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SCIENTIFIC OPINION

Safety of 'Lipid extract from *Euphausia superba*' as a novel food ingredient [1]

Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2008-027)

Adopted on 22 January 2009

This opinion, published on 26 June 2009, replaces the earlier version published on 13 February 2009[2].

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver a scientific opinion on the safety of 'Lipid extract from Euphausia superba' as food ingredient.

The novel food ingredient is an oil obtained by extraction of the crustacean Euphausia superba (Antarctic Krill) with acetone. Proteins and krill material are removed from the lipid extract by filtration. The acetone and residual water are removed by subsequent evaporation steps. The name proposed is Neptune Krill Oil (NKO™).

One of the main features of NKO™ is the low content of triglycerides and the high content of phospholipids (38 - 50 g/100g). Another characteristic property is the high content of polyunsaturated fatty acids, in particular eicosapentaenoic acid (EPA, C20:5n-3; 15 - 19 g/100g) and docosahexaenoic acid (DHA, C22:6n-3; 7-16 g/100g). The major amounts of these fatty acids are present in phospholipids. Compositional data on major components of NKO™ as well as on minor components (e.g. unsaponifiable matter) have been provided and a broad

[1] For citation purposes: Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission on the safety of 'Lipid extract from *Euphasia superba*' as food ingredient. *The EFSA Journal* (2009) 938, 1-17.

[2] The unit for the saponification value in Table 3 was corrected from "165 - 185 mg/kg KOH" to "165 - 185 mg KOH/g".

spectrum of potential contaminants has been analysed. The parameters presented sufficiently characterise the product and demonstrate its consistency and stability.

The applicant reviewed data on absorption, distribution and elimination, data from toxicity studies and data from clinical human studies performed with the major components of NKO™, i.e. phospholipids, EPA and DHA, and with the carotenoid astaxanthin.

The toxicological data provided for NKO™ are limited to a repeat-dose study (six months) conducted with C57BL/6 nude congenic mice (B6NU-T heterozygotes) in order to examine potential effects of oral, topical and topical/oral administration of NKO™ on UVB radiation-induced skin cancer. The animals treated orally received a diet containing 16.6 % NKO™. According to the study report, the histopathological examination of selected organs and tissues revealed no relevant differences between the treatment and control group, which received soybean oil. Body weight development, haematology, clinical chemistry and urine analyses as well as organ weight determinations were not performed.

Twenty-five healthy male and female volunteers consumed six 1g NKO™ gel capsules (6 g of NKO™) daily for a period of two months. Haematology and clinical chemistry analyses were performed at baseline, and after 1 and 2 months. One female withdrew from the study due to a known salt intolerance. Two other females withdrew because of rapidly increasing greasiness of their facial skin. No adverse effects were reported in the remaining 22 subjects.

In a trial designed to examine the effect of NKO™ on the clinical course of hyperlipidaemia, 120 patients (mean age 51 years) were administered up to 3 g NKO™/day for a period of 12 weeks, with one group continuing for an additional 90 days with a consumption of 500 mg NKO™/day. No adverse effects were reported.

The effects of NKO™ on the premenstrual syndrome and on dysmenorrhea were examined in a clinical trial. Seventy female adults of reproductive age consumed either NKO™ or fish oil (during the first month of the trial two 1 g capsules once daily; during the second and third month of the trial two 1 g capsules once daily for 8 days prior to menstruation and 2 days during menstruation). No adverse effects were reported by the subjects during the trial.

The effects of NKO™ on markers of chronic inflammation were examined in a clinical trial in which 300 mg NKO™/day or 100 mg of placebo were administered to 90 subjects. The subjects ranging from 50 to 68 years were diagnosed with cardiovascular disease or rheumatoid arthritis and osteoarthritis, and were reported to have C-reactive protein levels of > 1.0 mg/dL. No adverse effects associated with the consumption of NKO™ were reported.

NKO™ contains residual protein (0.8 - 3.0 g/100 g), the nature of which has not been further elucidated. In accordance with the Labelling Directive 2003/89/EC, all products containing NKO™ must be labelled as "contains crustaceans and fish". For food supplements, the applicant proposed the following additional warning to be put on all products containing NKO™: "Persons with coagulopathy or who are taking anticoagulants or other medications should discuss their situation with their doctor and submit to tests before taking nutritional supplements".

