Application for the Approval of Calanus® Oil as an Ingredient for Use in Food Supplements

Pursuant to

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 Concerning Novel Foods and Novel Food Ingredients

<u>Non-Confidential Dossier</u>

Calanus AS
Stakkevollv. 65
P.O. Box 2489
N-9272 Tromsø
Norway

November 10, 2011

Application for the Approval of Calanus® Oil as an Ingredient for Use in Food Supplements

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 Concerning Novel Foods and Novel Food Ingredients

Table of Contents

List of Appendices

List of Tables and Figures

GLOSSARY

AI	Adequate Intake
BCA	bicinchoninic acid
Calanus	Calanus AS
CVD	cardiovascular disease
DDT	dichlorodiphenyltrichloroethane
DHA	docosahexaenoic acid
DRV	Daily Reference Value
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbent Assay
EPA	eicosapentaeonic acid
EU	European Union
FKD	Norwegian Ministry of Fisheries and Coastal Affairs
GC	Gas Chromatography
GM	genetic modification
HACCP	Hazard Analysis and Critical Control Points
HCH	hexachlorocyclohexane
n-3 PUFAs	n-3 polyunsaturated fatty acids
NKO™	Neptune Krill Oil
PAHs	Polycyclic Aromatic Hydrocarbons
PBDEs	Polybrominated Diphenyl Ethers
PCBs	Polychlorinated Biphenyls
SCF	Scientific Committee on Food
SDA	Stearidonic acid
US	United States
VLDL	very low density lipoproteins

Application for the Approval of Calanus® Oil as an Ingredient for Use in Food Supplements

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 Concerning Novel Foods and Novel Food Ingredients

EXECUTIVE SUMMARY

Approval is sought under Regulation (EC) No 258/97 for use of Calanus® Oil, the oil obtained from the small crustacean, or copepod, *Calanus finmarchicus* for an ingredient in food supplement products. Although, historically there is some evidence for the limited consumption of *C. finmarchicus* by sailors on long expeditions or that were shipwrecked, the ingredient is best categorised under Article 1(2) of Regulation 258/97 as (e) "foods and food ingredients consisting of, or isolated from, plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe use." According to Section 4 of Commission Recommendation 97/618/EC Calanus® Oil belongs to Class 2.2: "the source of the novel food has no history of use in the Community".

Compositionally, the ingredient consists primarily of wax esters (>85%), or monoesters of fatty acids and fatty alcohols, with minor amounts of other lipid classes (*e.g.*, triglycerides). The fatty acid profile of Calanus® Oil is typical of existing oils of marine origin such as fish and krill oils, including significant levels of SDA (*ca*. 7%), EPA (*ca*. 7%) and DHA (*ca*. 5%). Similarly, the fatty alcohol profile is also consistent with that of other marine organisms and products derived thereof, containing significant levels of C20:1 n-9 and C22:1 n-11 species. The intact wax esters are generally between 30 and 44 carbons in length. Other minor components of the oil were identified to be astaxanthin and sterols with profiles typical of other marine oils. No residues of protein were detected in Calanus® Oil by gel electrophoresis and bicinchoninic acid (BCA) methods.

The absence of any external contaminants at levels of potentially toxicological concern has been confirmed analytically by external laboratories with the appropriate Member State or equivalent accreditation.

In terms of stability, while it is anticipated that the pathways of oxidative degradation commonly associated with long-chain polyunsaturated systems may occur, studies indicate that wax esters may represent a more stable form of these fatty acids than their triglyceride or ethyl ester counterparts. In this respect, wax esters may provide formulation advantages over other forms of long-chain polyunsaturated fatty acids when used as an ingredient in food supplement products.

The processes employed in the harvesting, extraction and refinement of oil from *C. finmarchicus* are conventional techniques commonly employed by the fats and oils industry. The entire production process is covered by a quality system based on good manufacturing practices and HACCP principles. The processes are not anticipated to alter the levels of substances in the novel ingredient that might have an adverse effect on human health.

C. finmarchicus is a herbivorous copepod or miniature shrimp with a lifespan of approximately one year and is the most abundant zooplankton species found in North Atlantic waters. Along with other marine zooplankton, *C. finmarchicus* plays an intermediary role in the food chain, and represents a major source of secondary energy to many marine organisms (*e.g.*, Atlantic herring, squid and salmon). Many of the marine organisms feeding on *C. finmarchicus* are consumed directly by humans or indirectly from marine-derived foods such as oils.

Calanus® Oil is intended for use as an ingredient in food supplements at levels not to exceed 250 mg of EPA plus DHA (*i.e.*, *ca*. 2.3 g of oil). The ingredient would be a direct replacement for other marine oil sources of EPA plus DHA such as fish, algal and krill oils. The proposed maximum use level permits the daily intake of Calanus® Oil to provide the Adequate Intake for EPA and DHA of 250 mg recently allocated by EFSA in the Opinion by the Scientific Panel on Dietetic Products, Nutrition and Allergies on DRVs for fats including saturated fats, polyunsaturated fatty acids, monosaturated fatty acids, trans fatty acids and cholesterol. Moreover, traditional fish oil supplement products currently on the market in the EU frequently deliver at least 250 mg of EPA plus DHA per daily serving.

There is a long history of consumption of wax esters of marine sources with similar fatty acid and fatty alcohol profiles to Calanus® Oil from the normal diet. Examples include deep water fish such as Orange Roughy that deposit the wax esters ingested *via* zooplankton in the diet, and mullet roe products (Botago or Greek Avgotaracho). Specifically, a typical 85 g serving of fish roe products may contain in the region of 13 to 71 g of wax or steryl esters. These intake estimates are at least 6-fold greater than the amount of wax esters delivered by a daily serving of a food supplement product containing Calanus® Oil (*i.e.*, up to *ca*. 2.3 g of oil). Wax esters *per se* are normal components of the diet with significant levels present in honeycomb, cereal grains, vegetables, fruits, nuts and seeds.

The absence of any micro-organisms at levels of potentially toxicological concern has been confirmed analytically by an external laboratory with the appropriate Member State or equivalent accreditation.

There are literature studies to suggest wax esters are only partially hydrolysed and assimilated in mammals, and mammalian lipases hydrolyse wax esters at a slower rate compared to triglycerides *in vitro*, suggesting the capacity to digest wax esters may also be limited in humans. However, there is evidence to suggest that following ingestion of wax esters, EPA and DHA are incorporated into plasma phospholipid to a similar extent as when these PUFAs are ingested in the form of fish oils or ethyl esters in rats (Gorreta *et al*., 2002). This study suggests consumption of wax esters in Calanus® Oil at the levels of intended use (*i.e.*, no more than 2.3 g per day with meals) is likely to produce reasonable systemic incorporation of EPA and DHA in humans. The fatty acid and fatty alcohol components of Calanus® Oil would be expected to undergo the normal metabolic fate of these substances from other dietary sources.

While focussing specifically on safety, there were no adverse effect reported in a number of studies in rats and mice supplemented with Calanus® Oil at levels of up to 1.5% or 1%, respectively of the diet. In a 112 day rat study, an intake level of 1.5% of the diet was determined to be equivalent to approximately 770 mg/kg body weight/day, which is far greater than the intended levels of exposure of humans to wax esters from food supplements containing Calanus® Oil.

The safety of Calanus® Oil is further supported by a study in humans in which participants' ingested doses of between 1 g, 2 g and 4 g of oil per day over a 4 week period. The highest dose tested is approximately 2-fold greater than the proposed maximum use level of Calanus® Oil in food supplement products. Overall, Calanus® Oil was well tolerated by study subjects and no significant adverse effects were reported.

Calanus® Oil is not anticipated to be associated with any allergenicity although products will be labelled in accordance with Annex IIIa of Directive 2000/13/EC to indicate the source. While it is recognised that intolerance to Oilfish and Escolar is generally attributed, at least in part, to their wax ester content, there are many examples of other significant sources of wax esters not associated with gastrointestinal effects at levels exceeding (>6-fold) the anticipated use of Calanus® Oil (*i.e.*, fish roe products, Orange Roughy). Moreover, the gastrointestinal effects commonly associated with Oifish and Escolar were not reported by participants of the human study consuming Calanus® Oil at levels equal or approximately 2-fold greater than the intended use of the novel ingredient .

INTRODUCTION

Calanus AS (Calanus) wishes to market the oil obtained from the small crustacean or copepod, *Calanus finmarchicus*, which is the dominant marine zooplankton species found in North Atlantic waters, as a food ingredient in the European Union (EU). The oil primarily contains wax esters comprised of typical long-chain fatty alcohols and long-chain polyunsaturated fatty acids of marine origin. The oil is intended to be marketed under the trade name Calanus® Oil as an ingredient in food supplement products.

Although, historically there is evidence for the limited consumption of marine zooplankton species by coastal communities in Europe, the oil and its source have not been previously marketed as food ingredients in the EU to a significant extent. Consequently, Calanus® Oil should be considered “novel” falling under category (e) of Article 1(2) of Regulation (EC) No 258/97: “foods and food ingredients consisting of, or isolated from, plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe use.” Furthermore, according to Section 4 of Commission Recommendation 97/618/EC (Commission of the European Communities, 1997) Calanus® Oil belongs to Class 2.2: “the source of the novel food has no history of use in the Community”.

The dossier herein follows the structured schemes required to establish the safety of a Class 2.2 novel food ingredient:

I Specification of the novel food
II Effect of the production process applied to the novel food
III History of the organism used as the source of the novel food
IX Anticipated intake/extent of use of the novel food
XI Nutritional information on the novel food
XII Microbiological information on the novel food
XIII Toxicological information on the novel food

At the start of each section the information that must be addressed in that structured scheme is specified in more detail.

A glossary is provided at the end of the document to explain the abbreviated terms referred to in the dossier.

1. ADMINISTRATIVE DATA

Applicant Details

Calanus AS
Stakkevollv. 65
P.O. Box 2489
N-9272 Tromsø
Norway

I SPECIFICATIONS OF THE NOVEL FOOD

Based on the Scientific Committee on Food (SCF) guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- “Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?”
- “Is the information representative of the novel food when produced on a commercial scale?”
- “Is there an appropriate specification (including species, taxon *etc.* for living organisms) to ensure that the novel food marketed is the same as that evaluated?”

These questions have been addressed collectively in the section that follows.

I.1 Description and Specification of the Novel Ingredient

I.1.1 Description of the Novel Ingredient

Calanus® Oil is ruby coloured, slightly viscous oil with a slight shellfish odour extracted from the marine zooplankton, *Calanus finmarchicus*. The ingredient consists primarily of wax esters (>85%) with minor amounts of triglycerides and other neutral lipids (<10%), and polar lipids (<5%).

I.1.2 Chemical Name

"oil from *Calanus finmarchicus* (marine zooplankton)"

I.1.3 Trade Name

Calanus® Oil

I.1.4 Specification of the Novel Ingredient

A specification for Calanus® Oil is proposed in Table I.1.4-1 and includes measure of the composition, purity and oxidative quality of the material. Where applicable the specification is consistent with those reported for other marine oils approved as novel food ingredients as source of fatty acids in the EU, for example krill and algal oils (Decision 2009/752/EC – Commission of the European Communities, 2009; and Decision 2003/427/EC – Commission of the European Communities, 2003).

Table I.1.4-1 Specification of the Novel Ingredient

Parameter	Specification	Method of Analysis
Appearance	Ruby, slightly viscous liquid	Visual inspection
Odour	Light shellfish odour	Olfactory
Water	<1%	Gravimetric determination
Wax esters	>85%	HPLC-ELSD
Total fatty acids	>50%	GC-FID
Eicosapentaenoic acid (EPA)	>3%	GC-FID
Docosahexaenoic acid (DHA)	>5%	GC-FID
Total fatty alcohols	>36%	GC-FID
C20:1 n-9 fatty alcohol	>12%	GC-FID
C22:1 n-11 fatty alcohol	>16%	GC-FID
Trans fatty acids	<1%	GC-FID
Peroxide value	<3 meq. O_2/kg	AOCS Cd 8b-90

HPLC-ELSD = high performance liquid chromatography-evaporative light scattering detection; GC-FID = gas chromatography-flame ionisation detection; AOCS = American Oil Chemists Society.

Note: where appropriate all analyses relating to the composition and levels of undesirable substances are performed by laboratories with the appropriate Member State or equivalent accreditation. Details on the laboratory accreditation and methodologies employed are provided in Appendices A-8 and B-5.

I.2 Composition of the Novel Ingredient

I.2.1 Lipid Profile

The lipid profiles of 3 batches of Calanus® Oil are provided in Table I.2.1-1 and confirm that the ingredient is primarily comprised of wax esters (>86%) with minor amounts of free fatty acids, free fatty alcohols, and glycerides. No individual minor component represents greater than 3% of the oil. Certificates of Analysis are provided in Appendix A-1.

