, 1985; Nickell and Bromage, 1998). Torrissen *et al.,* (1990) concluded that improved digestive absorption by increased dietary lipid level could explain the observed higher flesh deposition. This increased absorption with increased dietary lipid level indicates that solubilisation of the carotenoids (astaxanthin or canthaxanthin) in the digestive lumen is a limiting factor. The limited ability to absorb carotenoids due to solubilization in the digestive lumen explains the levelling off at dietary doses above 40-60 mg kg^{-1} in Atlantic salmon.

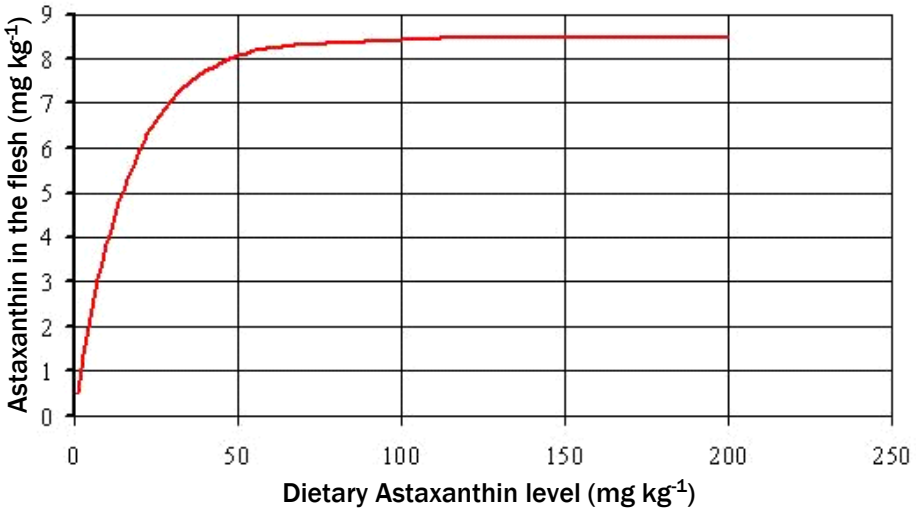

Figure 3. Astaxanthin deposition in the flesh of Atlantic salmon in relation to dietary astaxanthin level

Limited data is available on dose-response relationship for rainbow trout, and no data exist for a complete production cycle (Choubert and Storebakken, 1989; Bjerkeng *et al*., 1990). Figure 4 shows results from two papers. Choubert and Storebakken (1989) fed 135 g rainbow trout 6 dietary levels of astaxanthin and canthaxanthin from 12.5 mg kg^{-1} to 200 mg kg^{-1} for 6 weeks (100 g increase in fish weight) and Bjerkeng *et al.,* (1990) fed three levels of astaxanthin supplementation from 25 to 100 mg kg^{-1} to 460 g rainbow trout for 4 months (450 g weight increase). Both experiments show a decrease deposition efficiency by increased dietary level of astaxanthin and canthaxanthin. Experience from the Norwegian production of large, highly pigmented trout for the Japanese market shows a similar plateau for trout but this plateau is at a significant higher level, 20-25 mg kg^{-1}. The practical experience from the industry is that trout levels off at a similar level as for Atlantic salmon (40-60 mg kg^{-1} feed), but the results from Choubert and Storebakken, (1989) and Bjerkeng *et al*., (1990) indicate a higher dietary dose before levelling off.

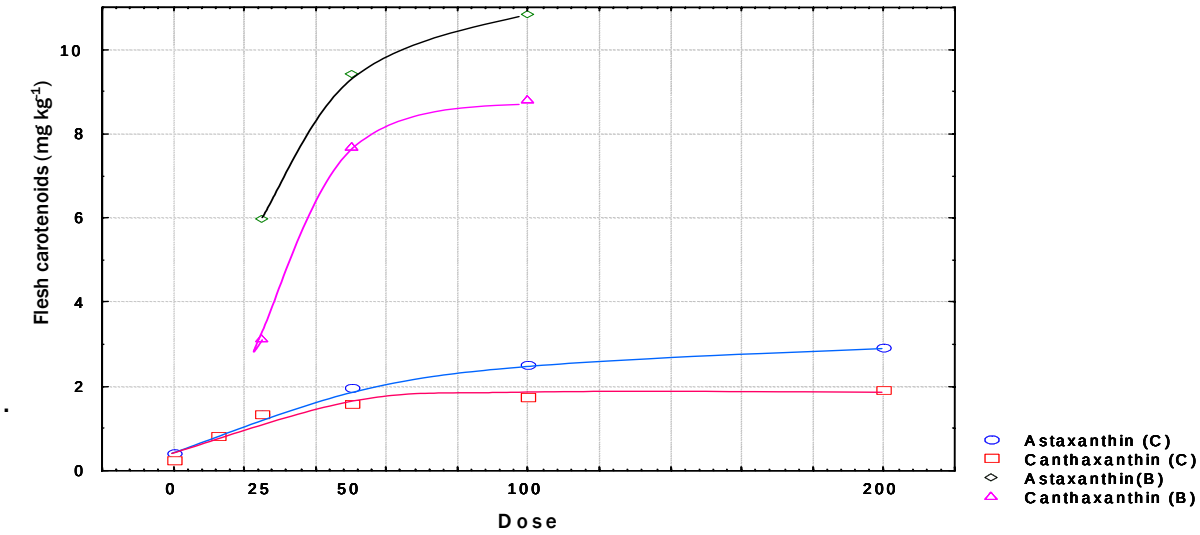

Figure 4. **Dose response curves for astaxanthin and canthaxanthin in rainbow trout** (Choubert and Storebakken, 1989; Bjerkeng *et al.*, 1990)

2.3.5. Astaxanthin tolerance in fish

Up to 200 mg astaxanthin kg^{-1} feed no adverse effects have been reported for Atlantic salmon throughout a whole production sycle (Torrissen *et al.,* 1995) and rainbow trout for 6 weeks (Choubert and Storebakken, 1989)

2.4. Astaxanthin in Poultry

Although, astaxanthin is not currently approved as a colouring feed additive for poultry, some experiments have been conducted to test the pigmenting efficiency of astaxanthin, mainly derived from crustaceans, fish, algae and yeast.

2.4.1. Sources

The yeast *Phaffia rhodozyma* is proven to be a suitable source of astaxanthin for poultry (Akiba *et al*., 2000a). Carranco *et al*. (2003) reported some colouring efficiency of shrimp meal, which contains considerable amounts of astaxanthin. Experiments with layers revealed that the pigmenting efficiency of astaxanthin is about 5 times less than of canthaxanthin (Marusich and Bauernfeind, 1981) and 1.5 to 2 times better than for capsanthin/capsorubin (Akiba *et al*., 2000b).

2.4.2. Effects in Poultry

Waldenstedt *et al.* (2003) reported a total digestive tract apparent digestibility of astaxanthin in broilers of 94%, whereas, Akiba *et al*. (2000a) found a lower digestibility of Astaxanthin from *Phaffia* yeast.