The applicant provided data on the use of Antarctic krill as human food and data on the sales of capsules containing NKO™ outside the EU.

The food groups to which NKO™ is intended to be added and the maximum levels of DHA and EPA proposed by the applicant are in accordance with the Commission Decision of 5 June 2003 on the use of oil rich in DHA from the microalga Schizochytrium.

The maximum use levels of EPA and DHA as proposed by the applicant and food consumption data obtained from the National Diet and Nutrition Survey (NDNS) program in the United

Kingdom were used as a basis to estimate the daily intakes of EPA and DHA. Mean estimated daily intakes of EPA and DHA range from 210 mg/person/day for children to 363 mg/person/day for male adults. The 97.5th percentile intakes range from 490 mg/person/day for children to 976 mg/person/day for male adults.

The toxicological and clinical data provided for NKO™ are limited. However, in combination with the data available for the main constituents, they support the safety of the novel food ingredient under the proposed conditions of use.

The Panel concludes that the lipid extract from Euphausia superba (Antartic Krill, NKO™) as a novel food ingredient is safe under the specified conditions of use.

Key words: *Euphausia superba*, Antartic Krill, lipid extract, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), phospholipids

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BACKGROUND AS PROVIDED BY THE COMMISSION

On 7 November 2006, Neptune Technologies & Bioresources Inc. submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the competent authorities of Finland for placing on the market a 'Lipid extract from *Euphausia superba*' as food ingredient.

On 29 January 2007, the competent authorities of Finland forwarded to the Commission its initial assessment report, which came to the conclusion that the research results presented by the applicant do not raise any doubts about the product's safety.

On 19 February 2007, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted additional comments or raised objections.

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

- Further specification of the phospholipids.
- Qualitative and quantitative data on the presence of non-lipid constituents.
- Data on the acid value.
- Data on the purity of the oil.
- Data on the potential accumulation of marine biotoxins in krill and data on the analysis of these toxins in the final product.
- Data on the presence of organic arsenic.
- It is questioned whether the limited history of consumption of krill is sufficient to demonstrate the safety of the oil.
- Quantitative information on the content of similar organisms of the family Euphausiidae in the fished krill.
- A more detailed description of the production process (e.g. separation of proteins and fats, purification steps).
- Stability tests with products from the proposed food categories to which krill oil is added.
- The number of products which can be enriched with NKO™ should be limited
- No estimates on the intakes by children have been provided.
- The addition of such cholesterol-rich oil to foods is considered inappropriate because it reduces the nutritional quality.
- The data are considered insufficient to conduct a safety assessment.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'Lipid extract from *Euphausia superba*' as food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to consider the elements of scientific nature in the comments raised by the other Member States.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: Jean-Louis Bresson, Karl-Heinz Engel, Marina Heinonen, Pagona Lagiou, Bevan Moseley, Andreu Palou, Annette Pöting, Seppo Salminen, Hendrik Van Loveren and Hans Verhagen.

ASSESSMENT

The novel food ingredient is an oil extracted from the crustacean *Euphausia superba* (Antarctic Krill). The name proposed is Neptune Krill Oil (NKO™).

NKO™ is a complex novel food ingredient derived from an animal, which has not been genetically modified. It has, therefore, been assigned to class 2, as defined in the recommendations of the Scientific Committee for Food (SCF) concerning the assessment of novel foods (European Commission, 1997). It belongs to sub-category 2.2, because the source of the novel food has no history of food use within the Community. Accordingly, information related to the structured schemes I, II, III, IX, XI, XII and XIII has been submitted.

I. Specification of the novel food (NF)

One of the main features of NKO™ is that the content of triglycerides is low (approximately 37 %) whereas the content of phospholipids is rather high. For three batches the average content of total phospholipids was shown to be 39.5 % (Table 1). According to data provided for two samples, the phospholipids are composed of approximately 72 % phosphatidylcholine, 22 % phosphatidylethanolamine, 1 % phosphatidylinositol and 5 % cardiolipin.

Another characteristic property is the high content of polyunsaturated fatty acids, in particular eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). As shown in Table 1, the major amounts of these fatty acids are present in phospholipids.

Table 1. **Phospholipid contents of NKO™ and proportions of EPA and DHA present in phospholipids**

Batch	**060116**	**060127**	**060822**
		Phospholipids (%)	
	39.2	39.7	39.5
		% of total fatty acids present in phospholipids	
EPA	69.2	70.5	68.2
DHA	68.4	71.2	66.3

Compositional data resulting from the analysis of three batches of NKO™ are shown in Table 2. The analyses were performed by experienced external laboratories, using standard methods.