Table I.2.1-1 Lipid Profiles of 3 Batches of Calanus® Oil

Lipid Class	Analytical Data (% of oil)			Mean[1]
	Batch 1	Batch 2	Batch 3	
Wax ester	**86.2**	**86.3**	**89.0**	**87.2**
Free fatty acid	0.7	1.9	1.6	1.4
Free fatty alcohol	1.1	1.0	1.2	1.1
Triglyceride	3.3	2.7	2.4	2.8
Diglyceride	0.3	0.3	2.1	0.9
Monoglyceride	0.4	0.9	0.5	0.6
Cholesterol	0.3	0.3	0.5	0.4
Phospholipids	-	-	-	-
Sum of neutral lipids	>91	>91	>96	>91
Total lipids	92.3	93.5	97.3	-

[1] Mean of 3 batches of oil.

I.2.2 Fatty Acid Profile

The fatty acid profiles of 3 batches of Calanus® Oil are summarised in Table I.2.2-1 and Certificates of Analysis are provided in Appendix A-1. The fatty acids identified are those typically obtained in species of marine origin, including significant amounts of stearidonic acid (SDA; *ca*. 7%), eicosapentaeonic acid (EPA; *ca*. 7%) and docosahexaenoic acid (DHA; *ca*. 5%). A comparison of the fatty acid profile of Calanus® Oil with that of other marine oils is provided in Section XI.

Table I.2.2-1 Fatty Acid Profiles of 3 Batches of Calanus® Oil

Fatty Acid (Common Name)[1]	Analytical Data (% of oil)			Mean[2]
	Batch 1	Batch 2	Batch 3	
C14:0 (myristic acid)	8.3	8.2	7.9	8.1
C14:1 n-5	0.2	0.3	0.3	0.3
C15:0	0.3	0.4	0.4	0.4
C16:0 (palmitic acid)	4.6	5.3	4.7	4.9
C16:1 n-9	0.1	0.1	0.3	0.2

Table I.2.2-1 Fatty Acid Profiles of 3 Batches of Calanus® Oil				
Fatty Acid (Common Name)[1]	**Analytical Data (% of oil)**			**Mean**[2]
	Batch 1	**Batch 2**	**Batch 3**	
C16:1 n-7 (palmitoleic acid)	2.9	1.8	2.2	2.3
C16:1 n-5	0.2	0.3	0.3	0.3
C17:0	0.1	0.1	0.1	0.1
C17 undefined	0.2	0.2	0.2	0.2
C16:2 n-7	0.4	0.2	0.3	0.3
C16.3 n-4	1.1	0.3	0.4	0.6
C18:0 (oleic)	0.4	0.4	0.4	0.4
C16:4 n-1	1.8	0.5	1.1	1.1
C18:4 n-9	1.3	1.6	1.4	1.4
C18:1 n-7	0.4	0.3	0.3	0.3
C18:2 n-6	0.5	0.7	0.6	0.6
C18:3 n-6	0.5	0.3	0.5	0.4
C18:3 n-3	0.8	1.4	1.0	1.1
C20:0	0.1	0.1	0.1	0.1
C18:4 n-3 (stearidonic acid, SDA)	**6.7**	**7.3**	**7.1**	**7.0**
C20:1 n-11	0.8	0.7	0.7	0.7
C20:1 n-9 (gadoleic acid)	2.5	2.6	2.4	2.5
C20:1 n-7	0.7	0.8	0.7	0.7
C18:3 n-3	-	-	0.4	<0.1
C20:2 n-6	0.1	0.1	0.1	0.1
C20:3 n-6	0.1	0.1	-	0.1
C20:4 n-6	0.2	0.1	1.3	0.5
C20:3 n-3	0.1	0.1	-	0.1
C22:0	-	-	0.1	<0.1
C20:4 n-3	0.6	0.6	0.7	0.6
C22:1 n-11 (cetoleic acid)	4.8	5.1	5.3	5.1
C22:1 n-9	0.2	0.2	-	0.1
C20:5 n-3 (eicosapentaenoic acid, EPA)	**7.0**	**5.6**	**7.2**	**6.6**
C21:5 (undefined, assumed n-3)	0.4	0.3	0.3	0.3
C24:0	-	-	0.1	<0.1
C24:1 n-9	0.3	0.3	0.5	0.4
C22:5 n-3	0.4	0.3	0.5	0.4
C22:6 n-3 (docosahexaenoic acid, DHA)	**4.2**	**4.8**	**4.6**	**4.5**
Sum of fatty acids	53.3	51.5	54.5	53.1
Sum of saturated fatty acids	14.0	14.7	14.1	14.3
Sum of monosaturated fatty acids	14.4	14.0	14.4	14.3
Sum of polyunsaturated fatty acids	24.7	22.6	26.2	24.5
Sum of n-3 fatty acids	19.8	20.0	21.5	20.4
Sum of EPA plus DHA	**11.2**	**10.4**	**11.8**	**11.1**

[1] Common name of all fatty acids typically present at levels of 2% oil or greater are indicated;
[2] Mean of 3 batches of oil.

I.2.3 Fatty Alcohol Profile

The fatty alcohol profiles of 3 batches of Calanus® Oil are provided in Table I.2.3-1 and Certificates of Analysis are provided in Appendix A-1. The primary fatty alcohols identified are C20:1 n-9 (*ca*. 13%) and C22:1 n-11 (*ca*. 20%). These fatty alcohols are consistent with those obtained from other marine sources and data for a range of species or their products that form part of the normal diet are provided in Section XI.

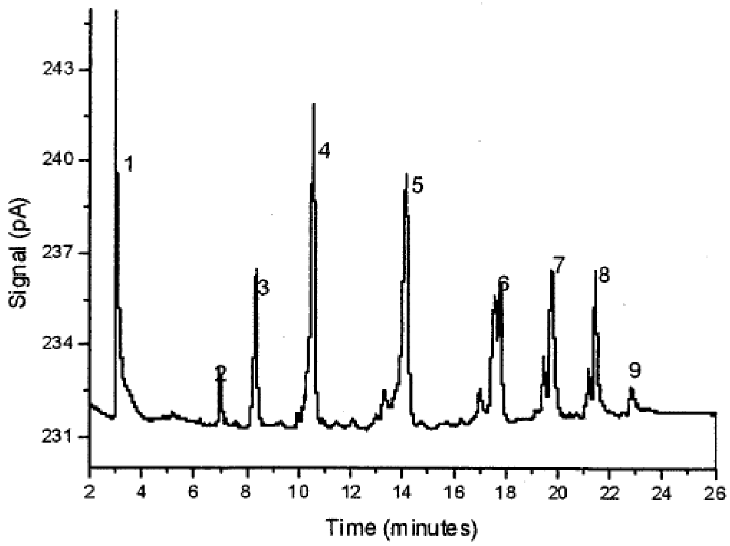
Table I.2.3-1 Fatty Alcohol Profiles of 3 Batches of Calanus® Oil

Fatty Alcohol	Analytical Data (% of oil)			Mean
	Batch 1	Batch 2	Batch 3	
C14:0	8.3	8.2	7.9	8.1
C16:0	3.6	2.8	2.7	3.0
C16:1 n-7	1.8	0.6	0.9	1.1
C18:1 n-9	1.1	1.0	1.0	1.0
C20:1 n-9	**13.3**	**12.9**	**13.1**	**13.1**
C22:1 n-11	**17.3**	**18.8**	**22.3**	**19.5**
C22:1 n-9	1.0	1.0	1.3	1.1
Sum of fatty alcohols	46.4	45.3	49.2	46.9
Sum of monosaturated fatty alcohols	34.5	34.3	38.6	35.8

I.2.4 Wax Ester Profile

A representative Gas Chromatography (GC) trace of Calanus® Oil displaying the intact wax esters is presented in Figure I.2.4-1 and the full study report is provided in Appendix A-2. The peaks may be assigned according to the chain length from the fatty acid and fatty alcohol moieties with species containing between 30 and 44 carbons identified.

Figure I.2.4-1 GC Trace of Intact Wax Esters of a Representative Batch of Calanus® Oil



Peak	Chain Length (Fatty Acid + Fatty Alcohol)
1	Internal standard (hexadecane)
2	C30
3	C32
4	C34
5	C35
6	C38
7	C40
8	C42
9	C44

I.2.5 Trans Fatty Acid Content

The results of trans fatty acid analyses on 3 batches of Calanus® Oil are summarised in Table I.2.5-1 and the Certificate of Analysis provided in Appendix A-3. Although, a maximum level for the trans fatty acid content of foods has not been set by the European Commission to date, Denmark has established a maximum level of 2 g per 100 g of oil under Executive Order 160 (Danish Veterinary and Food Administration, 2003) and levels of less than 1% are generally considered desirable. The level of trans fatty acids falls well below 1% for all the batches tested.

Table I.2.5-1 Trans Fatty acid Contents of 3 Batches of Calanus® Oil

Component	Analytical Data (% of oil)		
	Batch 1	Batch 2	Batch 3
Total trans fatty acids	0.3	-	-

I.2.6 Astaxanthin

The astaxanthin contents of 3 batches of Calanus® Oil are presented in Table I.2.6-1 and Certificates of Analysis are provided in Appendices A-4. Calanus® Oil contains in the region of 0.8% astaxanthin primarily in the esterified form.

Table I.2.6-1 Astaxanthin Content of 3 Batches of Calanus® Oil			
Component	**Analytical Data (% of oil)**		
	Batch 1	**Batch 2**	**Batch 3**
Total astaxanthin	0.081	0.094	0.078
Esterified astaxanthin	0.075	0.086	0.070

I.2.7 Sterol Content

In addition to the cholesterol content of Calanus® Oil, which is measured routinely as part of the lipid profile of the ingredient with levels of up to 0.5% reported in Table I.2.1-1 above, minor amounts of other sterols may also be present. The sterol profile of a representative batch of Calanus® Oil is provided in Table I.2.7-1 and low levels of other compounds. A Certificate of Analysis is provided in Appendix A-5.

Table I.2.7-1 Sterol Profile of a Representative Batch of Calanus® Oil			
Component	**Analytical Data**		
	% by weight of sterols	**mg/g of oil**	**% of oil**
Cholesterol	45.3	2.1	0.21
Brassicasterol	53.6	2.5	0.25
Campesterol	0.9	0.04	0.004
Sitosterol	0.2	0.01	0.001
Sum sterols (with cholesterol)	100	4.7	0.47

I.2.8 Water Content

The moisture contents of 3 batches of Calanus® Oil are presented in Table I.2.8-1 and all values reported fall below 1%. Certificates of Analysis are provided in Appendices A-4.

Table I.2.8-1 Water Content of 3 Batches of Calanus® Oil			
Component	**Analytical Data (% of oil)**		
	Batch 1	**Batch 2**	**Batch 3**
Water	0.6	0.8	0.8

I.2.9 Protein Content

The protein content of Calanus® Oil has been investigated by gel electrophoresis and by high salt extraction followed by detection using the bicinchoninic acid (BCA) assay and gel electrophoresis techniques, as described in Appendix A-6. No residues of protein were detected in Calanus® Oil in either of the methods described.

I.3 Potential for Presence of External Contaminants

I.3.1 Heavy Metals

The results of heavy metals analyses for 3 batches of Calanus® Oil are presented in Table I.3.1-1 and Certificates of Analysis provided in Appendices A-4. All values reported for the metals tested are below detection limits or fall well below the maximum limits specified by Commission Regulation (EC) No 1881/2006 (Commission of the European Communities, 2006) as amended by Commission Regulation (EC) No 629/2008 (Commission of the European Communities, 2008a) of 0.1 mg/kg wet weight in fats and oils for lead, and 1.0 and 0.10 mg/kg wet weight respectively, for cadmium and mercury in food supplements as sold.

Table I.3.1-1 Results of Heavy Metals Analysis for 3 Batches of Calanus® Oil

Parameter	Maximum Limits (mg/kg wet weight)[1]	Analytical Data (mg/kg of oil)		
		Batch 1	Batch 2	Batch 3
Arsenic (inorganic)[2]	-	<0.1	<0.1	0.1
Cadmium	1.0[3]	0.026	0.29	0.73[4]
Lead	0.10[5]	<0.02	<0.02	<0.02
Mercury	0.10[3]	<0.02	<0.02	<0.02

[1] Maximum limits as specified under Regulation (EC) 1881/2006 (Commission of the European Communities, 2006) or its amendment, Regulation (EC) 629/2008 (Commission of the European Communities, 2008a) considered applicable to Calanus® Oil for the intended use as an ingredient in food supplements – limits are set for wet weight but given the reported values for Calanus® Oil fall well below the maximum levels no adjustment for the moisture content was considered necessary;
[2] Only inorganic arsenic measured on the basis that this is the form of arsenic of toxicological concern, which provisional tolerable weekly intake of 2.14 µg/kg bw/day set by the Joint WHO/FAO Expert Committee on Food Additives (JECFA);
[3] Limit applies to food supplements as sold;
[4] In Calanus experience, levels are not usually this high with variability between batches typically ranging from 0.02 to 0.5 mg/kg (not adjusted for moisture content);
[5] Limit applies to fats and oils, excluding milk fat.