Some experiments with the use of astaxanthin as a red pigment revealed significant increases in yolk colour as measured by the DSM-YCF (Hammershoj, 1995; Gouvea *et al*., 1996; Elwinger *et al*., 1997; Akiba *et al*., 2000a, 2000b, 2000c). The relationship between the dietary astaxanthin content and yolk colour was linear up to the highest tested supplementation level of 16 mg kg^{-1} feed (Akiba *et al*., 2000a).

2.4.3. Deposition of astaxanthin in the egg and tissues

Deposition rates in literature are difficult to summarize and to compare as many different factors have been included in the experiments and not all information is provided to calculate deposition rates from the measured contents in egg yolks with sufficient accuracy. An egg yolk with a colour score of 13 DSM-YCF may contain about 55 µg astaxanthin when supplementing the diet with 16 mg astaxanthin kg^{-1} by the use of *Phaffia rhodozyma* (Akiba *et al.,* 2000a). Meal of shrimp heads as a source of astaxanthin included at levels of 25 % results in roughly 4 mg astaxanthin in the yolk (Carranco *et al.*, 2003). Calculated deposition rates for astaxanthin vary highly between literature sources (Table 4). The reason may be that the sources of astaxanthin (*Phaffia*, shrimp meal and synthetic) differ markedly in purity and bio-availability.

Waldenstedt et al. (2003) observed a linear increase of astaxanthin concentrations in broilers tissues when supplementing 0, 7, 36 and 179 mg astaxanthin kg^{-1} diet, respectively. Tissue concentrations of astaxanthin were 400 mg kg^{1} dry matter of breast meat and 91 mg kg^{-1} dry matter of subcutaneous fat, when diets supplemented with 36 mg kg^{-1} diet were fed.

Table 4. **Deposition rates (%) of astaxanthin to egg yolks as derived from literature (based on own calculations)**

Deposition rate %	Reference
<10	Marusich and Bauernfeind, 1981
8	El Boushy and Raterink, 1992
3	Akiba *et al.,* 2000a
7-11	Akiba *et al.*, 2000b
25-37	Carranco *et al.,* 2003

Deposition of canthaxanthin to tissues (skin and subcutaneous fat) was calculated to be less than 10%. The deposition of astaxanthin to broilers tissues is not available from literature. Considering a linear relation between feed and tissue concentration the deposition for astaxanthin to fat tissues is estimated to be less than 2%.

2.5. Metabolism of Asthaxanthin

2.5.1. Fish

The reported quantitative values for apparent digestibility coefficients (ADC) of astaxanthin show huge variations. Due to methodological insufficiencies (difficulty to collect quantitatively the faeces but also the unstability of astaxanthin in faeces which results in an overestimation of the true digestibility coefficient), a high variability is observed and therefore results must be interpreted cautiously. Astaxanthin apparent digestibility, defined as the difference between ingested astaxanthin and the amount recovered in the faeces, is given in Table 5 for various salmonids species.

Table 5. **Apparent digestibility (%) of astaxanthin in different salmonid species**

Astaxanthin source	Rainbow trout	Atlantic salmon	Sea trout	Reference
Shrimp meal	79	–	–	Choubert, 1977
Capelin red oil	19-74	–	–	Choubert, 1977
Mixture (50:50) of synthetic astaxanthin + canthaxantin	91-97	–	74-96	Foss *et al*., 1987
Synthetic	51-70	–	–	No and Storebakken, 1991
Synthetic	–	45-74	–	Storebakken *et al.,* 1987
Synthetic	–	46-59	–	Bjerkeng and Berge, 2000

The significantly higher faecal carotenoid concentration (ca. 50%) of trout fed an all-E/Z astaxanthin diet when compared to those receiving an all-E diet was due to a higher concentration of astaxanthin Z isomers. Together with a similar faecal all-E- astaxanthin concentration for both treatments, it suggests that intestinal absorption of 9Z– and 13Z-astaxanthin might be lower than for the all-E isomer (Osterlie *et al*., 1999). Similarly, other authors have found that the apparent digestibility coefficient of all-E- astaxanthin in salmon was higher ($P < 0.05$) than that of the 9Z isomer but not different from the 13Z isomer (Bjerkeng and Berge, 2000). The literature also shows lower apparent digestibility for astaxanthin esters compared to the free astaxanthin (Torrissen and Brækkan, 1979; Storebakken *et al*., 1987; Whyte *et al*., 1998; Bowen *et al*., 2002). It appears that the hydrolysis of the ester linkage is a limiting step of the bioavailability of astaxanthin for trout and salmon (Torrissen and Braekkan, 1979; Schiedt and Leuenberger, 1981; Storebakken *et al*., 1987). However, the hydrolysis step is more efficient for (3R, 3'R)- than for (3S, 3'S)-astaxanthin esters. Foss *et al*. (1987) reported that all three astaxanthin isomers, e.g. (3S,3'S), (3R,3'S) and (3R,3'R) as well as their 1:2:1 mixture were utilized at the same level and that no epimerization took place at the chiral centers of C-3 and C-3' in astaxanthin.

It has been shown that unchanged astaxanthin and metabolites are excreted through the bile of the Atlantic salmon to a very significant extent. The nature of these metabolites has not been established but no astaxanthin conjugate have been found (Xu and Ding, 2004; Torrissen and Ingebrigtsen, 1992). Similar results are also reported for ^{14}C-labeled canthaxanthin fed to rainbow trout (Hardy *et al*, 1990)

Astaxanthin is metabolised in fish through oxidative and reductive pathways. However, no oxidation occurs in «sea bream type» of fish like salmonids. A double step reduction at the 4 and 4'-oxo groups initiates a metabolic process leading to idoxanthin then to adonixanthin and zeaxanthin. No cleavage of the polyene chain is observed. The configuration of astaxanthin does not influence the reduction of the 4'–oxo group, but the enzymatic reduction is stereospecific leading to the 4'R-hydroxy group irrespective of the configuration at C(3'). Therefore, the reduction of the 3 astaxanthin stereoisomers leads to 4 idoxanthin isomers instead of 8 theoretically, i.e. the Z forms (3S, 3'S, 4'R) and (3R, 3'S, 4'R) and the E forms (3S, 3'R, 4'R) and (3R, 3'R, 4'R), which proportions are identical to those of the original astaxanthin. Racemic astaxanthin (1 :2 :1) gives rise to (3S, 3'S)-zeaxanthin and to a lesser extent (3R, 3'S)-zeaxanthin, while the (3R, 3'R) isomer appears at very low level (Schiedt *et al*., 1988). In the trout, the (3S, 3'S)-astaxanthin leads to (3R, 3'R)-zeaxanthin. These data indicate that the epimerization from 3S- to 3R- and *vice versa* occurs *in vivo* (Katsuyama *et al*., 1987). No significant *in vivo* E/Z isomerization has been observed (Schiedt *et al*., 1981; 1989; Osterlie *et al*., 1999). Due to the absence of carotenoid oxidative pathways in salmonids the conversion of zeaxanthin to astaxanthin does not occur.