Table 2. **Compositional data of three batches of NKO™**

	060116	060519	060224
Total phospholipids	47.3 g/100g	44.8 g/100g	43.8 g/100g
Total lipids as fatty acids	88.5 g/100g	81.2 g/100g	88.2 g/100g
Total saturated fatty acids	29.8 g/100g	29.2 g/100g	28.9 g/100 g
Total monounsaturated fatty acids	22.1 g/100 g	21.6 g/100 g	21.9 g/100 g
Total polyunsaturated fatty acids	36.7 g/100 g	34.2 g/100 g	37.4 g/100g
Total omega-3 fatty acids	33.6 g/100 g	31.5 g/100 g	34.3 g/100 g
Eicosapentaenoic acid (EPA)	17.2 g/100 g	16.6 g/100 g	18.0 g/100 g
Docosahexaenoic acid (DHA)	11.3 g/100 g	10.3 g/100 g	11.4 g/100 g
Docosapentaenoic acid (DPA)	0.6 g/100 g	0.5 g/100 g	0.6 g/100 g
Total omega-6 fatty acids	1.6 g/100 g	1.4 g/100 g	1.6 g/100 g
Linoleic acid	1.4 g/100 g	1.2 g /100 g	1.3 g/100 g
Total omega-9 fatty acids	9.6 g/100 g	9.3 g/10 g	9.4 g/100 g
Oleic acid	8.6 g/100 g	8.5 g/100 g	8.2 g/100 g
Total *trans* fatty acids	< 0.01 g/100 g	< 0.02 g/100 g	not detected
Vitamin A	260 IU/g	337 IU/g	270 IU/g
Vitamin E	0.65 IU/g	0.67 IU/g	0.70 IU/g
Esterified astaxanthin	163 mg/100 g	161 mg/100 g	154 mg/100 g
Total protein	3.0 g/100 g	0.7 g/100 g	0.8 g/100 g
Total cholesterol	1.1 g/100 g	1.0 g/100 g	0.9 g/100 g
Saponification value	175 mg KOH/g	171 mg KOH/g	178 mg KOH/g
Iodine value	138 g I_2/100 g	136 I_2/100g	134 I_2/100g
p-Anisidine value	1.0	0.9	0.9
Peroxide value	< 0.1	< 0.1	< 0.1
Moisture	0.1 %	0.2 %	0.1 %
Viscosity	564 cP	477 cP	586 cP

The specifications of NKO™ proposed by the applicant are shown in Table 3

Table 3. **Specifications of NKO™ proposed by the applicant**

Components / Parameter	Specification
Total phospholipids	38.0 - 50.0 g/100g
Total lipids as fatty acids	73.0 - 93.0 g/100 g
Total saturated fatty acids	22.0 - 30.0 g/100g
Total monounsaturated fatty acids	12.0 - 28.0 g/100g
Total polyunsaturated fatty acids	32.0 - 44.0 g/100g
Total omega-3 fatty acids	28.0 - 41.0 g/100g

Eicosapentaenoic acid (EPA)	15.0 - 19.0 g/100g
Docosahexaenoic acid (DHA)	7.0 - 16.0 g/100g
Docosapentaenoic acid (DPA)	0.4 - 0.7 g/100g
Total omega-6 fatty acids	1.4 - 2.6 g/100g
Linoleic acid	1.3 - 2.3 g/100g
Total omega-9 fatty acids	6.0 - 13.0 g/100g
Oleic acid	5.0 - 11.0 g/100g
Total *trans* fatty acids	0 - 0.1 g/100g
Vitamin A	100 - 450 IU/g
Alpha-Tocopherol	0.5 - 0.9 IU/g
Esterified astaxanthin	150 - 190 mg/100g
Astaxanthin	110 - 150 µmoles/100 g
Moisture	0.1 - 0.9 %
Acetone	0 - 10 mg/kg
Characteristics	
Saponification value	165 - 185 mg KOH/g
Iodine value	130 - 145 g I_2/100g
p-Anisidine value	0 -1.6
Peroxide value	0 - 0.2 meq O_2/kg
Contaminants	
Arsenic (inorganic)	< 0.1 mg/kg
Cadmium	< 0.1 mg/kg
Mercury	< 0.1 mg/kg
Lead	< 0.1 mg/kg
Copper	< 3 mg/kg
Tin	< 10 mg/kg
Antimony	< 1 mg/kg
Aldrin	< 0.1 mg/kg
Dieldrin	< 0.1 mg/kg
Chlordane (alpha and gamma)	< 0.05 mg/kg
Sum of DDT	< 0.5 mg/kg
Endrin	< 0.05 mg/kg
Heptachlor	< 0.1 mg/kg
Toxaphene	< 0.01 mg/kg
Hexachlorobenzene	< 0.1 mg/kg
Alpha-HCH	< 0.1 mg/kg
Beta-HCH	< 0.05 mg/kg
Lindane	< 1 mg/kg