Although, meeting legislative requirements for cadmium food supplements, it is noted that the levels of this metal in Calanus® Oil can vary between batches. Cadmium is known to concentrate in marine animals with bioaccumulation factors ranging from 5 to 3160 reported for saltwater aquatic species compared to their water (ASTER, 1994; EFSA, 2009). Reflecting the varying accumulation of cadmium in marine organisms, under Regulation 629/2008 (Commission of the European Communities, 2008a) fish species were re-grouped from previous legislation into different categories and maximum levels. Additionally, levels are recognised to be relatively high in certain marine products with maximum levels of 3.0 mg/kg wet weight set for food supplements exclusively or mainly comprising of dried seaweed or products derived from dried seaweed.

I.3.2 Dioxins and Dioxin-Like Polychlorinated Biphenyls (PCBs)

The results of dioxin and dioxin-like PCBs analyses for 3 batches of Calanus® Oil are presented in Table 1.3.2-1 and the Certificates of Analysis provided in Appendices B-1. The values reported for Calanus® Oil fall well below the limits specified for marine oils by

Commission Regulation (EC) No 1881/2006 (Commission of the European Communities, 2006) of 2.0 pg/g fat for dioxins and 10 pg/g fat for dioxins and dioxin-like PCBs.

Table I.3.2-1 Results of Dioxin and Dioxin-Like PCBs Analysis for 3 Batches of Calanus® Oil

Parameter	Maximum Limits (pg/g fat)[1]	Analytical Data (pg/kg fat)		
		Batch 1	Batch 2	Batch 3
Sum of dioxins (WHO-PCDD/F-TEQ)	2.0	1.55	1.09	0.74
Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)	10.0	2.43	2.00	1.66

[1] Marine oils intended for human consumption.

I.3.3 Polycyclic Aromatic Hydrocarbons (PAHs)

A screen for PAHs has been performed on 3 batches of Calanus® Oil and the Certificates of Analysis are provided in Appendix B-2. The results for benzo(a)pyrene, as a marker for other PAHs, are presented in Table I.3.3-1 where the values reported fall well below the limit specified for this substance in oils by Commission Regulation (EC) No 1881/2006 (Commission of the European Communities, 2006) of 2.0 µg/g wet weight. In general, the levels of all PAHs detected in the 3 batches of Calanus® Oil were considered to be acceptable by the independent testing laboratory.

Table I.3.3-1 Benzo(a)pyrene Analysis for 3 Batches of Calanus® Oil

Parameter	Maximum Limits (µg/g wet weight)[1]	Analytical Data (µg/kg)		
		Batch 1	Batch 2	Batch 3
Benzo(a)pyrene	2.0	0.27	0.33	0.27

[1] Oils and fats intended for direct human consumption or as ingredients in food – limits are set for wet weight but given the reported values for Calanus® Oil fall well below the maximum levels no adjustment for the moisture content was considered necessary.

I.3.4 Polybrominated Diphenyl Ethers (PBDEs)

A screen for residues of PBDEs has been performed on 3 batches of Calanus® Oil and the Certificates of Analysis are provided in Appendices B-3. The concentrations of PBDEs for all the batches tested are below detection limits or at levels which do not pose a toxicological concern under the conditions of intended use of Calanus® Oil.

I.3.5 Pesticides

Calanus® Oil is harvested from natural marine environments and the potential for contamination with pesticides is low. A pesticide screen has been performed on 3 batches of Calanus® Oil and the Certificates of Analysis are provided in Appendices B-4. The screen included hexachlorocyclohexane (HCH) isomers, dichlorodiphenyltrichloroethane (DDT) and related substances/isomers and chlorinated pesticides. The levels of residues in the batches tested were found to either be below detection limits or at levels which do not pose a toxicological concern under the conditions of intended use of Calanus® Oil.

I.4 Oxidative Quality and Stability of the Novel Ingredient

I.4.1 Oxidative Quality

Analytical data for 3 batches of Calanus® Oil relating to measures of the oxidative quality of the ingredient are presented in Table I.4.1-1. The peroxide, *p*-anisidine and totox values reported for all batches of Calanus® Oil fall within the range considered acceptable for marine oils intended for human consumption, as illustrated by the maximum levels specified for fish oils rich in omega-3 fatty acids by the *European Pharmacopeia* (Ph. Eur., 2007).

Table I.4.1-1 Oxidative Quality of 3 Batches of Calanus® Oil

Parameter	Units	Ph. Eur.	Internal Specification	Analytical Data		
				Batch 1	Batch 2	Batch 3
Peroxide value	meq O_2/kg	Max. 10.0	<3	2	<1	<1
p-Anisidine value	-	Max. 30.0	<4	3.3	<2	2.6
Totox value (calculation)[1]	-	Max. 20.5	<10	7.3	<4	<4.6

[1] 2 x peroxide value + anisidine value.

I.4.2 Shelf-Life and Stability

1.4.2.1 Shelf-Life and Storage Conditions

The shelf-life of Calanus® Oil is 3 years when stored under an inert (N_2) atmosphere in sealed unopened containers below 25 °C in the absence of light.

I.4.2.2 Anticipated Degradation Pathways

On the basis that Calanus® Oil is comprised of fatty acids and fatty alcohols typically identified in marine organisms, it is anticipated that the oxidative degradation pathways commonly associated with long-chain polyunsaturated fatty acids might occur during prolonged exposure. These pathways are well established and associated with detrimental effects on the organoleptic properties of the product.

The degree of susceptibility to oxidation of long-chain polyunsaturated fatty acids extracted from fish oils has been compared for the triglyceride, ethyl ester and wax ester forms (Gorretta *et al*., 2002). The wax ester form was reported to have a peroxide value of less than1 meq. O_2/kg of oil on production, 5 to 7 times lower than for the ethyl ester form and which did not increase after exposure to air for 1 week. Similarly, production of malonyldialdehyde used as a marker of peroxidation was significantly lower in wax esters than fish oils (triglyceride form) exposed to air for 1 week. These results indicate that generally, wax esters may represent a more stable form of long-chain polyunsaturated fatty acids than their triglyceride or ethyl ester counterparts. In this respect, wax esters may provide formulation advantages over other forms of long-chain polyunsaturated fatty acids in the production of food supplement products.

I.4.2.3 Stability Studies

Stability studies have been performed on Calanus® Oil and the study report is provided in Appendix C-1.

The stability of Calanus® Oil has been monitored by measuring the total oxidation (totox) value for representative batches of Calanus® Oil stored from between 1 to 4 years under the recommended conditions. The results are summarised in Table I.4.2.3-1 and demonstrate that the oxidative quality of the material remains acceptable during storage falling well within the limit of 10 laid down by the internal product specification (see Table I.4.1-1).

Table I.4.2.3-1 Total Oxidation Values (Totox) of Representative Batches of Calanus® Oil After Storage

Storage Duration (days)	Totox (calculated)
412	4
730	7.3
1275	7.4
1640	6

Astaxanthin present naturally in Calanus® Oil will act as an antioxidant (oxygen scavenger) and be consumed during any oxidation processes that might occur over time. The levels of astaxanthin in 2 batches of Calanus® Oil after storage for over 3 years are presented in Table I.4.2.3-3. The results indicate that no more than 14% of the astaxanthin is consumed during the storage period measured. Other antioxidants approved for use in food by Council Directive No 95/2/EC (European Parliament and Council of the European Union, 1995; as amended or replaced) might also be added to Canalus® Oil if necessary to prolong the shelf-life of the ingredient used in food supplement products.

Table I.4.2.3-2 Astaxanthin Content of Representative Batches of Calanus® Oil After Storage

Batch	Storage Duration (days)	Total Astaxanthin[1] Content (mg/kg oil)	
		Initial Content	Final Content
1	1221	1700	1450
2	1221	1700	1700

[1] Free plus esterified astaxanthin.

Additionally, the degree of oxidation may also be monitored over time by measuring the ratio of EPA and DHA to palmitic acid, commonly referred to as the polyene ratio. The change in polyene content of a representative batch of Calanus® Oil stored for approximately 1 year is provided in Table I.4.2.3-3. Data for other marine oils commonly consumed in food supplement products are also provided in Table I.4.2.3-3. The data for the different marine oils are not directly comparable because the ingredients have not been subjected to identical conditions and periods of storage, but overall the results demonstrate that the changes in polyene ratio observed in the Calanus® Oil sample over 1 year are consistent with the effects generally exhibited by marine oils.

Table I.4.2.3-3 Polyene Ratio of Representative Batches of Calanus® Oil and Other Marine Oils After Storage			
Oil	**Storage Duration (days)**	**Polyene Ratio[1]**	
		Initial Ratio	**Final Ratio**
Calanus® Oil	360	174	172
Krill oil	15	238	225
Squid oil	40	187	55
Sardine oil	31	180	145

[1]Palmitic acid/DHA+EPA.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

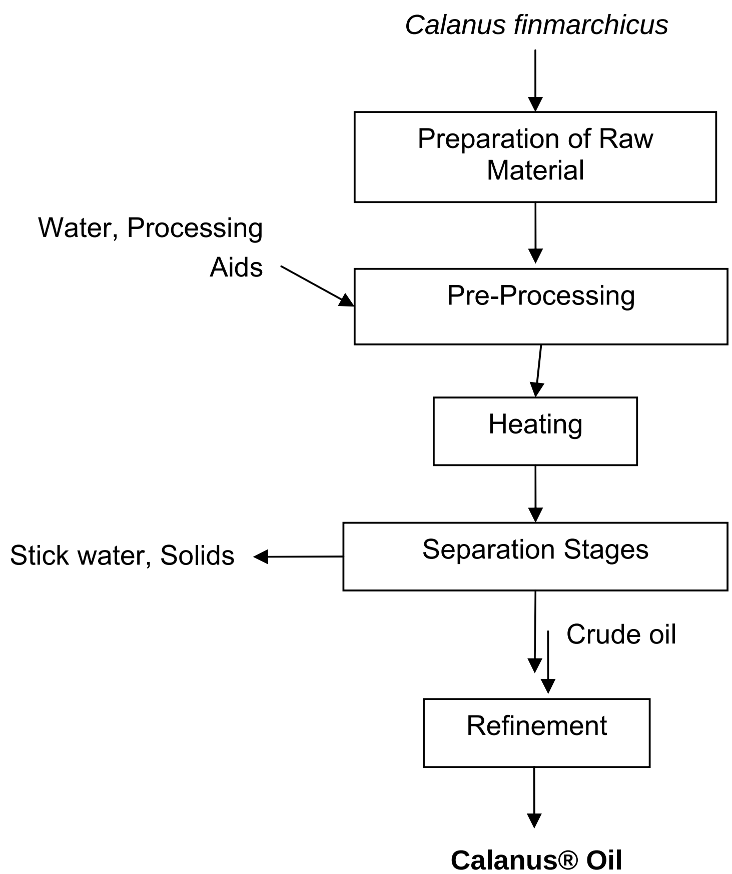
- “Does the novel food undergo a production process?”
- “Is there a history of use of the production process for the food?” If no, “does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?”
- “Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?”
- “Are the means identified for controlling the process to ensure that the novel food complies with its specification?”
- “Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?”
- “After processing is the novel food likely to contain microorganisms of adverse public health significance?”

These questions have been addressed collectively in Sections II.1.

II.1 Overview of the Production Process

An overview of the production process to Calanus® Oil is provided in Figure II.1-1. The full details of the manufacturing process are considered proprietary but involve separation of the oil from the zooplankton raw material followed by standard refinement processes.

Figure II.1-1 Overview of the Production Process



II.2 History of Use of the Production Process

II.2.1 Production Processes

The processes employed in the harvesting, extraction and refinement (purification) of oil from *Calanus finmarchicus* are conventional techniques of the fats and oils industry and commonly utilised in the production of fish and other marine oils for consumption as ingredients in foods or food supplements in the EU (Smith and Hong-Sum, 2003; Ackman, 2005).

II.2.2 Processing Aids

All processing aids are suitable for food use but this information is considered proprietary to the manufacturer.

II.3 Production Controls and Identification of Potential Hazards

II.3.1 Production Controls and Hazard Identification

The entire production process to Calanus® Oil including harvesting of the raw material, storage, transport, extraction and packaging is covered by a quality system based on Hazard Analysis and Critical Control Points (HACCP) principles.

II.3.2 Quality Controls and Compliance with Specification

Quality control procedures are in place in order to ensure that Calanus® oil complies with the product specification (see Section I.1.4-1) and these details are considered proprietary to the manufacturer. Where the novel ingredient does not meet the product specification, if the deviations are minor the material is reprocessed but where major deviations are identified, the product is discarded.

II.3.3 Potential to Alter Levels of Substances in the Novel Ingredient with an Adverse Effect on Public Health

The production of Calanus® Oil is not anticipated to be associated with the potential formation or accumulation of substances hazardous to health. Detailed analysis on the composition and potential for levels of undesirable substances in the novel ingredient are provided in Sections I.2 and I.3. Furthermore, Canalus® Oil is manufactured consistent with good manufacturing practices using food grade quality processing aids and conventional techniques of the marine oils industry as discussed in Section II.2 above. The testing regime for the raw material and Calanus® Oil in terms of compositional parameters and potential contaminants is summarised in Table II.3.3-1.