The composition of astaxanthin deposited in fish muscle, skin and ovaries reflects the feeding sources of the animal. Wild Pacific salmon flesh contains predominantly the (3S, 3'S) and (3R, 3'R) steroisomers (78-85 % and 12-17 % respectively) depending on the dietary prey organism, while only a small percentage of the (3R, 3'S) isomer is present. The percentage of all-E and Z-forms (structural) of each isomer varies slightly with fish species, feeding regimes and environment, the Z form representing less than 15% of the E one.

Following the administration of astaxanthin of either yeast or synthetic origin at 50 mg kg^{-1} diet for rainbow trout and Chinook salmon for 84 and 150 consecutive days respectively, 95% and 96% of the pigments deposited in the flesh have been found as unchanged astaxanthin, with very minor quantities of idoxanthin and zeaxanthin (FEEDAP, 2004). In the Artic charr (*Salvelinus alpinus*) fed racemic (1:2:1) synthetic astaxanthin under similar experimental conditions, free (non esterified) astaxanthin comprised 64-79% of flesh carotenoids, idoxanthin accounting for 20-35%. The corresponding figures for the skin were 85% and 10%, but astaxanthin and idoxanthin consisted mainly of esters (diesters: 82-87%, monoesters: 7-13%). Minor amounts of tunaxanthin, lutein and zeaxanthin were present in the ovaries of sexually maturing or immature female charr, idoxanthin was the major carotenoid (56%) followed by

crustaxanthin (20%) and astaxanthin (<5%) (Bjerkeng *et al*., 2000). Genus-specific variations in the ratio of astaxanthin to reductive metabolites in muscle and ovary have been reported. Physiological variations have been observed also, namely related to stressful conditions (review from Schiedt, 1998).

The incidence of the distribution of the stereo and geometrical isomers of astaxanthin of either *Phaffia Rhodozyma* yeast (R,R'; E/Z ratio 60:40) or synthetic origin (R/S racemic 1 :2 :1; E/Z ratio 75:25) on the composition of the deposits in the flesh of the Chinook salmon is indicated on Table 6.

Table 6. **Percentage of astaxanthin isomers in the flesh of Chinook salmon fed different astaxanthin sources supplying 50 mg kg^{-1} diet**

Isomer	*Phaffia rhodozyma*	Synthetic astaxanthin	Not supplemented (natural control population)
All-E; R, R'	42	23	23
All-E; R, S' and R', S	30	42	40
All-E; S, S'	19	29	29
Z; R, R'	4	1	2
Z; S, S'	2	2	3

Both the Z-(3R, 3'R) and Z-(3S, 3'S) geometrical astaxanthin isomers do not accumulate in the flesh. No major change in the distribution of the all-E–(R, S and S, S) -astaxanthin isomers is observed when comparing the composition of the flesh of fish receiving a non supplemented diet to that of those fed the two different pigment sources. Only increase of the all-E-(3R, 3'R) isomer content is observed with the yeast that corresponds to the specific composition of the derived astaxanthin.

2.5.2. Laboratory Animals

Very limited information is available concerning the metabolic fate of astaxanthin in the rat. It has been shown recently that astaxanthin biotransformation by rat hepatocyte primary cultures consists in the asymetrical cleavage of the polyene chain of the molecule at the C9 position (Wolz et al., 1999). One main metabolite, (rac)-3-hydroxy-4-oxo-β-ionone, and its reduced form (rac) -3-hydroxy-4-oxo-7,8-dihydro-β-ionone, were identified as glucuroconjugates. These preliminary results indicate a metabolic pathway which is very different from that described in fish.

2.5.3. Humans

The in vitro metabolic study of astaxanthin in primary human hepatocytes allowed the identification of 3-hydroxy-4-oxo-beta-ionol and 3-hydroxy-4-oxo-beta-ionone as the main free metabolites. The same compounds and their reduction products 3-hydroxy-4-oxo-7,8-dihydro-beta-ionol and 3-hydroxy-4-oxo-7,8-dihydro-beta-ionone were also present as glucuronides (Kistler *et al*., 2002). The same authors identified these four metabolites in the plasma taken from two human volunteers 24 hours after oral administration of 100 mg astaxanthin (8% gelatine formulated beadlets). They showed that the C9,C9' cleavage of the astaxanthin molecule and reduction of the polyenic 7,8-double bond are common to the human and the rat whereas the reduction of β-ionone to β-ionol is specific to the human. Moreover, in human hepatocytes, in contrast to what was observed in the rat, astaxanthin appeared as an inducer of CYP3A4 and CYP2B6 but not of CYP1As (Kistler *et al*., 2002).

2.6. Human Exposure Assessment

Astaxanthin is present in seafood but also in algae and krill at varying concentrations (Bell *et al.,* 1998; Guerin *et al.,* 2003). The astaxanthin intake varies per country but the major foods are salmon and trout. Some limited exposure can be attributed to astaxanthin containing supplements produced from krill or algae. Astaxanthin intake from supplements varies from 1.5 to 6 mg per day.

The European consumers' ingestion of astaxanthin will originate from both wild and farmed salmonids and shrimps. The shrimp's contribution to the astaxanthin ingestion will not be discussed as the majority of the astaxanthin in shrimps is located to the exoskeleton and lesser in the edible muscle and since there is no significant aquaculture production of shrimps in Europe.

The data available on intake of trout and salmon are insufficient for this reason the FEEDAP Panel used three different models to estimate human intake, based on i) salmon and trout production in Europe, ii) WHO GEMS figures and iii) the SCOOP data.

In 2003, the consumption of Atlantic salmon in Europe (EU-25) was 579 600 tonnes (Kontali analyse 2004), corresponding to 0.9 kg per head and year.

Considering the figure above and the maximum content of astaxanthin in salmon flesh an annual astaxanthin intake of 9 mg could be calculated from salmon consumption. Production of pigmented trout in Europe (see Annex 1A) is about one third of Atlantic salmon, and using the same figures as for Atlantic salmon not more than 0.3 kg trout per head per year could be consumed. This corresponds to a maximum intake of 7.5 mg astaxanthin. From both sources, salmon and trout, a mean daily astaxanthin intake of 0.045 mg would result.

The following worst case calculations are based on the alternatives of an exclusive salmon (10 mg astaxanthin kg^{-1}) or trout (25 mg astaxanthin kg^{-1}) intake, resulting in a range of values. WHO GEMS figures for Europe[1] indicate a mean daily fish consumption of 34 g per person. The figure includes seafood as well and results in a total annual fish consumption of 12.41 kg, equivalent to about 120 - 310 mg astaxanthin per year (0.34–0.85 mg day^{-1}). This is a considerable overestimation of the potential astaxanthin intake assuming that all fish is salmon or trout all fed with astaxanthin at the highest level approved or from wild catch.