Benzo(a)pyrene	< 0.2 µg/kg
Sum of PCDD/PCDF	< 2.0 pg/g fat WHO-PCDD/F-TEQ/g fat
Sum of PCDF/F/PCBs	< 10 pg/gfat WHO-PCDD/F-TEQ/g fat
Microbial parameters	
Total coliforms and *E. coli*	< 10 CFU/g
Yeasts and moulds	< 10 CFU/g
Staphylococcus aureus	< 10 CFU/g
Listeria monocytogenes	Not detectable/25 g
Pseudomonas aeruginosa	Not detectable/25 g
Salmonella spp.	Not detectable/25 g

The fatty acid distributions in the total product as well as in the phospholipids were shown to be consistent in these batches.

The unsaponifiable matter of NKO™ was determined to be 2.7 % (by mass). Separation by silica gel chromatography and analysis of the fractions obtained by GC/MS resulted in the identification of the components shown in Table 4.

Table 4. **Components of the unsaponifiable matter of NKO™ (batch 070219)**

Component	**% of unsaponifiable matter**	**% of NKO™**
Fatty alcohols		
Myristyl alcohol	0.4	0.009
Palmityl alcohol	0.8	0.02
Stearyl alcohol	0.1	0.0003
Eicosyl alcohol	0.4	0.01
Docosyl alcohol	0.2	0.004
Mixture of long-chain polyunsaturated alcohols	11.9	0.32
Hexadecanediol	1.8	0.05
Cis-9,10-Epoxyoctadecan-1-ol	1.2	0.03
Octadecandiol	4.7	0.13
Docosanediol	20.4	0.55
Hydrocarbons		
Mixture of long-chain polyunsaturated hydrocarbons	1.8	0.05
Fatty acid amides		
N-(2-hydroxyethyl)-dodecaneamide	0.6	0.02
Palmitamide	0.5	0.01

Oleamide	3.0	0.08
Erucamide	3.2	0.09
Terpenoids		
Squalene	0.1	0.001
Phytol	17.3	0.47
Phytenol	0.6	0.17
Sterols		
Cholesterol	20.3	0.55
Desmosterol	4.3	0.12
Campesterol	0.5	0.01
Cholest-4-ene-3-one	0.6	0.02
Ergosta-5,24(28)-dien-3-ol	0.3	0.009
12-Hydroxyergostan-3-one	0.1	0.003
Stigmast-7-ene-3-ol	0.3	0.007
Unknown sterol	1.8	0.05
Carotenoids		
Astaxanthin	2.0	0.053
Total	98.9	2.7

For the compound classes determined in the unsaponifiable matter, the applicant demonstrated and discussed that these are not unique to NKO™ but naturally occurring in various fats of marine origin.

Krill oil has been developed from a laboratory product to commercial scale over the past ten years. According to the applicant, crude oil obtained upon extraction of crushed Antartic krill contains a number of compounds, such as carotenoids and flavonoids typically associated with marine oils. The continuous adaptation and improvement of the various technologies employed during the stages of product development resulted in a significant reduction or complete removal of these substances in the commercial product. According to a certificate provided, the Canadian Food Inspection Agency has been performing toxin-producing marine algae tests on Canadian as well as imported krill (raw material) since December 2004. Every lot that is imported has to be declared. The results of all tests (Amnesic Shellfish Poisoning, ASP; Paralytic Shellfish Poisoning, PSP; domoic acid) have been acceptable.