Table II.3.3-1 Sampling and Analysis Plan for Raw Material and Calanus® Oil in Terms of Composition and Levels of Undesirable Substances

Test	Raw Material	Calanus® Oil
Composition		
Protein	X	
Fat	X	
Water	X	X
Fatty acid profile		X
Lipid classes		X
Fatty alcohols		X
Astaxanthin (esterified and total)		X
Contaminants		

Table II.3.3-1 Sampling and Analysis Plan for Raw Material and Calanus® Oil in Terms of Composition and Levels of Undesirable Substances

Test	Raw Material	Calanus® Oil
As (arsenic)		X
Cd (cadmium)		X
Pb (lead)		X
Hg (mercury)		X
Oxidative Quality		
Peroxide		X
p-Anisidine		X

II.3.4 Potential for Microbiological Contamination

Cold water-adapted bacteria found naturally in the raw material will not grow under the conditions employed during the production of Calanus® Oil. Microbiological contamination from other sources should be controlled during the heating process (90 °C for at least 20 minutes). The sampling and analysis plan for the raw material and Calanus® Oil in terms of microbiological contamination is summarised in Table II.3.4-1. Analysis confirming the absence of any microbiological contaminants is provided in Section XII.

Table II.3.4-1 Sampling and Analysis Plan for Raw Material and Calanus® Oil in Terms of Microbiological Hazards

Test	Raw Material	Calanus® Oil
Microbiology		
Aerobic microorganisms	X	X
Coliforms and *Escherichia coli*	X	X
Staphylococcus spp.		X
Salmonella spp.		X
Listeria monocytogenes		X
Yeast and mould		X

III HISTORY OF THE SOURCE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

- "Is the novel food obtained from a biological source, *i.e.*, a plant, animal or microorganism?"
- "Has the organism used as the source of the novel food been derived using GM?"
- "Is the source organism characterised?"
- "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

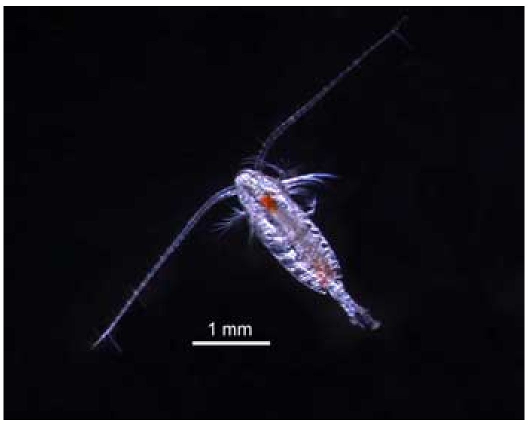
These questions have been addressed collectively in the following section.

III.1 Source of the Novel Ingredient

III.1.1 Identification and Characterisation of the Source

Calanus® Oil is extracted from *Calanus finmarchicus*, a herbivorous copepod or miniature shrimp (3 to 4 mm in length) that feeds on phytoplankton, and to a smaller extent, proto-zooplankton (see Figure III.1.1-1). It has a lifespan of approximately one year, and is one of the most abundant species of zooplankton found in the North Atlantic Ocean. In the Nordic Seas, it is the most abundant zooplankter by biomass and the major contributor to the estimated annual production of zooplankton biomass of 200 to 400 million tons wet weight (Skjoldal *et al*., 2004).

Figure III.1.1-1 Image of *Calanus finmarchicus*[1]



The scientific classification of the source is summarised in Table III.1.1-1. As expected for a natural marine organism, *C. finmarchicus* is not derived using genetic modification (GM).

Table III.1.1-1 Scientific Classification of *Calanus finmarchicus*

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustacea
Class	Maxillopoda
Subclass	Copepoda
Infraclass	Neocopepoda
Superorder	Gymnoplea
Order	Calanoida
Family	Calanidae
Genus	Calanus
Species	*Calanus finmarchicus*

[1] Reproduced from http://www.coastalwiki.org/coastalwiki/Image:Calanus_finmarchicus.jpg

III.1.2 Nutritional Significance of the Source

In terms of the aquatic ecosystem, marine zooplankton generally play an intermediary role between the primary organisms in the food chain, *e.g.*, phytoplankton and those of higher tropic level *e.g*., fish and marine mammals. *C. finmarchicus* specifically represents a major source of secondary energy to red fish (*Sebastes sp.*, Atlantic herring and salmon (Yusuf and Webster, 2008; FAO, 2011). As such, *C. finmarchicus* is a major contributor to the lipid stores in the muscles and fat deposits of many marine organisms commonly consumed as part of the normal diet of the European population, and from which marine oils are traditionally derived.

III.1.3 Storage of Lipids in the Source

Marine zooplankton, and particularly copepods from cold water habitats, are characterised by their ability to feed on phytoplankton and generate large lipid stores (Lee *et al*., 2006). These lipids stores are typically located in the oil sacs, oil droplets, and gonadal tissues of the organism, and primarily take the form of wax esters or triglycerides. Triglycerides are generally considered to serve as an immediate energy source to the zooplankton with wax esters providing an energy reserve for periods of reproduction or starvation. *C. finmarchicus* are typically referred to as lipid-rich zooplankton, and it is reported that the species can withstand several months of starvation (diapause) due to its lipid rich nature. In addition to providing an energy source, lipids play other important roles in the zooplankton including buoyancy and vertical migration.

Whilst lipid stores will vary depending on season, food availability, life cycle stage, habitat *etc*., the lipid content reported for female *C. finmarchicus* found in the North Atlantic and Arctic are summarised in Table III.1.3-1. Overall, lipids represent a significant portion of the dry mass of *C. finmarchicus* with a significant portion in the wax ester form as reflected in the oil extracted from the source and the focus of this novel food ingredient application (see Section I.2).

Table III.1.3-1 Total Lipids and Storage Lipid Class in *Calanus finmarchicus* from Different Habitats (Lee *et al*., 2006)

Species	Lipid Mass (µg/individual)	Total Lipids (% dry mass)	Wax Ester Content (% of total lipids)	Triglyceride Content	Location	Source
C. finmarchicus female	50	31	62	6	Arctic (78-79°N)	Scott *et al*., 2000
C. finmarchicus female	80	40	60	10	North Atlantic (60°N)	Jónasdóttir (1999)

III.1.4 Comparison with Other Marine Oil Sources

C. finmarchicus represents an alternative source of marine lipids to traditional fish stocks such as cod, herring, sardine, salmon *etc*. In the past the small size of lower trophic organisms such as krill and copepods made their harvesting impractical but more recently economically viable methods have become available that make the production of marine oils from these sources possible on a commercial scale. Whilst beyond the scope of the novel food ingredient application, the rationale for the use of marine zooplankton is based on the growing concerns regarding the sustainability of current fish stocks to meet the increasing demands for long-chain polyunsaturated fatty acid-rich oils, and the need to identify alternative sources (FAO, 2006). The annual production of zooplankton biomass from the North Atlantic Seas of 200 to 400 million tonnes wet weight is tens of times greater than the accumulated biomass of all fish species from these same waters. The Norwegian Ministry of Fisheries and Coastal Affairs (FKD) is in the process of allowing a trial quota for *C. finmarchicus* harvesting as part of ongoing collaborative efforts between various research organisations and authorities to develop ecologically-friendly and sustainable methods of utilising this zooplankton in food production.

III.1.5 History of Consumption of Marine Zooplankton

For the purposes of this novel food ingredient application Calanus® Oil has been considered Class 2.2 on the basis that the source organism is not consumed to a significant extent by the European population at this time. However, the source organism <u>does</u> have a history of consumption that is relatively well documented both in Europe and the rest of the world including, Russia, China and the United States (US), and has been reviewed in detail by Schwimmer and Schwimmer (1955).

It is generally acknowledged that the use of zooplankton as a food source was first documented by Herdman (1891), where yachtsmen off the coast of Norway were reported to consume cooked red copepods (most likely *C. finmarchicus*) for breakfast. Subsequently, there have been a number of reports on the use of zooplankton as an important food source for sailors, particularly when shipwrecked or on long expeditions where supplies are low. In the 1930s and 1940s there were publications in both *Science* and *Nature* on the use of zooplankton, including *C. finmarchicus* specifically as a food source for the general European population. These publications were supported by various methods for potentially capturing the zooplankton from lakes using nets and filtration systems.

III.1.6 Potential Nutritional and Safety Concerns

Calanus® Oil has been extensively characterised and data pertaining to its composition and levels of undesirable substances are provided in Sections I.2 and I.3. Furthermore, given the position of *C. finmarchicus* at the lower trophic level of the food chain and the relatively short life-span compared to traditional fish sources of marine oils, the potential for accumulation of persistent organic pollutants should not pose a concern.

C. finmarchicus contributes significantly to the lipid profile of the marine organisms for which they are a significant food source. Many of these fish or related species are consumed directly by humans or indirectly from marine-derived products such as oils (*e.g.*, salmon, herring and squid). As such, the fatty acid and fatty alcohol profile of Calanus® Oil is typical of many marine organisms which are commonly consumed as part of the normal diet in the EU. The nutritional value of Calanus® Oil is discussed in detail in Section XI.

IX ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the intake/extent of use of the novel food:

- "Is there information on the anticipated uses of the novel food based on its properties?"
- "Is there information to show anticipated intakes for groups predicted to be at risk?"
- "Will introduction of the novel food be restricted geographically?"
- "Will the novel food replace other foods in the diet?"

These questions have been addressed collectively in the section below.

IX.1 Anticipated Use of the Novel Ingredient

IX.1.1 Supplementation of the Diet with EPA and DHA

Calanus® Oil is intended for use as an ingredient in food supplement products in the EU providing an alternative source of EPA and DHA to other marine oils such as fish, algal or krill oils. The current permitted uses and use levels for krill oil as an ingredient in food supplement products in the EU are those authorised under Commission Decision 2009/752/EC (Commission of the European Communities, 2009). These use levels were based on those authorised for DHA from DHA-rich algal oil at the time under Commission Decision 2003/427/EC (Commission of the European Communities, 2003) and include a maximum limit of 200 mg of EPA plus DHA per daily dose in food supplement products as recommended by the manufacturer. The previous dietary recommendations for EPA plus DHA on a Union-wide basis were essentially those of the SCF in 1993 which proposed an average requirement of 200 mg of long chain n-3 polyunsaturated fatty acids for adults (SCF, 1993). It was on the basis of the SCF advice that the maximum content of DHA from algal oils, and EPA plus DHA from krill oil products was set during the respective novel food assessments. Since this time, the European Food Safety Authority (EFSA) has re-evaluated the dietary reference values of fats including EPA and DHA (EFSA, 2005 and 2010). The Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies regarding the DRVs for fats (EFSA, 2010) including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids and cholesterol concluded that an Adequate Intake of 250 mg EPA plus DHA be set and acknowledged the cardiovascular benefits of intakes of between 250 to 500 mg and in excess of 1 g of these fatty acids on a daily basis (see Section XI.2.2 for further details).

IX.1.2 Anticipated Use and Use Levels

Taking into account the most recent EU dietary guidance outlined above, Calanus® Oil is proposed for use as an ingredient in food supplement products up to a daily maximum intake of EPA plus DHA of 250 mg (equivalent to *ca*. 2.3 g of Calanus® Oil). Practically, however, it is anticipated that intakes would be in the region of 1 g of Calanus® Oil per day provided in the form of two (2) 500 mg capsules and delivering *ca*. 111 mg of EPA plus DHA. Typically, the food supplement products would be in capsule or tablet form, but other delivery methods such as liquids may also be used.

The proposed maximum level would permit Calanus® Oil to provide the AI for EPA plus DHA and make a significant contribution towards the intakes required to achieve the anticipated health benefits of these fatty acids. This is logical considering that food supplements are a recognised alternative to the intake of meals containing fatty fish or foods fortified with marine or algal oils. Additionally, sources of EPA plus DHA that were marketed prior to 1997 and not subject to evaluation under Regulation (EC) No 258/97 (European Parliament and Council of the European Union, 1997), are not restricted to any maximum levels when used in food supplements and are typically available at equivalent or higher contents per daily serving in products across the EU (*i.e.,* fish oils; see Section IX.2.3). As discussed in Section XIII.5, the safety of Calanus® Oil is supported by a study in humans in which participants consumed between 1 and 4 g of Calanus® Oil (*ca*. 111 to 444 mg of EPA plus DHA) for 4 weeks. These treatment levels are equivalent to the maximum proposed use level of Calanus® Oil (*ca*. 2 g) and also provide a margin of safety of 2-fold compared to the high-dose group (4 g).