Another estimation is based on the indicative country based consumers only total intake of fish and seafood products primarily derived from EU reports, respectively on heavy metals (SCOOP Task 3.2.11) and Organotin compounds (SCOOP Task 3.2.13) (EC 2003, 2004). The Table is given in the Annex (Table 2A). The data enclose 12 EU Member States and Norway, the averages vary between 13 (fish only) and 80 g day^{-1} adult $person^{-1}$ (fish and seafood). The high intake (in general the 97.5th percentile) amounts to 103 (fish) and 165 g day^{-1} adult $person^{-1}$ (fish and seafood). A worst case scenario (all fish is salmon or trout all fed with astaxanthin at the highest level approved) based on these data for fish and seafood intake results in an asthaxanthin ingestion of 0.8-2.0 mg asthaxanthin for average fish consumption and of 1.6 - 4.1 mg astaxanhtin day^{-1} for high consumers. It should be noted that the same calculation based on asthaxanhin concentration found in Sockeye salmon would lead to asthaxanthin intakes of 3.0 and 6.3 mg day^{-1} adult $person^{-1}$ for the average and the high intake, respectively.

Based on this worst case scenario the higher daily asthaxanthin intake from farmed salmonids, would not exceed 4.1 mg astaxanthin day^{-1}.

2.7. Safety studies

Astaxanthin is one of the carotenoids that has been mentioned in relation to cancer prevention (Nishino, 1998; Mori *et al.,* 1997; Tanaka *et al.*, 1994; Tanaka *et al.*, 1995a; Tanaka *et al.*, 1995b). These reports suggesting possible cancer prevention by astaxanthin can not be used to

[1] http://www.who.int/foodsafety/chem/gems/en/

assess the safety of astaxanthin for humans, since these studies were not designed to investigate possible adverse effects of astaxanthin. Due to the scarceness of published studies on astaxanthin safety, information available in the internet is also referred as supporting documents.

2.7.1. Acute Toxicity

A safety summary on astaxanthin published on the internet stated that the acute toxicity of astaxanthin[2] in rats was determined following administration of 10 consecutive daily oral doses up to 2000 mg astaxanthin kg^{-1} bw. There was no mortality and no symptoms of toxicity were reported.

In a study reported as a summary on the internet the acute toxicity of *Haematococcus pluvialis* was tested.[3] The test article was given orally as a suspension in Intralipid^R solution 20% at the maximal administrable dose of 12 g kg^{-1} bw. The experimental group consisted of 5 male and 5 female CD(SD) BR rats. No clinical signs or behavioural alterations were noted during the 14 day post-treatment observation period. No rats died during the study. Body weight gain was unaffected and at autopsy at the end of the study there were no gross changes. It was concluded that the LD_{50} of *Haematococcus pluvialis* is higher than 12 g kg^{-1} bw. Assuming a minimum astaxanthin content of the red coloured powder of 1.4%[4] it could be estimated that the LD_{50} of astaxanthin in this rat study is higher than 168 mg kg^{-1} bw

Another summary[5] presents a technical report on *Haematococcus pluvialis* and astaxanthin safety for human consumption. The report refers to an acute toxicity study that reported that no lethality was seen in an oral acute rat study for *Haematococcus pluvialis* algae at doses up to 5000 mg kg^{-1} bw with a 13 day post treatment period. In this study three separate groups of 10 rats (5 males and 5 females per group) were fed 5000 mg algal meal kg^{-1} bw suspended in a 0.5% methylcellulose solution. The study found no remarkable differences in body weights or visible abnormalities. The post-mortem examination after sacrificing the animals at the end of the study revealed no abnormalities. Assuming [6] an astaxanthin content of the algal extract of 1.4% it could be estimated that the LD_{50} of astaxanthin in this rat study is higher than 70 mg kg^{-1} bw.

The same internet source[7] also mentions another acute toxicity trial with male and female mice. In this study *Haematococcus pluvialis* algal meal was suspended in distilled water for gavage to give a 30% solution (w/v). The solution was given in a single dose, at dosages ranging from 10417 to 18000 mg algal meal kg^{-1}. No mortalities occurred and no abnormalities were observed in the post-mortem examination. Assuming, as previously, an astaxanthin content of the algal extract of 1.4%[8] it could be estimated that the LD_{50} of astaxanthin in this mice study is higher than 252 mg kg^{-1} bw

Some studies on effects of astaxanthin on specific parameters in exposed experimental animals have been reported. Although these studies have not been designed to assess the safety of astaxanthin, they have not revealed adverse effects (e.g. Ohgami *et al*., 2003).

2.7.2. Genotoxicity

An Ames test on synthetic asthaxanthin published on the internet stated that: "Astaxanthin concentrations[9] ranging from 0.03 to 5.0 mg per plate did not induce mutations in *Salmonella*

[2] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-18-Appendix-3-vol170.pdf
[3] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-18-Appendix-3-vol170.pdf
[4] http://www.cfsan.fda.gov/~lrd/cfr73185.html
[5] htpp:/www.astafactor.com/techreports/tr3005-001.htm
[6] http://www.cfsan.fda.gov/~lrd /cfr73185.html
[7] htpp://www.astafactor.com/techreports/tr3005-001.htm
[8] http://www.cfsan.fda.gov/~lrd /cfr73185.html
[9] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-18-Appendix-3-vol170.pdf

typhimurium tester strains TA1535, TA1537, TA1536, TA98, and TA100 with or without activation by rat liver homogenate fraction".

The results of the micronucleus test are described in the internet: "Astaxanthin in the form of the 10% gelatin beadlet was administered orally to mice at 30 hours and again at 6 hours prior to sacrifice. The dosage was 500, 1000 and 2000 mg kg^{-1} bw of the 10% gelatin beadlets. No compound-related increase in micronuclei was observed. Astaxanthin at the doses tested induces neither chromosome breaks not mitotic disjunction in vivo".

Mutagenicity studies, in which astaxanthin was tested, focussed on the antimutagenic and in vivo anticlastogenic effects of series of carotenoids (Rausher *et al*., 1998). In these studies it appeared that astaxanthin was inactive or at best marginally active in inhibition of aflatoxin B1, benzo[a]pyrene, 2-amino-3-methylimidazole[4,5-f]quinoline (IQ) and cyclophosphamide induced mutagenicity in the histidine deficient strains of *Salmonella typhimurium* TA98, TA 98NR and/or TA100 (Ames test). In control incubations in which the carotenoids were tested for mutagenic activities in the absence of the model mutagens but in the presence of S9 mix, no mutagenic activities could be detected at concentrations up to 100 μg astaxanthin per plate (Rauscher *et al*., 1998).

Astaxanthin at 180 mg kg^{-1} bw (dosed orally dissolved in corn oil) also did not result in clastogenic effects in the in vivo micronucleus test in male NMRI mice (Rausher *et al*., 1998).

Haematococcus pluvialis was tested in an in vitro mammalian cell gene mutations assay using mouse lymphoma (L5178Y) cells with and wthout S-9 activation. The test was performed in accordance with OECD and EU guidelines.[10] No biologically or statistically increase in mutation frequency was observed in cultures teated with the test material (highest concentration: 5000 μg mL^{-1}) in either test, compared to the negative control cultures. The sensivitivity of the test was demonstrated by large increases in mutation frequency in the positive (ethylnitrosourea) control cultures.

