

## ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

## ZEAXANTHIN

**Issue**

The Committee is asked to consider an initial opinion from the Dutch Competent Authority on an application for the authorisation of Zeaxanthin as a novel ingredient, under the Novel Foods Regulation (EC) No. 258/97.

The Committee is asked whether it agrees with this initial opinion and whether they have any further comments to make on the application.

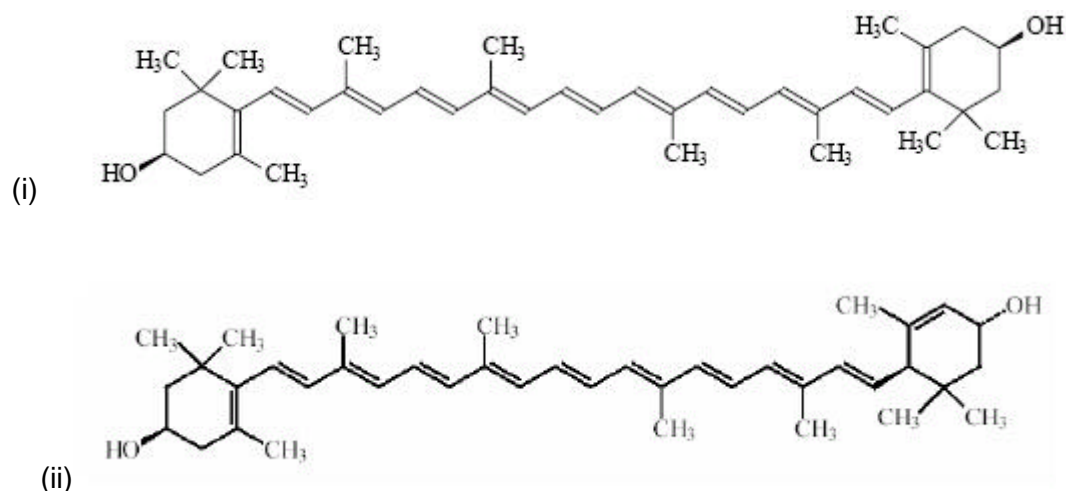
**Introduction**

1. On 1 August 2005, the European Commission forwarded the Dutch Competent Authority (CA)'s initial opinion on the application made under Article 4(1) of Regulation (EC) No 258/97 submitted by Bioresco, on behalf of DSM Nutritional Products for the authorisation of zeaxanthin as a novel food ingredient.
2. The Dutch CA have sought advice from the Committee on Safety Assessment of Novel Foods of the Health Council and their opinion concludes that additional control measures are needed to restrict and define the range of products in which zeaxanthin can be used. Whilst the Dutch CA endorse the applicant's proposal that based on the available scientific data the daily consumption should not exceed 20mg/day/person, they are concerned that the absence of information on intended products and levels of incorporation does not provide reassurance that this will not be exceeded. As this information is not available the Dutch CA have concluded that they have insufficient data to complete their safety assessment and are of the view that additional control levels are needed to ensure that the products in which the NI is to be used are precisely defined.
3. The dossier is therefore likely to be referred to the European Food Safety Authority. Although other Member States have not been formally asked to comment on the initial opinion, there is an opportunity to raise any additional points that need to be taken into account in EFSA's opinion.
4. A translation of the Dutch Initial Assessment Report and the full dossier, (including annexes) and additional information from the applicant are attached as Appendices **A**, **B** and **C** respectively. Members will wish to be aware that there are three typographical errors printed in the Initial Assessment report, these corrections are shown in the corrigendum which can be found on the last page on Appendix A.

**Background**

5. The application is for the placing on the market of a synthetic zeaxanthin as a novel food ingredient (NI) in the EU. Zeaxanthin is a fat-soluble xanthophyll pigment that is naturally present in some fruit, vegetables and flowers.

Zeaxanthin and the closely-related pigment lutein are the most common xanthophylls present naturally in food.