The food supplement products will be marketed in accordance with the risk management procedures laid down by Directive 2002/46/EC (European Parliament and Council of the European Union, 2002). Under Article 10 of the Directive, these procedures include the provision for Member States to require the manufacturer or persons placing the product on the market in their territory to notify the Competent Authority and provide a copy of the label. Such provisions allow another level of individual scrutiny by the Member States in addition to meeting the general requirements of EU-wide legislation.

IX.2 Estimated Intakes of the Novel Ingredient

IX.2.1 Extent of Use of the Novel Ingredient

Calanus® Oil is intended for use as a source of EPA plus DHA to help meet the daily recommendations for long-chain n-3 polyunsaturated fatty acids in the EU (see Sections IX.1.1 and XI). The ingredient is intended as a replacement for existing marine oils such as fish, krill or algal oils, and use would not result in any additional exposure by the general population to these fatty acids from food supplement products.

IX.2.2 Estimated Intakes

The estimated intakes of Calanus® Oil and its key compositional components from the proposed use in food supplement products is summarised in Table IX.2.2-1. Values are estimated taking into account typical daily consumption of 1 g of Calanus® Oil per day and also the proposed maximum daily intake of 250 mg of EPA plus DHA.

Table IX.2.2-1 Estimated Intakes of Key Components of Calanus® Oil from the Proposed Conditions of Use in Food Supplement Products

Component	Estimated Intakes in mg from 1 g of Calanus® Oil/Person/Day	Estimated Intakes in mg from 2.3 g of Calanus® Oil/Person/Day
Fatty Acids		
C18:4 n-3 (SDA)	70	161
C20:5 n-3 (EPA)	66	152
C22:6 n-3 (DHA)	45	104
EPA plus DHA	111	255
Fatty Alcohols		
C20:1 n-9	131	301
C22:1 n-11	195	449

The nutritional consequences of the estimated intakes of Calanus® Oil under the proposed uses are discussed in Section XI.

IX.2.3 General Consumption of Food Supplements by the EU Population

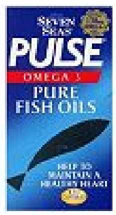
Limited information is available on the amounts and frequency of use of supplements in different EU countries, and sub-populations. Of the many studies that have been carried out, only a few provide suitable and recent food and supplement consumption data. The proportion of supplement users in selected European countries varies from about 20 to 40% in adults (Kiely *et al*., 2001; Henderson *et al*., 2002; Touvier *et al*., 2003; Beitz *et al*., 2004). In general, more women than men report taking supplements, and the percentage of supplement users in a population increases with a rise in educational level.

Given that food supplements containing Calanus® Oil are intended as a direct replacement for other marine or algal oil sources of EPA plus DHA, examples of these types of products identified on the UK market in 2011 are provided in Table IX.2.3-1. In these examples, the amount of EPA plus DHA delivered per daily serving are comparable to the anticipated and maximum proposed use levels of Calanus® Oil as an ingredient in food supplement products.

Table IX.2.3-1 Examples of Food Supplement Products Currently on the UK Market Containing Comparable Levels of EPA Plus DHA Per Daily Serving to the Proposed Use of Calanus® Oil as an Ingredient in the EU

Product	Description	Reference
Waitrose Capsules Omega 3 Fish Oil 	500 mg capsules containing 275 mg of EPA plus DHA	Waitrose Grocery Store, 2011: http://www.waitrose.com/shop/ProductView-10309-10001-98859-Waitrose+Capsules+omega+3+fish+oil+concen.html
Holland and Barratt Omega-3 Krill Oil 	1 g capsules containing 120 mg of EPA plus DHA Recommended daily intake of 2 capsules (2 g of oil) daily, preferably with meals	Holland and Barratt Health Foods Store, 2011: http://www.hollandandbarrett.com/pages/product_detail.asp?pid=2173&prodid=2421

Table IX.2.3-1 Examples of Food Supplement Products Currently on the UK Market Containing Comparable Levels of EPA Plus DHA Per Daily Serving to the Proposed Use of Calanus® Oil as an Ingredient in the EU

Product	Description	Reference
Seven Seas Pulse Omega-3 Pure Fish Oils with Vitamin E Capsules 	500 mg capsules providing 107 mg of EPA plus DHA Recommended daily intake 2 capsules per day (1 g of oil) with a cold drink	Boots Pharmacy Website, 2011: http://www.boots.com/en/Pulse-Omega-3-Pure-Fish-Oils-with-Vitamin-E-120-Capsules_1767/#detailedInfo

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- "Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"

This question has been addressed in this section.

XI.1 Nutritional Value of the Novel Ingredient and Comparison to Existing Foods in the Diet

Calanus® Oil is a source of fat, and in accordance with Council Directive 90/496/EEC (Council of the European Communities, 1990) the energy value will be declared as 9 kcal/g equivalent to 37 kJ/g of oil.

Calanus® Oil is intended for use as a replacement for existing marine oils such as fish, algal and krill oils as an ingredient in food supplement products. However, it is pertinent to this safety evaluation that the individual components of the oil, *i.e.*, long-chain n-3 polyunsaturated fatty acids and fatty alcohols, and wax esters of marine origin, are consumed from a variety of dietary sources in addition to marine oils. Moreover, esters *per se*, particularly ethyl esters, are a common delivery form of long-chain n-3 fatty acids from food supplement products. This Section considers the nutritional equivalence of the individual components of Calanus® Oil to existing foods in the diet, and also evaluates the impact of the proposed use of the novel food ingredient on the overall exposure by the EU population to these constituents.

XI.2 Fatty Acids

XI.2.1 Fatty Acid Composition

Table XI.2.1-1 provides a comparison of the fatty acid profile of Calanus® Oil to that of existing marine oils from fish sources that might be expected to feed on *C. finmarchicus*. Qualitatively, the fatty acids identified in Calanus® Oil are typical of those of the existing marine oils, although, the level of each component varies depending on the species. Whilst the levels of EPA and DHA in Calanus® Oil are comparable to herring, redfish and sardine oils, the SDA content is significantly higher in the novel ingredient. It is noted, however, that echium oil (plant-based oil) recently gained novel food approval in the EU under Commission Decision 2008/558/EC (Commission of the European Communities, 2008b) and has a content of at least 10% SDA on a total fatty acid basis. These data demonstrate that Calanus® Oil is comprised of fatty acids which form a normal part of the diet and under the intended conditions of use of the novel ingredient, will not make a significant impact on overall intakes of these components by the general population.

Table XI.2.1-1 Comparison of the Fatty Acid Profiles of Calanus® Oil and Existing Marine Oils (Firestone, 2006)

Fatty Acid (Common Name)	Typical Composition (% of oil)[1]			
	Calanus® Oil[2]	Herring Oil[3]	Redfish Oil[3]	Sardine Oil[3]
C14:0 (myristic acid)	8.1	3-10	4-6	4-12
C16:0 (palmitic acid)	4.9	8-25	10-14	9-22
C15:0	0.4			0-0.6
C16:1	2.8	3-12	7-14	6-13
C16:3	0.6	-	-	-
C17:0	0.1	-	-	0-1
C18:0 (oleic acid)	0.4	1-4	1-3	2-7
C16:4 n-1	1.1	-	-	-
C18:4 n-9	1.4	-	-	-
C18:1	0.3	-	17-22	7-17
C18:2 n-6	0.6	0.1-2	0.6-2	1-3
C18:3	1.5	0-2	0.2-1	0.1-4
C18:4 n-3 (SDA)	**7.0**	**1-5**	**1-3**	**2-3**
C20:1	3.9	-	11-20	1-8
C20:2 n-6	0.1	0.5-0.7	-	-
C20:4	0.8	0.3-0.5	0.1-0.5	9-35
C22:1	5.2	4-31	-	1-8
C20:5 n-3 (EPA)	**6.6**	**4-15**	**5-10**	**1-4**
C24:1 n-9	0.4	0.2-1.3	-	
C22:5 n-3	0.4	-	0.1-1	-
C22:6 n-3 (DHA)	**4.5**	**2-10**	**2-6**	**4-13**

[1] Only fatty acids with typical composition of at least 0.5% included in table, where content is not listed for a given fatty acid the component may well be present albeit at very low levels;
[2] Mean over 3 batches, see Section I.2;
[3] Typical ranges for Herring oil, Redfish (*Sebastes marinus*) and Sardine oil (Firestone, 2006).

XI.2.2 Supplementation of the Diet with Long-Chain n-3 Polyunsaturated Fatty Acids

The nutritional value of EPA and DHA is well-established and has been reviewed by the European Food Safety Authority in 2005 and 2009 (EFSA, 2005, 2010). In particular, in 2010, the Scientific Panel on Dietetic Products, Nutrition and Allergies issued an Opinion regarding the DRVs for fats (EFSA, 2010) including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids and cholesterol. In this Opinion, the Scientific Panel reported that intervention studies have demonstrated the beneficial effects of pre-formed long-chain n-3 polyunsaturated fatty acids on a number of recognised cardiovascular risk factors at intake levels exceeding 1 g per day. At the same time it was noted that epidemiological studies indicate far lower levels were associated with a reduced risk of cardiovascular disease (CVD). The Scientific Panel also found that prospective epidemiology and dietary intervention studies demonstrated oily fish consumption or dietary long chain n-3 polyunsaturated fatty acid supplements, delivering between 250 to 500 mg of EPA plus DHA, decreased the risk of mortality from coronary heart disease and sudden cardiac death. Taking into account the cardiovascular information summarised above, the Scientific Panel concluded that whilst there were insufficient data to derive an Average Requirement for EPA plus DHA, an Adequate Intake (AI) of 250 mg should be set for adults. It was also proposed that the dietary advice for children should be consistent with that for the adult population (*i.e.*, 1 to 2 meals containing fatty fish per week or alternatively approximately 250 mg per day of EPA plus DHA).

XI.2.3 Overview of Dietary Recommendations Set by Different Organisations

A number of dietary recommendations for the intakes of n-3 polyunsaturated fatty acids have been set by different organisations, often including values for long chain n-3 polyunsaturated fatty acids (*i.e.*, EPA plus DHA), as part of overall strategies for dietary fat consumption. These recommendations have been reviewed in detail by EFSA in the recent Opinion by the Scientific Panel on Dietetic Products, Nutrition and Allergies regarding the DRVs for fats (EFSA, 2010) including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids and cholesterol. A summary of the recommendations detailed in the Opinion specifically relating to n-3 polyunsaturated fatty acids and long chain n-3 polyunsaturated fatty acids are provided in Tables XI.2.3-1 and XI.2.3-2 for adults and children, respectively.

Table XI.2.3-1 Summary of the Dietary Recommendations for the Intakes of n-3 Polyunsaturated Fatty Acids (n-3 PUFA) for Adults Set by Various Countries and Organisations (EFSA, 2010)

Country/ Organisation	Recommendation for n-3 PUFA	Body	Reference
Germany, Austria and Switzerland	7-10 E% Total PUFA of which 0.5 E% n-3 PUFA (all population groups) 0.25-1 g/day LC n-3 PUFA for primary and secondary prevention of coronary heart disease, respectively, adequate, whilst 3 g/day safe 200 mg/day DHA (pregnant and lactating women)	German, Austrian and Swiss Recommendations (D-A-CH)	D-A-CH, 2008
Nordic countries	5-10 E% Total PUFA of which 1 E% n-3 PUFA (all adults) Ratio n-6 to n-3 of 3-9 adequate	Nordic Nutrition Recommendations (NNR)	Nordic Council of Ministers, 2004
United Kingdom	COMA: 6 E% Total PUFA (max. 10 E%) SACN: 450 mg n-3 LC PUFA (all population groups)	Committee on Medical Aspects of Food and Nutrition Policy (COMA) Scientific Advisory Committee on Nutrition (SACN)	Department of Health, 1991; SACN, 2004
France	500 mg/day (0.2 E%) of which 120 mg DHA (0.05 E%) (general population) 0.4 E% for LC PUFA, 0.1 E% DHA (pregnant and lactating women)	Nutritional Recommendations for the French Population; French Food Safety Agency (AFSSA)	AFSSA, 2001

Table XI.2.3-1 Summary of the Dietary Recommendations for the Intakes of n-3 Polyunsaturated Fatty Acids (n-3 PUFA) for Adults Set by Various Countries and Organisations (EFSA, 2010)

Country/ Organisation	Recommendation for n-3 PUFA	Body	Reference
The Netherlands	<12% E% Total PUFA 450 mg/day LC n-3 PUFA from fish adequate (adults, pregnant and lactating women)	Health Council of the Netherlands (GR)	Gezondheidsraad, 2001
World Health Organisation (WHO)/Food and Agricultural Organisation (FAO)	6-10 E% Total PUFA of which 1-2 E% n-3 PUFA	WHO/FAO	WHO/FAO, 2003

PRI=population reference intakes.