A summary obtained from the internet[11] presents a technical report on *Haematococcus pluvialis* and astaxanthin safety. This report states that no mutagenic effect of *Haematococcus pluvialis* algae was found using a mutagenicity test with *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, TA1538 and *E.coli* WP2 uvr A.

2.7.3. (Sub)chronic Toxicity Studies

A summary obtained from the internet presents a technical report on *Haematococcus pluvialis* and astaxanthin safety. In this summary it is mentioned that a 28-day sub-acute rat toxicity study, with *Haematococcus pluvialis* algal meal and astaxanthin, did not result in any sign of toxicity. In this study groups of 20 rats (10 females/10 males) were fed daily by gavage 0, 5, or 50 mg algal meal in a corn oil suspension kg^{-1} bw or 1.15 mg astaxanthin kg^{-1} bw for 28 consecutive days.

A study reported a 13 week subchronic oral toxicity study of *haematococcus colour*, a food additive containing astaxanthin, in male and female F344 rats (Ono *et al.,* 1999). Rats were randomly divided into 4 groups each consisting of 10 males and 10 females and given CRF-1 powder diet containing 0, 0.025%, 0.075%, and 0.25% haematococcus colour. None of the animals died during the administration. There were no exposure-related changes in body weight gain or food consumptions. Serum biochemical examinations showed a dose-related increase in cholesterol, but the differences were slight and not considered an adverse effect by the authors. No treatment related effects were noted in haematological examinations and organ weights, and no abnormalities were observed in histopathological examinations that could be ascribed to haematococcus colour. It was concluded by the authors that ingestion of haematococcus colour in the diet for 13 weeks at levels up to at least 0.25% does not cause

[10] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-20-Edwards-vol170.pdf
[11] htpp://www.astafactor.com/techreports/tr3005-001.htm

any toxicological changes in F344 rats. Assuming an astaxanthin content of the haematococcus colour equal to the minimum level requiered of 1.4%[12], 0.25% haematococcus color in the diet corresponds to at least 37 mg astaxanthin kg^{-1} diet, and this would give rise to a daily exposure of about 2 to 3.5 mg astaxanthin kg bw^{-1} day^{-1}.

Another 13 week study on rats with a HPP (*haematococcus pluvialis* product) at doses of 0, 1, 5 and 20% in the diet is reported[13] with 20 animales (10 males, 10 females) per group. Body weight was unaffected by treatment, feed consumption in females of the 20% HPP group was marginally lower than in the control. There were no ophtalmic conditions that could be attributed to the administration of HPP. The highest HPP group showed higher serum AP activity and total cholesterol, also kidney weigth was increased and pigment deposition in the straight proximal tubule (in five females). The stomach and to a lesser extent the duodenum and the caecum showed an orange appearance in the intermediate and the highest dose level group. Although there are no data on the astaxanthin content of HPP, it could be estimated (on the base of the above figures) that the 5% exposure corresponded to 40-70 mg astaxanthin kg bw^{-1} day^{-1} (20 fold higher than in the study of Ono *et al*., 1999).

A 13-week subchronic oral toxicity study of phaffia colour was performed in both sexes of F344 rats by feeding CRF-1 powder diet containing 0, 0.2, 0.6, 1.7 and 5% phaffia colour (Onodera *et al*., 1997). Rats were randomly divided in 5 groups each consisting of 10 males and 10 females. No animals died during the administration period. There were no treatment related changes in body weight gain, haematological and blood biochemical examination. No treatment related histopathological changes were observed. The authors conclude that 5% phaffia color in the diet for 13 weeks does not cause any toxicological changes in rats. Assuming an astaxanthin content of the phaffia colour equal to the minimum level approved for the product on the European market of 4.5 g kg^{-1} (FEEDAP, 2004), 5% phaffia colour in the diet corresponds to at least 225 ppm, and this would give rise to a daily exposure of about 11 to 22.5 mg kg bw^{-1} day^{-1}.

Some studies on effects of astaxanthin on specific parameters in exposed experimental animals have been reported (Uchiyama *et al.,* 2002; Gradelet *et al.,* 1997; Gradelet *et al.,* 1998; Jewell and O'Brien, 1999; Kang *et al.,* 2001; Jyonouchi *et al*., 2002; Tanaka *et al*., 1994). Although these studies have not been designed to assess the safety of astaxanthin, they have not revealed adverse effects.

With the aim to investigate the chemoprevention of rat oral carcinogenesis by naturally occurring astaxanthin a study was conducted by Tanaka *et al.* (1995a). 4-Nitroquinoline 1-oxide exposed male F344 rats were given diets containing 100 mg astaxanthin kg^{-1} for 32 weeks. One group (12 rats) was exposed to the 100 mg astaxanthin kg^{-1} diet alone for 32 weeks. The astaxanthin intake was estimated to be 15.2-15.9 g per rat and day. Given the average body weight at the end of the experiment of 313 gram per rat this amounts to an intake of at least 50 mg kg bw^{-1} day^{-1}. All rats were carefully observed daily. Mean body, liver and percentage liver weight in the groups fed astaxanthin alone were significantly lower than those from the untreated control. Dietary administration of astaxanthin caused no clinical signs of toxicity, low survival, poor condition or histological changes suggesting toxicity in liver, kidney and lung.

Tanaka *et al.* (1995b) also reported the suppression of azoxymethane-induced rat colon carcinogenesis in male F344 rats fed a diet containing 100 mg astaxanthin kg^{-1} for 34 weeks during the post-initiation period. One group (15 rats) was exposed to the 500 mg astaxanthin kg^{-1} diet alone for 37 weeks. All rats were carefully observed daily, weighed weekly until they reached 14 weeks of age and then every 4 weeks. Mean body, liver and percentage liver weight in the groups fed astaxanthin alone did not significantly differ from the untreated control. Dietary administration of astaxanthin caused no clinical signs of toxicity, low survival, poor condition or histological changes suggesting toxicity in liver, kidney and lung. The intake of 500

[12] http://www.cfsan.fda.gov /~lrd/cfr73185.html
[13] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-25-Final-Report-vol170.pdf

mg astaxanthin kg^{-1} diet amounts to an intake of approximately 25 to 50 mg astaxanthin kg^{-1} bw day^{-1}.

2.7.4. Reproductive Toxicity Studies, including developmental toxicity

A summary of a reproductive stuydy of astaxanthin in rats is published on the internet.[14] The following cites this internet source:

"Astaxanthin was tested in accordance with the guidelines of the American FDA and the English CSM for effects on fertility and general reproductive performance of the rat and on the in utero and postnatal development of the F1-offspring to time of weaning. The study includes the assessment of later development and of the reproductive capability of selected F1-offspring retained beyond weaning.