Chemical structures of (i) zeaxanthin (ii) lutein

6. According to the applicant the primary purpose for placing the NI on the market is because of perceived health benefits thought to be due to its antioxidant properties. However, the Dutch CA has correctly stated that such health claims are outside the scope of the Regulation (EC) 258/97. The NI could conceivably have a use as a food colour although the Secretariat notes that such a use would require authorisation in accordance with Council Directive 94/36/EC<sup>1</sup>
7. Members will also wish to note that zeaxanthin was evaluated in 2004 by JEFCA<sup>2</sup> when a group ADI of 0 – 2 mg/kg/ bodyweight was allocated for lutein and zeaxanthin combined. (Appendix D)
8. In accordance with the Novel Foods Regulation (EC) 258/97, zeaxanthin has been classified as a food produced using a novel process (Class 6). The application has been prepared pursuant to Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel ingredients. The requirements for the submission for this class are:

I	Specification of the NF	X	VIII	Ability to survive in and colonise the human gut	-
II	Effect of the production process applied to the NF	X	IX	Anticipated intake/extent of use of the NF	X
III	History of the organism used as the source of the NF	X	X	Information from previous human exposure to the NF or its source	X
IV	Effect of the genetic modification on the	-	XI	Nutritional information on the NF	X

<sup>1</sup> European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs, Official Journal L 237 , 10/09/1994 p.13 -29

<sup>2</sup> JECFA: Joint FAO/WHO Expert Group on Food Additives

	properties of the host organism	
V	Genetic stability of the GMO	-
VI	Specificity of expression of novel genetic material	-
VII	Transfer of genetic material from GM microorganisms	-

<b>XII</b>	<b>Microbiological information on the NF</b>	<b>X</b>
<b>XIII</b>	<b>Toxicological information on the NF</b>	<b>X</b>

## I Specification of the Novel Ingredient (NI)

Appendix B p.13-20 **CONFIDENTIAL**

9. The NI is described as an orange-red crystalline powder, with little or no odour with a chemical structure of C<sub>40</sub>H<sub>56</sub>O<sub>2</sub> (also known as (3R, 3'R)-dihydroxy-β-carotene), and a molecular weight of 568.87 Daltons. The NI contains a minimum of 96% of (3R, 3'R)-zeaxanthin and no more than 2% of cis-zeaxanthin.
10. According to the specification (Appendix B, annex 3) the NI contains small quantities of other carotenoids, thought to be by-products of the production process. These have been identified by the applicant, as diatoxanthin, parasiloxanthin and 12'-apo-zeaxanthinal. The NI also contains traces of triphenylphosphine oxide.
11. The applicant has provided compositional data on five representative batches of their NI, which was produced between 1977-1985, and on additional batches produced in 2001. These data demonstrate that the NI meets the specification and purity criteria prepared at the 63<sup>rd</sup> meeting of JECFA in June 2004. The methods of analysis for determining the parameters in the specification are detailed in Appendix B, annex 4.
12. In its crystalline form zeaxanthin is unstable as it is susceptible to light and oxygen. The applicant intends to sell the product as a food ingredient, and will sell the NI in two stable forms, as a powder preparation ("beadlets") with either a modified starch or corn-starch base (5% zeaxanthin content), or as a corn oil suspension (20% zeaxanthin) - (Appendix B p 16).
13. The applicant has provided supporting data to demonstrate the stability of the NI in the forms described in para 12 and in typical food matrices. These studies (Appendices B & C) indicate the zeaxanthin content in the oil suspension remained constant when stored at 15°C for 48 months, whilst the zeaxanthin contents in the powder based preparation with a gelatine base remained unchanged when stored at 15°C for two years. Stability data for the NI in a range of food matrices shows that it is generally stable with minor degradation during the shelf life of the product.
14. The Dutch CA concluded that the compositional analysis of several batches of the NI were sufficient to demonstrate that the product can be produced in accordance with the specification. However the Dutch CA note that whilst the stability of the product is acceptable, the applicant should advise manufacturers who intend to incorporate the product into foodstuffs of the relevant information regarding shelf life of foods containing the NI.

## **II Effect of the production process applied to the NF**

Appendix B p. 21-26 **CONFIDENTIAL**

15. The NI is synthesised in a multi-step chemical process. The production process is described and a flow chart provided in Appendix B pages 21-26 and figure 1 respectively. After each step of the production process the product is purified, filtered and washed with an organic solvent and/or water.
16. Each step of the production process is carefully controlled to ensure that the final ingredient meets the company's specification and purity requirements.
17. The Dutch CA are satisfied that the applicant has sufficient measures in place to ensure that the product is produced in accordance with the specification.