Table XI.2.3-2 Summary of the Dietary Recommendations for the Intakes of n-3 Polyunsaturated Fatty Acids (n-3 PUFA) for Children Set by Various Countries and Organisations (EFSA, 2010)

Country/ Organisation	Recommendation for n-3 PUFA	Body	Reference
Germany, Austria and Switzerland	7-10 E% Total PUFA of which 0.5 E% n-3 PUFA	German, Austrian and Swiss Recommendations (D-A-CH)	D-A-CH, 2008
Nordic countries	5-10 E% Total PUFA of which 1 E% n-3 PUFA (6-23 months) Ratio n-6 to n-3 gradual increase 5-15 to 3-9 adequate (6-11 months)	Nordic Nutrition Recommendations (NNR)	NNR, 2004
The Netherlands	150 to 200 mg/day (1 E%) adequate (0-6 months) 20 mg/kg body weight/day DHA adequate (0-5 months)	Health Council of the Netherlands (GR)	GR, 2001

Collectively, these recommendations form part of the evidence supporting the role of Calanus® Oil as an alternative source of EPA and DHA to existing marine and algal oils to help achieve the desired daily intakes of n-3 long-chain polyunsaturated fatty acids. As discussed in Sections IX.1 and IX.2, the intended use of Calanus® Oil as an ingredient in food supplements of 111 mg of EPA plus DHA (*ca*. 1 g of oil) but not exceeding 250 mg (*i.e*., *ca*. 2.3 g of oil), will contribute significantly to the AI of 250 mg recommended by EFSA, in an analogous manner to existing products containing marine or algal oils on the market.

XI.3 Wax Esters and Fatty Alcohols

XI.3.1 Commercial Fish Rich in Wax Esters

The majority of fish convert wax esters ingested *via* zooplankton to triglycerides (Ratnayake and Ackman, 1979a; Sargent, 1981; EFSA, 2004). As part of this process, fatty alcohols are converted directly to fatty acids by the fish. Thus, commercial fish with high triglyceride contents and their extracted oils may contain fatty alcohols as part of their unsaponifiable matter, but the levels are relatively low, typically representing less than 0.5% by weight of the total lipids (*e.g*., herring and mackerel and their oils; Ratnayake and Ackman, 1979b).

However, there are a number of fish in which dietary wax esters from zooplankton are stored in the flesh and are not metabolised.

Examples of commercial fish species with a long history of consumption by the EU population and high wax ester contents are the deep-water fish species Orange Roughy (*Hoplostethus atlanticus*) and Black Oreo (*Allocyttus sp*.). Evidence for the availability of these fish species and their use in cooking by the UK population is provided in Table IX.3.1-1.

Table IX.3.1-1 Evidence for Orange Roughy and Black Oreo Consumption by the UK Population

Description/Evidence of Consumption	Reference
Orange Roughly is available for sale in the UK at grocery stores although, recently environmental (fish stocks) issues have led to efforts to reduce consumption	UK Food Standards Agency Survey of Mercury in Imported Fish and Shellfish and UK Farmed Fish and the Products (July 2003): http://www.food.gov.uk/multimedia/pdfs/fsis40_2003annex.pdf Bite-Back campaign regarding the availability of Orange Roughy in the UK: http://www.bite-back.com/bb_factsheets/bb_roughy.pdf Greenpeace summary of efforts across the EU to remove Orange Roughy from grocery store shelves due to environmental concerns: http://www.greenpeace.org/raw/content/new-zealand/press/reports/orange-roughy-rejection.pdf
Recipes for Orange Roughy are available on the websites established British television channels Grilled Orange Roughy recipe	Grilled Orange Roughly recipe on the “Food Network” channel: http://www.foodnetwork.co.uk/recipes/grilled-orange-roughy-ru307626.html Orange Roughy with warm noodles recipe on the “Good Food” channel: http://uktv.co.uk/food/recipe/aid/516867
Black Oreo fillets are available for sale in the UK and supported by examples of home recipes	Regal Fish Supplies Ltd (UK fishmongers): http://www.regalfish.co.uk/seafood/exotic-fish/oreo-fillets-(frozen)/

<u>Lipid Profiles of Orange Roughy and Black Oreo and Comparison with Calanus® Oil</u>

The lipid profiles reported for Orange Roughy and Black Oreo are summarised in Table XI.3.1-2 and indicate that, similar to Calanus® Oil, the lipids are primarily in the form of wax esters with minor amounts of triglycerides (Buisson *et al*., 1982).

Table XI.3.1-2 Comparison of the Lipid Profiles of Calanus® Oil and the Total Lipids of Orange Roughy and Black Oreo (Whole Fish) (Buisson *et al*., 1982)

Component	Typical Composition (%)		
	Calanus® Oil[1]	Orange Roughy	Black Oreo
Wax esters	**87.2**	**94.9**	**91.5**
Triglycerides	2.8	3.1	4.8
Diglycerides	0.9	-	-
Monoglycerides	0.6	-	-
Cholesterol	0.4	1.0	2.7
Phospholipids	None	1.0	1.0

[1] Mean over 3 batches, see Section I.2.

Analysis of the wax ester fractions of Orange Roughy and Black Oreo indicate that the chain lengths from the fatty acid and fatty alcohol moieties are typically between 30 and 46 carbons in length, comparable to the distribution of between 30 and 44 carbons observed in Calanus® Oil of (see Section I.2.4-1).

Fatty Acid and Fatty Alcohol Profiles of Orange Roughy and Black Oreo

The fatty acid and fatty alcohol profiles of the lipids (primarily wax esters) identified in Orange Roughy and Black Oreo are outlined in Tables XI.3.1-3 and XI.3.1-4, respectively. The levels of the individual fatty acids and alcohols identified in the 2 fish species are also compared with those in Calanus® Oil [only the fatty acids or fatty alcohols present in significant amounts (>0.5%) are shown for the novel ingredient; for the full profiles see Tables I.2.2-1 and I.2.3-1]. Although, there are differences in fatty acid and fatty alcohol compositions of Calanus® Oil and these 2 commercial fish species, overall the profiles contain many of the same components.

Table XI.3.1-3 Comparison of Fatty Acid Components of Calanus® Oil with Whole Fish Wax Esters of Orange Roughy and Black Oreo (Buisson *et al*., 1982)

Component	**Typical Composition (% of fatty acids)[1]**		
	Calanus® Oil[2]	**Orange Roughy**	**Black Oreo**
Saturated Fatty Acids			
C14:0	15.4	1.2	4.1
C15:0	0.7	Trace	0.8
C16:0	**9.2**	**1.0**	**15.5**
C17:0	0.2	0.7	1.1
C18:0	0.8	0.3	3.2
C20:0	Trace	Trace	0.2
C22:0	Trace	Trace	Trace
C24:0	Trace	Trace	Trace
Unsaturated Fatty Acids			
C14:1	Trace	0.5	0.3
C15:1	-	Trace	0.7
C16:1	5.2	11.8	7.9
C16:3	0.6	-	-
C16:4	1.1	-	-
C17:1	Trace	1.0	0.8
C18:1	**3.3**	**56.0**	**26.9**
C18:2	1.1	1.9	1.0
C18:3 n-3	2.0	-	-
C18:4 n-9	2.6	-	-
C18:4 n-3	13.2	-	-
C20:1	**7.4**	**17.8**	**15.8**

Table XI.3.1-3 Comparison of Fatty Acid Components of Calanus® Oil with Whole Fish Wax Esters of Orange Roughy and Black Oreo (Buisson *et al*., 1982)

Component	Typical Composition (% of fatty acids)[1]		
	Calanus® Oil[2]	Orange Roughy	Black Oreo
C20:4 n-3	1.1	-	-
C20:5 n-3	12.5	-	-
C22:1	**9.9**	**7.8**	**11.6**
C22:6 n-3	8.5	-	-
C23:1	-	Trace	1.8
C24:1	0.8	Trace	8.3

[1] Major fatty acids identified in Orange Roughy and/or Black Oreo are **bold**;
[2] Mean over 3 batches, see Section I.2, values adjusted to give % of total fatty acids.

Table XI.3.1-4 Comparison of Fatty Alcohol Components of Calanus® Oil with Whole Fish Wax Esters of Orange Roughy and Black Oreo

Component	Typical Composition (% of fatty alcohols)		
	Calanus® Oil[1]	Orange Roughy	Black Oreo
Saturated Fatty Alcohols			
C14:0	1.6	-	1.9
C15:0	-	-	Trace
C16:0	7.8	7.3	20.8
C17:0	-	-	0.8
C18:0	-	8.1	2.3
C20:0	-	-	0.6
Unsaturated Fatty Alcohols			
C16:1	2.8	-	-
C17:1	-	-	0.8
C18:1	**2.7**	**34.6**	**19.0**
C20:1	**33.6**	**30.6**	**29.6**
C22:1 (total/undefined)	**49.9**	**13.8**	**20.3**
C24:1	-	5.4	3.9

[1] Major fatty alcohols in Orange Roughy and Black Oreo are **bold**;
[2] Mean over 3 batches, see Section I.2, values adjusted to give % of total fatty alcohols.

<u>Distribution of Lipids in Orange Roughy and Black Oreo</u>

The distribution of lipids in these 2 fish species are presented in Table XI.3.1-5 and indicate that significant amounts are present in the skin and muscle, a portion of which at least, would be consumed as part of the normal diet by the EU population (it is assumed that the head, guts, frame and potentially the skin and muscle immediately adjacent to the skin would be discarded; see Section XIII.6).

Table XI.3.1-5 Distribution of Lipids in Orange Roughy and Black Oreo (Buisson *et al*., 1982)

Fish Part	Fish Species (% of oil or lipids)	
	Orange Roughy	Black Oreo
Whole fish	100	100
Head	44.1	49.7
Gut	11.0	12.0
Skin	8.4	8.4
Frame	15.9	15.3
Muscle	20.4	14.7

Consumption of Lipids from Commercial Fish Rich in Wax Esters

The muscle of Orange Roughy and Black Oreo are reported to comprise around 9.9 and 7.6% lipids, respectively. In any serving of these fish, the muscle will represent the major part of the dish, although some of the oil content may have been removed as part of the flesh adjacent to the skin (Shadbolt *et al*., 2002). However, on the basis of the total lipid content of the muscle meat, it can be estimated that a single serving of 180 g fillet of Orange Roughy or Black Oreo (FSA, 2002) would provide up to 17.8 g or 12.7 g of lipids, respectively. Even if these levels were reduced in the preparation of the fish (*i.e.*, removal of the skin and adjacent flesh) it would still be anticipated that the levels of wax ester intake would be far greater than the proposed daily intakes of Calanus® Oil of no more than 2.3 g from food supplement products. As discussed in Section XIII.6, these 2 fish species are well tolerated by humans and are not associated with any adverse effects.

XI.3.2 Fish Roe

Lipid Content of Fish Roe Products

Another significant source of exposure of the general population to wax esters of marine origin is from the consumption of fish roe products. Generally, fish roe lipids are predominantly in the form of triglycerides or phospholipids but there are some exceptions. Examples of fish roe where the wax or steryl esters represent a significant fraction of the total lipid content include mullet (*Mugil cephalus*; 64%), burbot (*Lola lola*; 80 to 83%), perch (*Perca fluviatilis*; 80 to 83%), kahawai (*Armpits trutta*; 26%), hoki (*Macruronis novaezelandiae*; 32%), red cod (*Pseudomonas bacchus*; 26%), hake (*Mercluccius hubbsi*; 28%) and haddock (*Melanogramus aeglefinus*; 15%) (Bledsoe *et al*., 2003; Kalogeropoulos *et al*., 2008).

Fatty Acid and Fatty Alcohol Profiles of Mullet Roe

Wax-covered, salted and dried roe from striped Mullet in particular, has a history of traditional use dating back to at least the 17th century as botago in Italy and avgotaracho in Greece (Bledsoe *et al*., 2003; Bernasconi *et al*., 2007; Kalogeropoulos *et al*., 2008). Typical fatty acid and fatty alcohol profiles of the wax/steryl ester fractions of commercial mullet roe products are summarised in Tables XI.3.2-1 and XI.3.2-2. The levels of the individual fatty acids and alcohols identified in the wax/steryl ester fractions[2] of 2 commercial mullet roe products are also compared with those in Calanus® Oil [only the fatty acids or fatty alcohols present in significant (>0.5%) are shown for the novel ingredient; for the full profiles see Tables I.2.2-1 and I.2.3-1]. Qualitatively, the fatty acid and fatty alcohol profiles contain many of the same components and unlike Orange Roughy or Black Oreo (see Section XI.3.1), are significant sources of EPA and DHA. Similarly, other roe products with high wax ester contents are reported to contain EPA and DHA including hake, with values of 0.5 and 1.3 g per 100 g of roe reported, respectively (Bledsoe *et al*., 2003). These findings are consistent with other studies on roe which indicate that the fatty acid profiles often mimic that of the corresponding fish oil for a given species (Bernasconi *et al*., 2007).