Doses of 25, 100 or 400 mg astaxanthin (96% pure) kg^{-1} day^{-1} were administered by oral gavage to 12 male rats per group, and to 32 females/group beginning 14 days prior to mating and continuing through gestation until sacrifice or weaning. Control animals (32 per sex) received the vehicle (rape-oil, 2 mL kg^{-1}) in a comparable regimen.

About half of the mated females in each group were sacrificed on about gestation day 14, while the remaining females were allowed to litter. F_1-pups of selected litters were evaluated for developmental indices during lactation. On lactation day 23, selected weanlings were retained for learning and memory testing or the assessment or their reproductive capability.

The results of the study can be summarized as follows: P-generation: No substance related mortality in males or females was observed in any of the dosage groups. The body weight gain of both P-males and P-females in all experimental groups matched that of the concurrent controls. The percentage of males which mated their partners, as well as the ratio of mated to pregnant females and the median precoital time were comparable between all groups. Up to 400 mg kg^{-1}, the highest dose tested, the reproductive parameters of females sacrificed between gestation days 14 and 16 were within normal limits.

In the F_1-generation: all experimental groups, the litter parameters such as the body weight gain of pups, the time of onset of developmental landmarks and the learning and memory ability matched that of the controls. The neonatal mortality of the F_1-generation in the highest dosage group (400 mg kg^{-1}) was at the upper limit of the biological range (25.6%). However, there was no statistical significance for this finding and no dose-relationship was evident. Therefore a substance-related impairment of pup viability was considered to be very unlikely. In all dosage groups, the macroscopic and soft tissue examination of pups found dead during lactation showed isolated findings which were not considered to be substance-related. These included hematoma in the lung, empty stomach, and dilated renal pelvis and ureter. The gross examination of weanlings for malformations, as well as for liver and kidney weights, yielded normal findings. One neonate in the low-dose group (25 mg kg^{-1}) exhibited unilateral anophtalmia. This isolated anomaly was not considered to be substance-related."

2.7.5. Human data

A safety report of an astaxanthin-rich *Haematococcus pluvialis* algal extract on the basis of a randomized clinical trial was published by Spiller *et al*., (2003). The authors conducted a human safety study with a *Haematococcus pluvialis* algal extract with high levels of astaxanthin Thirty-five healthy adults aged 35-69 years took part in a randomized, double-blind, placebo-controlled trial of 8 weeks. All participants took three gel caps per day, one at each meal, with nineteen participants taking gel caps with an algal extract in safflower oil containing 2 mg of astaxanthin, and sixteen participants receiving the control gel caps containing safflower oil only. No significant differences were detected between the treatment and placebo groups after 8

[14] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-18-Appendix-3-vol170.pdf

weeks of supplementation in cell blood counts, blood pressure and blood chemistry tests, including a comprehensive metabolic panel, except for small increases in serum calcium, total protein and eosinophils. The authors stated however, that although these differences were statistically significant, they were very small and considered to be of no clinical importance. Altogether it was concluded that 6 mg of astaxanthin per day from a *Haematococcus pluvialis* algal extract can be safely consumed by healthy consumers.

In a study reported on the internet[15] healthy Japanese volunteers between 40 and 50 years of age, 15 males and 10 females took two placebo and astaxanthin capsules containing 0, 1, 2, 4 and 6 mg ashtaxathin, respectively, extracted from *Haematococcus pluvialis* each, orally after dinner, for 4 weeks. Venous blood samples were taken 22 days in advance of receiveing astaxanthin and after four weeks of supplementation, 2-3 hours after lunch. The examination of biochemical and haematological parameters revealed no significant or adverse effects after receiving up to 12 mg of astaxanthin per day. Some significant changes were observed in biochemical blood parameters after astaxanthin intake, but these changes were not dose related. The authors conclude that extracted asthaxanthin consumed in the range of 2 to 12 mg per day up to four weeks displayed no undesirable reactions and deemed safe for human consumption.

A summary obtained from internet[16] presents a technical report on *Haematococcus pluvialis* and astaxanthin safety for human consumption. This report summarizes results from a clinical trial with *Haematococcus pluvialis* algal meal. In this study 33 human volunteers (15 males and 18 females age 28 to 62) ingested on a daily basis for 29 consecutive days, either a low dose supplements containing 228 mg algal meal and 3.85 mg astaxanthin, or a high dose supplement containing 1140 mg algal meal and 19.25 mg astaxanthin. Medical examination included the weight, skin coloration, general appearance, blood pressure, vision and eye (near and distant vision, color vision, depth perception, eye condition), ears and nose, mouth, throat and teeth, chest and lungs and reflexes for each volunteer. This medical examination was complemented by haematology, urine analyses (color, appearance, blood, specific gravity, pH, protein, glucose, ketones, nitrite, bilirubin, urobilinogen, leukocyte esterase), and biochemical blood parameters (SGPT, LDH, glucose, total protein, total bilirubin, BUN, creatinine, total, HDL and LDL cholesterol, triglycerides, albumin, and globulin). No ill effects or toxicity were observed. The report concludes that a supplement containing 5 mg astaxanthin derived from 250 mg, or less, of the specified *Haematococ-cus pluvialis* algal meal is safe for daily human consumption. This technical report[17] also mentions a study in which human volunteers ingested up to 14.4 mg astaxanthin day^{-1} for two weeks, with no ill effects reported.

2.8. Risk Assessment

Numerous in vitro and in vivo tests with astaxanthin (and astaxanthin containing *Haematococcus pluvialis*) show that astaxanthin is not mutagenic and not clastogenic.

The (sub)chronic and the reproductive toxicological data do not allow the identification of a NOEL because no satisfactory reassurance on the endpoints could be found (only summaries - abstracts- were available). In additon in the subchronic studies no information on the astaxanthin content of the test products *Haematococcus pluvialis* and *Phaffia rhodozyma* was given. Thus astaxanthin intake of the laboratory animals could only be estimated. Consequently no ADI can be proposed.

Three subchronic toxicity studies could be assessed. No effects were seen at about 2 and 11 mg astaxanthin kg^{-1} bodyweight, the highest doses tested in the studies of Ono *et al*., (1999) and Onodera *et al*., (1997) respectively and about 40 mg astaxanthin kg^{-1} bodyweight.[18]

[15] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-21-Shimada-vol171.pdf
[16] htpp:/www.astafactor.com/techreports/tr3005-001.htm
[17] htpp:/www. astafactor.com/techreports/tr3005-001.htm
[18] http://www.fda.gov/ohrms/dockets/dockets/95s0316 /95s-0316-rpt0236-25-Final-Report-vol170.pdf

The study of Tanaka *et al*., (1995b) is limited by the fact that only 15 rats per treatment were used. however, the authors used astaxanthin and fed the animals for 37 weeks. With at least 25 mg astaxanthin kg^{-1} bw no toxic effects could be observed.

Reproduction of rats and developmental toxicity is reported as not influenced up to 400 mg astaxanthin kg^{-1} bodyweight day^{-1}. However, neonatal mortality in the F_1 generation was high, however not statistically significant.