The specification (< 0.1 mg/kg) given for arsenic in Table 2 refers to inorganic arsenic. The predominant form of arsenic in fish and shell-fish derived products is organic. According to the applicant, NKO™ may contain up to 10 mg/kg of total arsenic. It is worthwhile to recall that in fish and seafood often more than 90 % of the total arsenic substances represent the non-toxic arsenobetaine and arsenocholine (Buchet and Lauwerys, 1994; EFSA 2005a).

Edible oils are normally characterized by low contents of free fatty acids, expressed by the acid value (typical specification for fish oil: 0-5 mg KOH/g). The acid value given for NKO™ is much higher (25.7-32.4 mg KOH/g) owing to the inherent content of free fatty acids. Therefore, this parameter is less appropriate as stability indicator.

The oxidative stability of NKO™ is reflected by the low peroxide and *p*-anisidine values. It has also been supported by stability data on NKO™ in soft gelatine capsules.

Similar to the provision in Commission decision 2003/427/EC of 5 June 2003 authorising the placing on the market of oil rich in DHA from the microalga *Schizochytrium* sp. as novel food (European Commission, 2003), the applicant suggests the following statement "All food products containing Neptune Krill Oil should demonstrate oxidative stability by appropriate and recognized national/international test methodologies (e.g. AOAC)".

II. Effect of the production process applied to the NF

According to the applicant, the krill swarms are so dense, that other food competitors, such as jellyfish, small fish or squid are usually excluded. On the rare occasions of by-catches, the contaminants (small species of fish and squid) can be readily observed by visual inspection and be discarded.

The deep-frozen raw Antarctic krill is crushed and subjected to an extraction with acetone. Proteins and krill material are removed from the lipid extract by filtration. The acetone and residual water are removed by subsequent evaporation steps. The product is then packaged in a modified atmosphere. Process parameters, such as particle size of the crushed material, ratio krill material/solvent, extraction temperature and duration, filtration and evaporation conditions have been given by the applicant. The production process is performed according to Good Manufacturing Practices (GMP).

III. History of the organism used as the source of the NF

NKO™ is produced from *Euphausia superba* (Antarctic Krill) which is native to the Atlantic section of the Austral-Antarctic Circumpolar Ocean. According to the applicant, Antarctic Krill has a history of consumption as food in Japan, Russia, Ukraine and France since the mid-1970´s. Of about 20,000 to 30,000 tons of products from krill used annually in Japan in the 1980´s, 4000 – 6000 tons have been utilized as human food (Suzuki and Shibata, 1990; Nicol and Endo, 1997).

IX. Anticipated intake/extent of use of the NF

The food groups to which NKO™ is intended to be added and the maximum levels of DHA and EPA proposed by the applicant are listed in Table 5. They are in accordance with the use of oil rich in DHA from the microalga *Schizochytrium* as given in the Commission Decision of 5 June 2003 (EU, 2003).

Table 5. **Uses of NKO™ and maximum levels of DHA and EPA proposed by the applicant**

Use group	Maximum level of DHA and EPA
Dairy products except milk-based drinks	200 mg/100g; 600 mg/100g for cheese products
Dairy analogues except drinks	200 mg/100g; 600 mg/100g for analogues of cheese products
Spreadable fat and dressings	600 mg/100g
Breakfast cereals	500 mg/100g
Food supplements	200 mg per daily dose as recommended by the

	manufacturer
Dietary foods for special medical purposes	In accordance with the particular nutritional requirements of the persons for whom the products are intended
Foods intended for use in energy-restricted diets for weight reduction	200 mg/meal replacement

The maximum use levels of EPA and DHA as proposed by the applicant and based on food consumption data obtained from the National Diet and Nutrition Survey (NDNS) program in the United Kingdom (Gregory et al., 1995; UKDA, 1995; UKDA, 2001; Office for National Statistics, 2005) were used as a basis to estimate the daily intakes of EPA and DHA (Table 6).