## **III History of the organism used as the source of the NF**

Appendix B p. 27 **CONFIDENTIAL**

18. The NI is produced by a chemical process and does not have a biological source

## **IX Anticipated intake and extent of use of the NF**

Appendix B p. 28-43 **CONFIDENTIAL**

19. The NI is to be sold as a compound food ingredient in the forms described above (Para 12) Representative examples of these are described in Appendix B, Table 1, although the applicant notes that there may be other nutrients e.g. lutein added. The applicant company will market these compound food ingredients for incorporation into food supplements, foods intended for a particular nutritional purpose (PARNUTS) and foods with added vitamins, minerals and/or other nutrients.
20. The Dutch CA have repeatedly requested that the applicant provide further information relating to the specific products they intend to incorporate their NI in, however, the applicant has declined to specify the types of products which might be used (Appendix B pp 28-30, Appendix C pp 10-12). In the applicants supplementary information they have stated that the intended use of the NI will mirror the use of vitamins, which have been authorised without the need to list its specific foods. The Dutch CA have stated that they do not agree with the applicants view that the NI should be regarded as a conditionally essential nutrient, and as no information regarding use groups and levels have been forthcoming are of are unable to complete the assessment of this NI.
21. The applicant notes that the perceived role of zeaxanthin in maintaining eye function is such that it could be considered a 'conditionally essential nutrient'. If categorised as such it is the applicant's view that the NI should be used in a manner that mirrors the current use of vitamins and minerals. The applicant notes that in the case of vitamins and minerals a restriction on the consumption of vitamins and minerals is achieved by consumers referring to Recommended Daily Allowance (RDA) labelling.
22. The Applicant intends that 'Adequate Intake' for zeaxanthin should be within the 2-20 mg/d, and as a group ADI for zeaxanthin and lutein has been set by JECFA at 0-2 mg/kg/bw<sup>3</sup> (Appendix D), is of the view that this gives an adequate safety factor. The Applicant will use the figure of 20mg/day as the maximum adequate

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<sup>3</sup> JECFA propose that the ADI for zeaxanthin is a group ADI for both lutein and zeaxanthin.

intake figure and indicate that products containing the NI are labelled in such a way that consumers will not exceed this figure

23. The Dutch CA consider that this is insufficient and are unable to complete their safety assessment until the applicant has provided additional information regarding the product range and at what concentration levels in which the NI will be added to, including the target group for which the final products will be intended.

## **X Information from previous human exposure to the NF or its source**

Appendix B p.44-45 **CONFIDENTIAL**

24. Zeaxanthin and lutein are [found naturally in green and yellow-orange fruit and vegetables such as broccoli, cabbage, kale, spinach, kiwi, honeydew melon and oranges. The ratio of lutein to zeaxanthin varies depending on the plant source. It has been estimated that the ratio of lutein to zeaxanthin in the US diet is 5:1 (Third National Health and Nutrition Examination Survey (1988-1994) from the United States).
25. The dietary intake of zeaxanthin in the US is estimated to be 0.6-1.2 mg/d (mean and 90<sup>th</sup> percentile). Dietary intake data have also indicated that the average intake of zeaxanthin in Europe varies between 0.2 and 0.9 mg/d, depending on the geographical location.
26. The applicant reports that synthetic zeaxanthin is used in dietary supplements in the United States, although there are no reliable data and it is thought that its actual amount is rather small. The applicant estimates that at present the daily intake of zeaxanthin from dietary supplements in various countries (such as Mexico, US, Australia, Taiwan, Brazil, South Africa, Indonesia and Vietnam) does not exceed 2mg.
27. The Dutch CA are satisfied that the applicant has demonstrated that natural zeaxanthin is a regular part of the daily diet. Zeaxanthin intake figures are derived from data for the consumption of lutein, or zeaxanthin and lutein and the Dutch CA are content that the ratio used to determine the consumption levels for of zeaxanthin in the US figures are reasonable, although they note that there are no data to support the assumption that these figures can be applied to the an average European diet.

## **XI Nutritional information on the Novel Food**

Appendix B p. 46-57 **CONFIDENTIAL**

28. Xanthophylls are found in the macula of the human retina and are thought to serve as an accessory to light gathering pigments and protect against phototoxic damage.
29. The applicant notes that there are minor compositional differences between naturally occurring zeaxanthin and synthetic zeaxanthin, with the latter comprising almost not less than 96% all-trans (3R, 3'R)-zeaxanthin, compared with 85% (all-trans) and 15% cis forms in the naturally occurring counterpart respectively. The applicant also notes that other optical isomers (the meso and (3S,3S') forms) that are found in the NI (2% and 01% respectively) are not found naturally but they have been detected in the retina of the human eye. The applicant has concluded that in terms of nutrition these differences are of little

significance as the isomers do not exert a different biological function and the meso and (3S,3S') forms are normal metabolites of (3R, 3'R)-zeaxanthin.