Two studies were performed with astaxanthin containing *haematococcus pluvialis* on human volunteers for 4 weeks and a third one for 8 weeks. In all three studies the respective highest doses tested did not show any adverse effects (12 and 19,25 mg asthaxanthin kg^{-1} bodyweight for 4 weeks and 6 mg asthaxanthin kg^{-1} bodyweight for 8 weeks)[19] [20] (Spiller *et al*., 2003).

Estimations of human astaxanthin intake, based on limited data available on fish consumption as a worst case scenario, show that 4,1 mg astaxanthin $person^{-1}$ day^{-1} would probably not be exceeded.

After all these considerations it is important to note, that the consumer exposure's to astaxanthin is independent of the type of fish -wild or farm fish- ingested.

The FEEDAP Panel considers therefore that the use of astaxanthin as feed additive to salmonid feed at the maximum level approved is not a safety concern for the human consumer. This is in agreement with FDA where astaxanthin is regulated as a color additive exempt from certification for use in fish feed under 21 CFR Section (73.35 synthetic astaxanthin, 73.185 as haematococcus algae, 73.355 as *Phaffia* yeast.[21] The maximum permitted level in feed in the US is 80 mg kg^{-1}.

The assessment undertaken by the FEEDAP Panel does not specify the source of astaxanthin, because it is based on the chemical similarity of the astaxanthins assessed. Potentially harmful substances not found in the habitat of salmonids may be included in fish feed from synthetic products and biomass products, the latter containing only a small amount of astaxanthin, the rest being material (residues) from the production organisms or production procedure. Approved astaxanthins should therefore be characterised by specification.

2.9. Environment

No data are available for a qualified assessment of the environmental impact of asthaxanthin in salmonid feed.

According to the Directive 2001/79/EEC an environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, the use of which will not alter the concentration or distribution in the environment. Astaxanthin is a substance naturally occurring in the habitat of fish (salmonids) (see table 2)

The FEEDAP Panel does not expect differences between different sources of astaxanthin feed additives (synthetic or biotechnical) in respect to their environmental impact. Astaxanthin as feed additive for farmed fish substitutes natural dietary sources from the habitat of wild living salmon and trout.

Comparing astaxanthin concentration in fish feed (100 mg kg^{-1}) in zooplankton *Bosmina* (9 mg kg^{-1} flesh matter) and the shrimp *Gammarus lacustris* (18 mg kg^{-1} flesh matter) shows approximately equal levels in the natural and manufactured dietary asthaxantin sources, based on dry matter.

Under normal practice a small proportion of feed administered will remain unconsumed and enter the surrounding environment of the fish farm. Because of the importance of efficient feed conversion on the economy of the fish farm, such feed loss are not expected to be high.

[19] htpp://www.astafactor.com/techreports /tr3005-001.htm
[20] http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0236-21-Shimada-vol171.pdf
[21] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm

Astaxanthin is susceptible to oxidation. Absorbed astaxanthin is metabolised, no conjugated astaxanthin was found in the bile of Atlantic Salmon (Xu and Ding, 2004). Idoxanthin and zeaxanthin are the main metabolic products of astaxanthin. These metabolites occur frequently and in considerable quantities in plants, where they –if not consumed – are subject of oxidation and decomposition together with the other organic material.

As astaxanthin is insoluble in water, it will mainly bound to faeces and sink to the seabed. The loading to the sediment below net cage area in open sea or lakes is likely to be higher than in the natural environment where wild fish are not geographically restricted and as such their faeces would be distributed over a larger area of seabed. Beside the fact that no data are available, a possible impact of this higher loading of astaxanthin and its metabolites can not be assessed without considering the environmental impact of the higher loading of fish faeces itself.

3. Conclusions and recommendations

Astaxanthin [(3S,3'S)-3,3'-dihydroxy-β,β-carotene-4,4'-dione) is a natural carotenoid with red pigmenting properties occuring in yeasts, algae, crustaceans, and predator fish like salmonids. It is approved at EU level as feed additive for Salmon and trout at 100 mg kg^{-1} complete feed from 6 months of age onwards without time limit (and for ornamental fish). No specifications could be found at EU level. Products on the market are either synthetically or biotechnically produced.

Astaxanthin forms three optical and eight geometric isomers, showing a species specific distribution pattern.

Astaxanthin is vitamin A precursor for fish, it is important for performance, specific functions in reproduction and metabolism, and health in salmonids.

Astaxanthin is the major carotenoid in supplementing salmonid feed. It is used to produce flesh with the desired red colour equal to flesh from wild catch. The absorption capacity is limited, atlantic salmon would built a plateau at about 10 mg astaxanthin kg^{-1} flesh, trout at a higher level of about 20-25 mg astaxanthin kg^{-1} flesh. Based on the literature the FEEDAP Panel concludes that the astaxanthin level in farmed and wild fish is at comparable levels. Up to 200 mg astaxanthin kg^{-1} fish feed no adverse effects on fish have been reported.

Absorption is determined by several factors, the occurrence of astaxanthin free or esterified, and dietary factors (lipid level), excretion of undigested astaxanthin amounts to about approximately 50 %.

Astaxanthin is metabolised in salmonids through reductive pathways, leading to idoxanthin, adonixanthin and zeaxanthin. No cleavage of the polyen chain is observed. Epimerization from 3S to 3 R and *vice versa* occurs. Metabolites were mainly excreted via the bile. After astaxanthin application, the pigments deposited in flesh of trouts and Chinook salmons are predominantly astaxanthin, in the arctic charr also idoxanthin. Preliminary results indicate a metabolic pathway in rats (including cleavage of the polyen chain) very different from fish, but essencially similar to that seen in humans.

Safety assessment was difficult due to the scarceness of fully published toxicity studies. FEEDAP Panel therefore decided to consider also summaries and abstracts as additional information.

Astaxanthin is not mutagenic and not clastogenic. Subchronic studies on rats were performed with astaxanthin rich algae *Haematococcus pluvialis* or yeast *Phaffia rhodozyma*. No effects were seen at about 2 and 11 mg astaxanthin kg^{-1} bodyweight, the highest doses tested, and about 40 mg astaxanthin kg^{-1} bodyweight. In a 37 week rat study the corresponding dose was 25 mg astaxanthin kg^{-1} bodyweight.

A reproductive toxicity study, including developmental toxicity, with astaxanthin up to 400 mg kg^{-1} bw did not show statistically significant adverse effects.

Carcinogenicity studies could not be found, however several subchronic studies showed an anticarcinogenic effect of astaxanthin in experimental models with different carcinogens.

The FEEDAP Panel could not establish a NOEL or an ADI for different reasons (astaxanthin content of the product not clearly stated, no chronic study available, no satisfactory reassurance on the endpoints).

In three studies on healthy human volunteers the highest doses tested did not show any adverse effects (12 and 19.25 mg astaxanthin day^{-1} for 4 weeks, and 6 mg astaxanthin day^{-1} for 8 weeks).