Table XI.3.2-1 Comparison of Fatty Acid Profiles of Calanus® Oil and the Wax and Steryl Ester Fraction of Greek Avgotaracho (Kalogeropoulos *et al*., 2008)

Fatty Acid (Common Name)[1]	Typical Composition (% of fatty acids)		
	Calanus® Oil[2]	Greek Avgotaracho[3]	Mullet Botago[4]
Saturated Fatty Acids			
C14:0 (myristic acid)	15.4	3.4	1.6
C15:0	0.7	0.2	-
C16:0 (palmitic acid)	9.2	7.4	9.5 (undefined)
C17:0	0.2	0.8	-
C18:0 (oleic)	0.8	1.4	1.8
Unsaturated Fatty Acids			
C14:1 n-5	0.64	-	-
C16:1 n-9	0.3	0.8	11.6 (undefined)
C16:1 n-7 (palmitoleic acid)	4.4	19.9	
C16:1 n-5	0.5	-	
C16:2 n-6	-	0.8	0.2 (undefined)
C16:2 n-7	0.6	-	
C16:3	-	0.1	-
C17 undefined	0.4	0.8	-
C16.3 n-4	1.1	-	0.3 (undefined)
C18:2 n-6	-	2.9	-
C18:3 n-3	-	6.1	-
C16:4 n-1	2.1	-	-
C18:4 n-9	-	-	-
C18:1 n-9	2.7	23.3	15.4 (undefined)
C18:1 n-7	0.6	7.8	
C18:1 others	-	-	-

[2] While the relative amounts of wax and steryl esters not generally reported, Bernasconi *et al*., 2007 indicate that wax esters represent at least 80% of the wax/steryl ester fraction in mullet roe.

Table XI.3.2-1 Comparison of Fatty Acid Profiles of Calanus® Oil and the Wax and Steryl Ester Fraction of Greek Avgotaracho (Kalogeropoulos *et al*., 2008)

Fatty Acid (Common Name)[1]	Typical Composition (% of fatty acids)		
	Calanus® Oil[2]	**Greek Avgotaracho[3]**	**Mullet Botago[4]**
C18:2 n-6	1.1	2.8	1.6
C18:3 n-6	0.8	-	0.7 (undefined)
C18:3 n-3	2.0	6.1	
C18:4 n-3 (stearidonic acid, SDA)	**13.3**	**4.5**	**0.7**
C20:1 n-11	1.4	-	0.4 (undefined)
C20:1 n-9 (gadoleic acid)	4.7	0.2	
C20:1 n-7	1.4	-	
C20:2 n-6	-	-	-
C20:3 n-6	0.1	0.3	0.3
C20:4 n-6	1.0	0.9	2.1
C20:4 n-3	1.2	-	-
C22:1 n-11 (cetoleic acid)	9.5	-	-
C20:5 n-3 (eicosapentaenoic acid, EPA)	**12.5**	**4.4**	**10.0**
C22:5 n-3	0.7	3.1	4.4
C22:6 n-3 (docosahexaenoic acid, DHA)	**8.5**	**9.4**	**23.7**

[1] Major or nutritionally significant fatty acids in mullet roe are **bold**;
[2] Mean over 3 batches, see Section I.2, values adjusted to give % of total fatty acids;
[3] Mean over 3 batches, expressed as % total fatty acids in the wax ester/steryl ester fraction;
[4] Mean over 3 batches, expressed as % total fatty acids in the wax ester fraction, while these results are those published by Bernasconi *et al*. (2007) they are comparable with the results reported by for Italian botago by Kalogeropoulos *et al*. (2008).

Table XI.3.2-2 Comparison of Fatty Alcohol Profiles of Calanus® Oil and the Wax and Steryl Ester Fraction of Greek Avgotaracho (Kalogeropoulos *et al*., 2008)

Fatty Alcohol[1]	Typical Composition (% of fatty alcohols)		
	Calanus® Oil[2]	**Greek Avgotaracho[3]**	**Mullet Botago[4]**
Saturated Fatty Alcohols			
C14:0	1.6	10.5	-
C15:0	-	1.5	-
C16:0	**7.8**	**65.5**	**13.7**
C17:0	-	0.7	-
C18:0	-	7.6	0.8
C20:0	-	0.3	-
Unsaturated Fatty Alcohols			
C14:1	-	0.2	-
C16:1	**2.8**	**5.1**	**66.2**
C17:1	-	0.7	-
C18:1	**2.7**	**7.0**	**14.0**

Table XI.3.2-2 Comparison of Fatty Alcohol Profiles of Calanus® Oil and the Wax and Steryl Ester Fraction of Greek Avgotaracho (Kalogeropoulos *et al*., 2008)

Fatty Alcohol[1]	Typical Composition (% of fatty alcohols)		
	Calanus® Oil[2]	Greek Avgotaracho[3]	Mullet Botago[4]
C18:2	-	0.1	-
C20:1 n-9	**33.6**	**0.1**	**1.4**
C22:1 n-11	**49.9**	**-**	**-**
C22:1 n-9	2.7	-	-

[1] Major or nutritionally significant fatty alcohols in mullet roe are **bold**;
[2] Mean over 3 batches, see Section I.2, values adjusted to give % of total fatty alcohols;
[3] Mean over 3 batches, expressed as % total fatty alcohols in the wax ester/steryl ester fraction;
[4] Mean over 3 batches, expressed as % total fatty alcohols in the wax ester fraction, while these results are those published by Bernasconi *et al*. (2007) they are comparable with the results reported by for Italian botago by Kalogeropoulos *et al*. (2008).

<u>Consumption of Lipids from Commercial Fish Roe Products Rich in Wax Esters</u>

Taking into account the typical neutral lipid contents of various fish roe product (see Lipid Content of Fish Roe Products), an average 85 g serving of roe would lead to estimated intakes of between 12.8 to 70.6 g of wax or steryl esters. These estimates are far greater than the intake of wax esters from the proposed use of Calanus® Oil as an ingredient in food supplement products at levels not exceeding 2.3 g per day. As discussed in Section XIII.6, fish roe products are generally well tolerated by humans with no associated adverse effects.

XI.3.3 Other Dietary Sources of Wax Esters and Fatty Alcohols

Wax esters from sources other than marine origins are also commonly consumed as part of the normal diet by the general population and examples, including compositional information are provided in Table XI.3.3-1. Many of the natural sources of waxes in the diet contain free fatty alcohols in addition to wax esters as demonstrated in the table. In some instances, wax esters are also hydrolysed to yield long-chain free fatty alcohols such as policosanol, an ingredient in food supplement products derived from sugarcane/spinach or beeswax by saponification to yield greater than 90% aliphatic alcohols (Hargrove *et al*., 2004).

Although, many of the wax ester examples in Table XI.3.3-1 are comprised of different long-chain fatty acids and alcohols compared to Calanus® Oil, they do provide evidence of the established role of this class of substances in the diet. In an analogous manner to ethyl esters of EPA and DHA used as ingredients in food supplement products, the wax esters of Calanus® Oil may be considered an alternative, recognised delivery method of these long-chain fatty acids to triglycerides or phospholipids.

Table XI.3.3-1 Other Dietary Sources of Wax Esters Commonly Consumed by the General EU Population

Source	Compositional Information	History of Use	Reference
Honeycomb	– From the honeybee (*Apis mellifera* L.) Wax esters represent in the region of 35 to 45% of honeycomb with lesser amounts of free acids (8 to 12% and alkanes (14 to 23%): Esterified alcohols are typically 25 to 33 carbons in chain length The major esterified fatty acid is palmitic (C16:0) acid	Historically consumed with honey (not separated) where a typical serving of honey would be anticipated to be in the region of 17 g (heaped teaspoon – FSA, 2002)	Hargrove *et al*., 2004
Beeswax	– Wax obtained by melting the walls of honeycomb made by the honeybee Typically comprises 57% total esters, of which 41% are monoesters, 9.2% monoesters, 9% hydroxymonoesters and 7% diesters; other components include hydrocarbons (16%), free fatty acids (18%) and free fatty alcohols (0.6%): Predominantly saturated alkyl palmitates (C38 to C52) and unsaturated alkyl esters of oleic acid (C46 to C54)	Authorised for use as a glazing agent *quantum satis* on food supplements, confectionary (excluding chocolate), small products of bakery wares coated with chocolate, snacks, nuts and coffee beans and for the surface treatment of certain fruits according to Directive 95/2/EC; it is also permitted as a carrier for colours and in 2006 EFSA issued a positive Opinion on the use its use as a carrier for flavours Typical use in foods estimated to be up to 60 g/kg in soft gelatine capsules, 0.5 g/kg for glazings and coatings and 0.2 g/kg in water-based flavoured drinks An exposure assessment based on data from the Netherlands estimated intakes from all proposed uses at the maximum use levels to be between 475 to 1290 mg/person/day	European Parliament and Council of the European Union, 1995; EFSA, 2007
Cereals grains	Oats (*Avena sativa*) The wax components of oats (0.4 to 0.9%) comprise in the region of 7 to 21% wax esters, 6 to 14% primary alcohols, 9 to 14% free acids and 9 to 15% alkanes Free alcohols are predominantly C16:0 and C18:0 Bran/Wheat Processing will remove the majority of wax esters with the greatest levels identified in whole grain foods	Wheat, oats *etc*. are normal components of the diet Estimated that if whole grains contain 0.1 to 0.5 g of wax per 100 g, then a 454 g intake would be equivalent to consumption of at least 1 g of wax containing wax esters and free fatty alcohols	EFSA, 2007
Plants, nuts, seeds	Fruits/Vegetables Majority of wax esters in the skin and content significantly reduced by peeling Cabbages The wax components comprise primarily of alkanes (36 to 40%) with wax esters representing around 4 to 22%: Major alcohol C16:0 Nuts Wax esters identified as a components of nuts and fruit in general: Free alcohols 26 to 30 carbons in chain length Apples Wax esters represent around 18%, primary alcohols 6%, free acids 20%, aldehydes 2% and alkanes 20%	Normal components of the diet where exposure to wax esters will depend on the processing used	Hargrove *et al*., 2004

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- "Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"

This question has been addressed below.

XII.1 Microbiological Information

Analyses for micro-organisms for 3 batches of Calanus® Oil are presented in Table XII.1-1 and the Certificates of Analysis provided in Appendix A-4. All levels of micro-organisms were below the limits of detection or at levels well below the internal specifications. As discussed previously in Section II.3.4, the results confirm there are no potential microbiological hazards associated with Calanus® Oil for use as an ingredient in food supplement products.

Table XII.1-1 Results of Microbiological Analysis for 3 Batches of Calanus® Oil

Parameter	Units	Internal Specifications	Analytical Data (mg/kg)		
			Batch 1	Batch 2	Batch 3
Aerobic plate count	cfu/g	<1000	10	40	360
Coliform and *Escherichia coli*	cfu/g	<10	<10	<10	<10
Staphylococcus spp.	cfu/g	<10	<10	<10	<10
Yeast and mould	cfu/g	<10	<10	<10	<10
Salmonella spp.	Per 25 g	Negative	Negative	Negative	Negative
Listeria monocytogenes	Per 25 g	Negative	Negative	Negative	Negative

XIII TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

- “Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?”
- “Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?”

or

- “Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?”
- “Is there information which suggests that the novel food might pose an allergenic risk to humans?”

These questions have been addressed collectively in this section.

XIII.1 Introduction

Calanus® Oil is intended as an alternative source of long-chain n-3 polyunsaturated fatty acids, particularly EPA and DHA to other marine and algal oils. As described in the preceding Sections, Calanus® Oil is proposed as an ingredient in food supplement products where the consumption of EPA and DHA would be consistent with current dietary guidance published in the recent EFSA Opinion by the Scientific Panel on Dietetic Products, Nutrition and Allergies regarding the DRVs for fats including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids and cholesterol that allocated an AI of 250 mg EPA plus DHA (EFSA, 2010). Consequently, Calanus® Oil is proposed for use at levels of no more than 250 mg EPA plus DHA per daily serving of food supplement products equating to an intake of approximately 2.3 g of oil. Accordingly, anticipated exposure to long-chain n-3 fatty acids by the general population under the proposed conditions of use in food supplement products would not be expected to differ from current levels obtained from alternative products such as fish oils or their concentrates. The major difference between Calanus® Oil and alternative EPA and DHA sources is that the novel ingredient provides these long-chain fatty acids predominantly in the wax ester form rather than traditional triglyceride, ethyl ester or phospholipid forms. The additional exposure of the consumer to the fatty alcohol moieties in the wax ester form must therefore, be addressed. As demonstrated in Section XI however, there is a long history of consumption of wax esters of marine source with similar fatty acid and fatty alcohol profiles to Calanus® Oil from the normal diet, at levels far exceeding (>6-fold) those from the intended use of the novel ingredient. Moreover, wax esters *per se* are a normal part of the diet from foods such as beeswax, grains, fruits, vegetables and nuts. This safety assessment of Calanus® Oil, therefore, is based on the following principles:

1. History of consumption of the main components (*i.e.* wax esters, fatty acids and fatty alcohols) of Calanus® Oil as established constituents of the normal diet (Section XI;

2. The anticipated absorption, metabolism and excretion of Calanus® Oil (Section XIII.2);

3. Toxicological data on the ingredient and its components (*i.e.* wax esters, fatty acids and fatty alcohols) of Calanus® Oil; (Section XIII.3)

4. Clinical studies on Calanus® Oil (Section XIII.4).

XIII.2 Absorption, Distribution, Metabolism, Elimination

XIII.2.1 Wax Esters

Following ingestion, lipases or carboxyl esterases hydrolyse wax esters into its fatty acid and fatty alcohol components, both of which are then readily absorbed into the intestinal epithelium (Hargrove *et al*., 2004). It has been reported that the digestion of wax esters in terrestrial organisms is not as efficient as those occurring in fishes and seabirds, which have developed adaptations to allow them to readily digest wax esters for use as their primary energy source. Place (1992) demonstrated that seabirds readily hydrolyse wax esters and assimilated the released fatty acids and long-chain fatty alcohols with approximately 85% efficiency. In contrast, the absorption of wax esters was reported to be less than 25% in dogs, and approximately 40 to 50% in rats (Hansen and Mead, 1965; Place, 1992). The rate of hydrolysis of wax esters by purified porcine pancreatic lipase was reported to be between 10 and 50 times slower than that for triacylglycerols (Savary, 1971).