30. In light of the information provided by the applicant the Dutch CA are satisfied that there is no difference between the synthetic and natural form of zeaxanthin in terms of the way they are metabolised by the human body and that the two substances should be regarded as equivalent in nutritional terms.

#### Bioavailability

31. The absorption of zeaxanthin mirrors other carotenoids in that it requires release from the food matrix, and passively diffuses through the intestinal mucosa in lipid micelles. Research cited by the applicant indicates that the bioavailability of zeaxanthin is variable and is influenced by the presence of other dietary constituents.
32. The applicant has stated that zeaxanthin is a conditionally essential nutrient, which plays an important, and indispensable function in the human eye as it cannot be synthesised by the body it must therefore, be ingested in food. The Dutch CA state that they have not evaluated the applicant's claim that zeaxanthin is a conditionally essential nutrient as it falls outside the remit of regulation (EC) 258/97, however they have commented that zeaxanthin is not yet regarded as an essential nutrient as are a range of vitamins, minerals or trace elements. (Appendix A p.31 of 45).
33. The applicant has determined that consuming zeaxanthin above 20mg/day does not result in a corresponding increase in circulating levels as the absorption is not linear. The applicant also notes that there are variations in the bioavailability of the NI and these may be attributed to the fact that the product has a long 'half-life', and raised levels of consumption take a significant period of time to be seen in blood. The Dutch CA noted that this information is from an unpublished study and that the evidential basis for this conclusion is limited. However, the Dutch CA do not anticipate that people who consume up to 20mg of the NI per day will suffer any adverse consequences in nutritional terms.

### **XII Microbiological Information**

Appendix B p. 58 **CONFIDENTIAL**

34. The production of the NI does not involve the use of a micro-organism and the applicant has stated that the low moisture content is not supportive to microbial growth. In response to a request from the Dutch CA the applicant has submitted additional data that showing that the NI is produced in accordance with the described purity criteria (Appendix C p 10, Annex 5). The Dutch CA were content that these additional data provided sufficient reassurance that the product was free from microbial contamination.

### **XIII Toxicological information**

Appendix B p. 59-87 **CONFIDENTIAL**

35. The dossier includes an extensive review of the toxicological safety of synthetic zeaxanthin. The material used in these tests was produced by the applicant but differed slightly from that which is now being produced for human consumption. The Dutch CA are satisfied that the materials tested were representative of the commercially produced ingredient. No adverse effects were documented in these studies.

36. Members will wish to be aware that the toxicological data reviewed by JECFA (Appendix D) are similar to that submitted for novel food approval and these are summarised in the table below.
37. Following its evaluation of the safety studies on synthetic zeaxanthin, JECFA concluded that the toxicological tests do not indicate its use may have any adverse effect. Rather than derive an upper safe limit for the intake of zeaxanthin from these studies, JECFA decided to adopt a group ADI for zeaxanthin and lutein of 0 – 2 mg/kg bodyweight. This value was derived from the toxicological database for lutein, which is more complete.
38. In their initial opinion the Dutch CA have concluded that they do not anticipate that the daily consumption of up to 20mg of the NI is liable to have any adverse effects on health.

### **Labelling information**

Appendix A p.23

39. The Dutch CA have highlighted the need for the applicant to comply with the labelling requirement as set out in Directive 2000/13/EC and in accordance with Article 8 of Regulation (EC) 258/97.
40. The proposal to label the NI in accordance with 'Adequate Intake' was not considered by the Dutch CA who have stated that this is discussed in the Regular Consultation on the Commodities Act. (This is presumably a reference to Dutch national legislation)

### **Committee Action Sought**

41. The Secretariat notes that whilst the Dutch CA are generally content that the safety of this NI has been demonstrated *per se*, they have concluded that in the absence of information regarding the intended food uses there are insufficient data to complete a safety assessment. The Dutch CA have therefore identified a need for additional assessment and it is likely that the Commission will refer the application to EFSA for an additional assessment.
42. The Committee is therefore asked whether there are any additional safety concerns arising from the dossier that should be addressed by EFSA. The UK will forward comments to the Commission immediately after this meeting.