Table 6. **Estimated daily intake of EPA and DHA based on the proposed maximum use levels applied to food consumption data obtained in the UK***

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Intake[a)]				All-Users Intake[b)]			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children	1½-4 ½	98,4	1,621	210	374	434	490	211	373	434	490
Young People	4-10	99,3	831	292	496	587	658	294	496	587	658
Female Teenagers	11-18	97,1	433	262	499	591	691	261	495	590	695
Male Teenagers	11-18	99,0	412	353	616	692	802	355	623	692	802
Female Adults	16-64	92,7	888	290	564	645	768	297	568	663	775
Male Adults	16-64	93,6	717	357	713	827	976	363	714	835	976

* The estimated daily intake in this table does not include the EPA and DHA occurring naturally in foods.

a) All-Person Intake refers to the estimated intake averaged over all individuals surveyed regardless whether they consumed products in which NKO™ is proposed for use

b) All-user Intake refers to the estimated intake by those individuals consuming food products in which NKO™ is proposed for use

Mean estimated daily intakes of EPA and DHA range from 210 mg/person/day for children to 363 mg/person/day for male adults. The 97.5th percentile intakes range from 490 mg/person/day for children to 976 mg/person/day for male adults.

This type of intake methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the 4-day children's survey, may overestimate consumption of food products that are consumed relatively infrequently, particularly when weighted to 7 days (Gregory *et al*., 1995).

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

Softgel capsules containing NKO™ have been available as dietary supplement products in North America and Asia for several years. In Canada and the United States, NKO™ has been available for purchase since October of 2002 and in Japan, Korea, Singapore and Hong Kong since December of 2004. The recommended intake of NKO™ in North America and Asia is 1 - 2 softgel capsules/day, each containing 500 mg of NKO™. According to the applicant, 120,000 kg of NKO™ has been sold in softgel capsule form between 2002 and 2007 (88% of sales in North America and 12% in Asia). This corresponds to 240 Million capsules in total and an average consumption of approximately 130,000 capsules per day. According to the applicant, no adverse effects have been reported over the marketing period.

XI. Nutritional information on the NF

NKO™ is intended as a source of the two omega-3 polyunsaturated fatty acids EPA and DHA which are contained in the oil mainly in phospholipids. EPA and DHA are present in the diet from several foods (EFSA, 2005b). Phospholipids as integral components of animal and plant cell membranes are also widely present in the diet from sources, such as wheat flour, milk, eggs, meat, fish, soybean and other plant oils.

Considering the low content of cholesterol (0.9 - 1.1 g/100g for the three batches listed in Table 2), the contribution of NKO™-containing foods to the dietary cholesterol intake is considered negligible.

XII. Microbiological information on the NF

According to the applicant, the microbiological quality of NKO™ is controlled according to the HACCP system. Limits for microbial parameters are given in Table 3.

XIII. Toxicological information on the NF

The applicant reviewed data on absorption, distribution and elimination, data from toxicity studies and data from clinical human studies performed with the major components of NKO™, i.e. phospholipids, EPA and DHA, and with the carotenoid astaxanthin.

Animal studies

A repeat-dose study using C57BL/6 Nude Congenic mice (B6NU-T heterozygotes) was conducted in order to examine potential effects of oral, topical and topical/oral administration of NKO™ on UVB-radiation induced skin cancer. The study duration was six months during which the animals were exposed daily to UVB radiation. The animals treated orally received a diet containing 16.6% NKO™. According to the study report, the histopathological examination of selected organs and tissues (brain, lungs, heart, stomach, pancreas, liver, kidneys, uterus or prostate and intestine) revealed no relevant differences between the treatment and control group, which received soybean oil. Body weight development, haematology, clinical chemistry and urine analyses as well as organ weight determinations were not performed (Neptune, 2002).

Human studies

Twenty-five healthy male and female volunteers between 25 and 53 years consumed six NKO™ gel capsules daily for a period of two months. Each capsule contained 1 g of NKO™ and provided 386 mg of omega-3-fatty acids, 416 mg of phospholipids and 0.16 mg of astaxanthin. Haematology and clinical chemistry analyses were performed at baseline, and after

1 and 2 months. The parameters examined included cells counts, PTT, creatinin, glucose, alkaline phosphatase, albumin, amylase, total bilirubin, total cholesterol, HDL and LDL cholesterol, triglycerides, urea and TSH levels. One female withdrew from the study due to a known salt intolerance. Two other females withdrew because of rapidly increasing greasiness of their facial skin. No adverse effects were reported in the remaining 22 subjects.