The data available on intake of trout and salmon flesh are insufficient. Worst case calculations indicate that the mean astaxanthin uptake of the European consumer would not exceed 4.1 mg day^{-1}.

Supplementing salmonid feed with astaxanthin would not increase flesh astaxanthin of farmed fish essentially compared to wild catches. The FEEDAP Panel considers therefore the use of astaxanthin as feed additive to salmonid feed at the maximum level approved safe for the human consumer. This is in agreement withcurrent FDA regulations.

No data are available for a qualified assessment of the environmental impact of asthaxanthin in salmonid feed. Astaxanthin occurs naturally in the habitat of wild living salmonids. Astaxanthin as feed additive for farmed fish substitutes natural sources. As astaxanthin is insoluble in water and susceptible to oxido-reduction it will mainly bind to faeces and sink to the seabed. Idoxanthin and zeaxanthin, the main metabolic excretion products of astaxanthin, occur frequently and in considerable quantities in the environment.

In that respect the FEEDAP Panel does not expect that the use of astaxanthin as feed additive to salmon and trout will not pose a significant risk to the environment.

3.1 Recommendations

The assessment undertaken by the FEEDAP Panel does not specify the source of astaxanthin, because it is based on the chemical similarity of the astaxanthins assessed The Harmful substances not found in the habitat of salmonids may be included in fish feed from synthetic products and biomass products, the latter containing only a small amount of astaxanthin, the rest being material (residues) from the production organisms or production procedure. The FEEDAP Panel recommends therefore a detailed specification of astaxanthin products approved. Specification should include identity and purity of the respective product. This information will also be necessary for safety for the user, which can only be assessed on a product specific basis.

The FEEDAP Panel also recommends an adjustment of the regulations set under “Other Provisions”, which at present allow the use of astaxanthin for salmon and trout only from an age of 6 months onwards. Common practice (outside EU) is feeding astaxanthin during the whole growout period (100 g to harvest). Body weight can be better controlled than age. Considering safety of astaxanthin for the target animal and fish physiology there is no serious reason to restrict the use of astaxanthin to a particular developmental stage.

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ANNEX

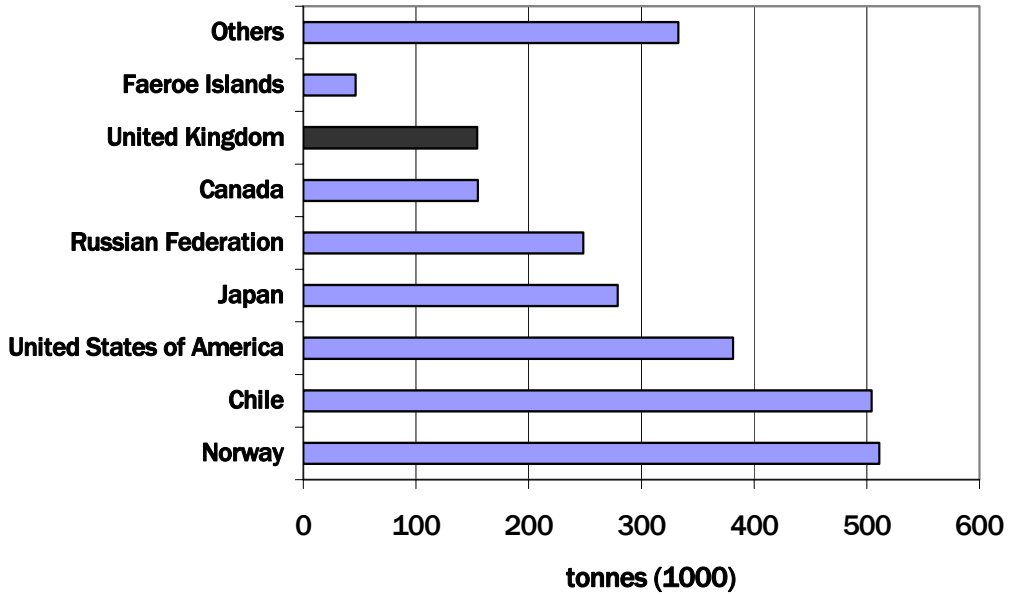

Figure 1A. World production of salmons, trouts and smelts (FAO, 2004) 2.6 million tones.

Table 1A. **Aquaculture production of salmonids in tonnes (Norsk havbruksnæring – tall og fakta 2003)**

Country	Atlantic salmon	Rainbow trout (L, Pi)	Rainbow trout (P, Pi,)	Rainbow trout (P, U,)	Chinook salmon	Coho salmon
Denmark		10,000	1,000	29,000		
Faroe Islands	46,000					
Finland		15,000				
France			40,000			
Germany			1,000	22,500		
Greece				2,300		
Iceland	3,500		1,000			
Ireland	22,000					
Italy			25,000	23,000		
Norway	450,000	82,000				
Poland				8,000		
Portugal			1,500			
Spain			16,000	10,000	6,000	2,000
Sweeden		4,000				111,000
U.K.	143,000	3,000	14,875			
Europe total	664,500	114,000	100,375	94,800	6,000	113,000
EU total	214,500	32,000	100,375	94,800	6,000	113,000
Japan						8,000
New Zealand					6,000	
Turkey				20,000		
USA	13,000					

L=large, P=portion size, Pi=pigmented, U=non pigmented

Portion size rainbow trout data is from 1998, while others are from 2002.

Table 2A. Worst case astaxanthin intake (mg adult person^{-1} day^{-1}) (range: Atlantic salmon – trout) based on a country based total intake of fish and seafood [1]

Country	Reference	Average Intake		High Intake [a]	
		Fish/seafood	Astaxanthin	Fish/seafood	Astaxanthin
Belgium	3.2.13	13 [b]	0.13-0.33	48[b]	0.48-1.20
Denmark	3.2.11	23 [b]	0.23-0.58	—	
Finland	3.2.11	53 [b]	0.53-1.33	—	
France	3.2.13	41 [b]	0.41-1.03	103 [b]	1.03-2.58
Germany	– [d]	30	0.30-0.75	50	0.50-1.25
Greece	3.2.13	37 [c]	0.37-0.93	95 [c]	0.95-2.38
Ireland	3.2.11	23	0.23-0.58	75	0.75-1.88
Italy	3.2.13	48	0.48-1.20	—	
Norway	– [e]	80	0.80-2.00	165	1.65-4.13
Portugal	3.2.11	40	0.40-1.00	—	
Sweden	3.2.11	30	0.30-0.75	—	
The Netherlands	3.2.11	10	0.10-0.25	—	
United Kingdom	3.2.11	14	0.14-0.35	—	

1 Indicative country based consumers only total intake of fish and seafood products primarily derived from EU reports, respectively on heavy metals (SCOOP Task 3.2.11) and OTC (SCOOP Task 3.2.13) (EC 2004, 2003)

a In general the 97.5th percentile

b Fish only

c Seafood other than fish only

d National Cosumption survey (1985-1988)

e Bergsten C. (2004) personal communication to the Scientific Panel on Contaminants in the Food Chain