**Secretariat  
September 2005**

Table 1 Summary of all toxicological studies provided by the applicant for novel food authorisation

Study	Test System	Strain(s) / Target cells	Genotoxicity		GLP	Reference
			Zeaxanthin test product	Concentration / Dose		
In vitro						
Ames	Salmonella typhimurium	TA 1535, 1537, 1538, 97, 98, 100, 102	Crystalline powder <sup>4</sup>	0, 2.4-1500 mg/plate*	Yes	Gocke, 1987
HGPRT	Chinese Hamster	V 79 cells	Crystalline powder <sup>4</sup>	0, 1-16 mg/ml*	Yes	Strobel, 1988
UDS	Rat	Hepatocytes	Crystalline powder <sup>4</sup>	0, 1-16 mg/ml*	Yes	Strobel, 1987
HLA	Human blood	Peripheral lymphocytes	Crystalline powder <sup>4</sup>	0, 60, 120 mg/ml*	Yes	Strobel & Bonhoff, 1987
In vivo						
Micro-nucleus	Mouse	Bone marrow cells	10% WS Beadletz	0, 44.5, 89.0, 178.0 mg/kg p.o.	Yes	Galandre, 1980

\* = With and without metabolic activation (S-9 mix)

Acute toxicity

Species	Route	Formulation	LD50 (mg/kg bw)	GLP audited	References
Mouse	Oral	Crystalline powder	> 8000	No	Baechtold, 1977a
Rat	Oral	Crystalline powder	> 4000	No	Baechtold, 1977b

Subchronic and chronic toxicity

Species	Route	Duration of treatment	Zeaxanthin test product	Animal nos. and sex, doses (mg/kg bw/day)	GLP	Reference
Mouse	Oral (Dietary)	13 weeks	10% WS Beadlets	10 m / 10 f 0, 0, 250, 500, 1000	No	Ettlin et al., 1980a
Rat	Oral (Dietary)	13 weeks	10% WS Beadlets	16 m / 16 f 0, 0, 250, 500, 1000	Yes	Ettlin et al., 1980b
Rat	Oral (Dietary)	13 weeks	10% WS Beadlets	16 m / 16 f 0, 0, 250, 500, 1000	Yes	Buser, 1985
Dog	Oral (Dietary)	13 weeks	10% WS Beadlets	3 m: 0, 123, 204, 422 3 f: 0, 104, 238, 443	Yes	Ettlin, 1985
Monkey	Oral (Gavage)	52 weeks	10% WS Beadlets	2 m / 2 f: 0, 0.2 3 m / 3 f: 20 Interim sacrifice 1 m / 1 f at 20 mg/kg/d	Yes	Pfannkuch et al., 2000

Reproduction toxicity

Species	Route	Type	Zeaxanthin test product	Doses (mg/kg bw/d)	GLP	Reference
Rat	Oral (dietary)	Segment II	10% WS Beadlet <sup>1</sup>	0, 250, 500, 1000	Yes	Kistler, 1984
Rabbit	Oral (gavage)	Segment II	Oily suspensions <sup>2</sup>	0, 100, 200, 400	Yes	Kistler, 1983

Footnotes

- <sup>1</sup> Zeaxanthin: Lot No 105003, Analysis No 464586; Beadlet Lot No 308001, Analysis No 471387.
- <sup>2</sup> Zeaxanthin: Lot No 01-9509/007, Analysis No 431571; Beadlet Lot No not reported, Analysis No 433973.
- <sup>3</sup> Zeaxanthin: Lot No 105004, Analysis No 464586.
- <sup>4</sup> Zeaxanthin: Lot No 504005, Analysis No 477480 (May 1985) and 6282/ZTN/06/B28/604/A05 (August 2001).

Abbreviations

n.a.	not available
HGPRT	Hypoxanthine Guanine Phosphoribosyl Transferase
UDS	Unscheduled DNA Synthesis
HLA	Human Lymphocyte Assay
WS	Water Soluble

Note: The results of the zeaxanthin analyses are shown in Annex 5 (Table 2).

## **Annexes attached**

**Appendix A:** Dutch Competent Authority's Initial Assessment report on an application for the authorisation of Zeaxanthin as a novel ingredient.

**Appendix B:** Application dossier for the evaluation of Zeaxanthin as a novel ingredient under Regulation (EC) 258/97 (confidential).

**Appendix C:** Supplementary information for the evaluation of Zeaxanthin as a novel ingredient under Regulation (EC) 258/97 (confidential).

**Appendix D:** Draft JECFA Monograph (restricted)

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

Dutch Competent Authority's Initial Assessment report on an application for the authorisation of Zeaxanthin as a novel ingredient.

This document has been published by the Dutch Competent Authority and is available at:

<http://www.cbg-meb.nl/nl/docs/nwvoeding/zeaxanthine.pdf>

**Secretariat  
September 2005**