In a trial designed to examine the effect of NKO™ on the clinical course of hyperlipidemia, 120 patients, with a mean age of 51 years, were randomized into 4 groups, and further subdivided according to their body mass index (BMI). Individuals in Group A were administered either 2 g NKO™/day (BMI < 30) or 3 g NKO™/day (BMI > 30) and individuals in Group B were administered either 1 or 1.5 g/day (BMI < 30 and BMI > 30). In Group C the subjects were administered a fish oil capsule providing 180 mg EPA and 120 mg DHA, while Group C received a placebo. All individuals consumed their treatments for a period of 12 weeks, with Group B continuing for an additional 90 days with a consumption of 500 mg NKO™/day. No adverse effects were reported to result from the consumption of NKO™, fish oil or placebo (Bunea et al., 2004).

The effects of NKO™ on the premenstrual syndrome and on dysmenorrhea were examined in a clinical trial. Seventy female adults of reproductive age were randomized to receive either NKO™ or fish oil treatments. During the first month of the trial the subjects consumed two 1 g capsules once daily with meals. During the second and third month of the trial the subjects consumed two 1 g capsules once daily for 8 days prior to menstruation and 2 days during menstruation. No serious adverse effects were reported by the subjects during the trial. Minor increases in the greasiness of the facial skin were reported by individual in the NKO™ treatment group (Sampalis et al., 2003).

The effects of NKO™ on markers of chronic inflammation were examined in a clinical trial in which 300 mg NKO™/day or 100 mg of placebo were administered to 90 subjects. The subjects ranging from 50 to 68 years were diagnosed with cardiovascular disease or rheumatoid arthritis and osteoarthritis, and were reported to have C-reactive protein levels of > 1.0 mg/dL. No adverse effects associated with the consumption of NKO™ were reported (Deutsch, 2007).

Allergenicity

NKO™ contains residual protein (0.8 - 3.0 g/100g for the three batches listed in Table 2; determined via the Kjeldahl method). The nature of this protein has not been further elucidated. Protein residues isolated from three batches of NKO™ were subjected to tests for the presence of shellfish allergens. The sandwich-type ELISA test applied detects shellfish protein using a polyclonal antibody to crustacean tropomyosin (limit of detection 1 mg/kg shellfish protein). According to the applicant, the kit employed an antibody capable of detecting intact tropomyosin as well as fragments formed during the processing of krill oil. The test showed negative results for three oil samples; positive results obtained for shellfish-spiked samples confirmed the suitability of the applied method.

According to PCR-based analyses, crustacean and fish DNA were not detectable in three lots of NKO™ (analytical details and limit of detection of the methods applied have not been reported).

Considering these inconclusive analytical results, the applicant agreed, in accordance with the Labelling Directive 2003/89/EC, that all products containing NKO™ are labelled as "contains crustaceans and fish".

For food supplements, the applicant proposed the following additional warning to be put on all products containing NKO™: "Persons with coagulopathy or who are taking anticoagulants or

other medications should discuss their situation with their doctor and submit to tests before taking nutritional supplements".

DISCUSSION

The data available on the use of Antarctic krill as human food and the experience gained from the marketing of NKO™ as capsules outside the EU are limited.

Compositional data on major components of NKO™ (e.g. phospholipids, EPA, DHA) as well as on minor components (e.g. unsaponifiable matter) have been provided and a broad spectrum of potential contaminants has been analysed. The parameters sufficiently characterize the product and demonstrate its consistency and stability.

The toxicological and clinical data provided for NKO™ are limited. However, in combination with the data available for the main constituents, they support the safety of the novel food ingredient under the proposed conditions of use.

The labelling provisions regarding allergy and coagulopathy are supported.

CONCLUSIONS AND RECOMMENDATIONS

The Panel concludes that the lipid extract from *Euphausia superba* (Antartic Krill, NKO™) as a novel food ingredient is safe under the specified conditions of use.

DOCUMENTATION PROVIDED TO EFSA

1. Application under Regulation (EC) N° 258/97 concerning novel foods and novel food ingredients concerning 'Lipid extract from *Euphasia suberba*' as food ingredient, "Krill Oil" (Neptune Technologies & Bioresources Inc.), February 2008.
2. Initial assessment report carried out by Finland:
   Asetuksen (EY) 258/97 artiklan 4 mukainen ensiarvioraportti: Antarktisesta krilliäyriäisesta (Euphasia superba) valmistettu öljy (Neptune Krill Oil™)
   Initial assessment report in compliance with Regulation (EC) No 258/97, Article 4: Oil prepared from antartic krill (Euphasia superba) – Neptune Krill Oil™
3. Member States' comments
4. Response to Member States Comments on the Finnish Assessment (by the applicant)

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