No specific wax ester digestive lipase exists in mammals (Ling *et al*., 2009); however, lipases and carboxyl esterases that act on triacylglycerols can hydrolyse wax esters, albeit at a much slower rate (Hargrove *et al*., 2004; Ling *et al*., 2009). Complete digestion of wax esters was reported to occur within 24 hours following *in vitro* incubation with human pancreatic lipase and porcine pancreatic colipase (Gorreta *et al*., 2002). In this experiment, wax esters enriched with EPA and DHA were incubated with 80U/L human pancreatic lipase, colipase from porcine pancreas, and 17.5mmol/L deoxycholic acid, for 4, 6 and 24 hours at 37°C. After 4 and 6 hours, approximately 80% of the wax esters had been hydrolyzed with approximately 40% to 50% of the total EPA and DHA content of the wax ester released after 6 hours. At the 24 hours mark all of the wax ester had been hydrolyzed and all of the EPA and DHA had been released. The authors employed jojoba oil as a basis for the comparison and concluded that the hydrolysis of wax esters containing EPA and DHA was not significantly different from that of jojoba oil *in vitro*.

Gorreta *et al*. (2002) compared the digestibility of fish oil (containing fatty acids in triglyceride form), ethyl esters derived from fish oil, and wax esters [ethyl esters trans-esterified with behenyl alcohol (C22:0)] in rats. The preparations tested contained the same content of EPA and DHA (33% and 22%, respectively). Administration of a single oral dose of wax esters [corresponding to 5 g/kg body weight of n-3 polyunsaturated fatty acids (n-3 PUFAs), or 1 g/animal assuming rats weighing 200 g] increased plasma triglyceride levels in a similar manner as ethyl esters, although plasma triglyceride levels were increased to the greatest extent in animals administered fish oil. Supplementation of DHA plus EPA (equivalent to doses of 150 mg/animal/day) in the form of wax esters, ethyl esters or triglycerides (fish oil) to the diets of rats for 4 weeks significantly increased the levels of EPA and DHA in plasma phospholipids compared to rats fed a standard control diet. The extent of enrichment of n-3 PUFAs in plasma phospholipids was similar for animals across the treatment groups regardless of the formulation (*i.e*. wax esters, ethyl esters or fish oil). Moreover, the ratio of n-6/n-3 PUFA in phospholipids was reduced to a similar extent in animals consuming fatty acids in the form of wax ester, ethyl esters, and fish oil, compared to controls. No gross toxic effects were observed, and gastrointestinal transit times did not differ between treatment groups. Together, this study suggests that DHA and EPA administered in the form of wax esters can be absorbed in rats to a certain degree.

The consumption of wax esters due to their presence in certain deep-sea fish in large amounts by humans has been associated with a condition known kriorrhea (Ling *et al*., 2009). Kirrohea occurs as a result of the passage of these oils through the gastrointestinal tract presumably undigested, resulting in their pooling in the rectum. As discussed in

Section XII.5.2, this effect has been observed at doses that are much larger than those provided by Calanus® Oil. Studies have not been conducted in humans to determine the bioavailability of EPA and DHA when these fatty acids are consumed in the form of wax esters. Earlier literature suggests wax esters are only partially hydrolysed and assimilated in mammals, and mammalian lipases hydrolyse wax esters at a slower rate compared to triglycerides *in vitro*, suggesting the capacity to digest wax esters may also be limited in humans. However, there is evidence to suggest that following ingestion of wax esters, EPA and DHA are incorporated into plasma phospholipid to a similar extent as when these PUFAs are ingested in the form of fish oils or ethyl esters in rats (Gorreta *et al*., 2002). This study suggests consumption of wax esters in Calanus® Oil at the levels of intended use (*i.e.*, no more than 2.3 g per day with meals) is likely to produce reasonable systemic incorporation of EPA and DHA in humans. Furthermore, results from an unpublished clinical trial (See Section XIII.4) indicate that at the intended level of use, Calanus® Oil was broken down as implied by the lack of any of the expected symptoms of malabsorption of waxy substances (*i.e.* oil stools, diarrhea).

XIII.2.2 Fatty Acids

The digestion and absorption of long-chain n-3 polyunsaturated fatty acids typically found in the diet in the form of triglycerides from marine sources are well established (Bézard *et al*., 1994). Partial hydrolysis of the triglycerides occurs in the stomach, and the free fatty acids and glycerides help disperse and emulsify fats. Further hydrolysis occurs in the small intestines, as mediated by pancreatic enzymes, the activity of which is enhanced by the detergent activity of bile salts that helps emulsify the oil droplets, and the increased luminal pH of the intestines (Carlier *et al*., 1991). Dietary cholesteryl esters, phospholipids and triglycerides are digested to release free fatty acids, free cholesterol, 2-monoglycerides and 1-lysophospholipids (Carlier *et al*., 1991).

Free fatty acids and 2-monoglycerides are solubilised by bile acids to form mixed micelles, which are essential in moving saturated and mono-unsaturated long chain fatty acids across the unstirred water layer and mucus lining of enterocytes. Dissociation of mixed micelles occurs prior to absorption in the proximal part of the small intestines, and only monomers are taken up by enterocytes (Carlier *et al*., 1991). The uptake of free long-chain fatty acids into enterocytes is thought to occur passively (Hargrove *et al.*, 2004), although the possibility that uptake may also occur through facilitated diffusion or even active transport has not been excluded (Carlier *et al*., 1991). It has been proposed that the specific long-chain fatty acid binding protein may act as a membrane receptor, in addition to helping transport these hydrophobic compounds throughout the cell (Hargrove *et al*., 2004). Short and medium-chain fatty acids are hydrophilic and fairly soluble; they are absorbed passively across the mucosa and are bound to albumin for transport to the liver through the portal vein (reviewed in Carey *et al*, 1983). In contrast, long chain fatty acids are re-esterified into triglycerides and enter the lymphatic system as very low density lipoproteins (VLDL) and chylomicrons prior to reaching systemic blood circulation (Carlier *et al*., 1991).

Upon absorption, long-chain n-3 polyunsaturated fatty acids are distributed to various tissues, including the eyes, liver, kidneys, red blood cells and adipose tissues where they are incorporated into the phospholipid membranes (Bézard *et al*., 1994). Within the cell, essential fatty acids released from the membrane can compete for several metabolic pathways, including anabolic (*i.e.* microsomal esterification*)* and catabolic pathways (*i.e.* mitochondrial and peroxisomal β-oxidation) (Bézard *et al*., 1994). EPA is a precursor for series 3 eicosanoids and series 5 leukotrienes, and can be converted to DHA or other more highly unsaturated fatty acids. Increased dietary intakes of EPA and DHA decreases tissue concentrations of arachidonic acid and alters the balance of eicosanoids synthesized from the long-chain n-6 and n-3 polyunsaturated fatty acids.

In summary, ingested long-chain polyunsaturated n-3 fatty acids undergo nearly complete absorption, and are either incorporated into tissue lipids, used to synthesize eicosanoids, or oxidized to carbon dioxide and water (IOM, 2005). It is expected that the fatty acids (EPA, DHA, and SDA) released from the wax esters of Calanus® Oil will undergo similar absorptive and metabolic processes as those described above.

XIII.2.3 Fatty Alcohols

Pharmacokinetic data on the oral absorption and bioavailability of ingested fatty alcohols are limited. Studies performed using policosanols (mixtures of aliphatic C24 to C34 fatty alcohols – see Section IX.3.3) suggest that they are poorly absorbed in the gastrointestinal tract (Pepping, 2003; Hargrove *et al*., 2004). Absorption of policosanols in rodents is estimated to range between 10 and 35% of an orally administered dose, while the bioavailability is estimated at only 5 to 12% (Gouni-Berthold and Berthold, 2002). Studies conducted in rats suggest that radio-labelled fatty alcohols ingested can be converted into fatty acids in the gastrointestinal tract, and appear in the circulation mostly as fatty acid esters in triglyceride and phospholipid form (Stetten and Schoenheimer, 1940; Friedberg, 1976). As mentioned above, fatty alcohols may also be esterified with fatty acids within the gastrointestinal tract of rats to produce wax esters (Friedberg, 1976; Place, 1992). The propensity to assimilate fatty alcohols appears to be independent on the efficiency of fatty acid hydrolysis. For example, the assimilation efficiency of fatty acids from wax esters in yellow-rumped warbler and tree swallows was >85%, but the absorption of fatty alcohols was only 40% (Place, 1992). It is expected that fatty alcohols (primarily C20:1 and C22:1) will undergo a similar fate and ultimately be metabolised into normal cellular constituents.

XIII.3 Toxicity Information

XIII.3.1 Animal Studies Using Calanus® Oil

The safety of Calanus® Oil was examined in rats fed a high-fat diet as a model for obesity-related (pre)diabetes (Larsen, 2011). Male Wistar rats (12/group) received high-fat diets supplemented with Calanus® Oil (0, 0.375, 0.75, or 1.5%), or a regular control diet for a period of 112 days. Animals fed the high-fat diets (with or without Calanus® Oil supplementation), had significantly higher body weights at the end of the study compared to animals fed the control diet. Supplementation of the diet with Calanus® Oil at up to 1.5% had no significant effect on body weight gain, or food and water intake. There were no significant differences in the absolute and relative weights of the liver and heart among all treatment groups, and no abnormal findings were found upon gross examination. Cardiac function was measured by left ventricular pressure development and cardiac output *ex vivo*, and no significant differences were found among treatment groups. The consistency and texture of faeces, as well as urine production, were normal and no unusual changes in animal behaviour or motor activity were observed. These results were confirmed in an additional follow-up study in male Wistar rats (12/group) receiving a high fat diet supplemented with 0 or 1.5% Calanus® Oil, or a regular control diet, for 125 days (results not provided). According to the study author, supplementation of Calanus® Oil at the 1.5% dose is equivalent to intakes of 770 mg/kg body weight/day assuming average body weight of 350 g and feed intake of 18 g/day. In comparison, Calanus® Oil is proposed for use by humans at levels not to exceed 250 mg per day, equivalent to 4.2 mg/kg body weight/day.

In another study, Calanus® Oil was administered to a *db/db* mouse model of diabetes (10/group; sex of animals not mentioned) (Larsen, 2011). Animals were fed diets containing 1.4% Calanus® Oil or diets supplemented with DHA plus EHA, providing equivalent amounts of PUFAs, for 56 days. Consumption of Calanus® Oil did not have any significant effects on body weight gain, food or water intake, or plasma levels of glucose. Plasma levels of glucose, free fatty acids, and triacylglycerol were also not significantly different among treatment groups. No abnormal clinical or behavioural signs were reported.

Eilertsen *et al.*, 2011 (submitted, in revision) conducted a study to investigate the effects of Calanus® Oil on the development of atherosclerotic plaques, plasma lipid levels and cytokines, and hepatic gene expression in mice. Six-week-old female apolipoprotein E-deficient mice (10/group) were fed lipid-rich diets (containing 21% fat and 0.2% cholesterol) enriched with corn oil (1% w/w), corn and Calanus® Oil (1% each w/w), or corn oil and EPA and DHA ethyl esters (1.88 and 0.12% w/w, respectively) for 13 weeks. All diets were isocaloric and isonitrogenous, and contained similar amounts of carbohydrates, proteins, fats, cholesterol, and vitamins. It was reported that all mice thrived well, no adverse effects were observed, and the faeces of the mice consuming the diet enriched with Calanus® Oil appeared normal. Furthermore, the mice consuming the diet enriched with Calanus® Oil gained significantly more weight compared to those given the corn oil-enriched diet (20.5±1.1 g *vs*. 16.0±0.8 g) ($p < 0.05$), but not more than animals fed EPA plus DHA diets (19.1±1.4 g). After 13 weeks of consuming the study diets, animals were fasted for 3 hours and then anesthetized with phenobarbitol and killed by exsanguination. EPA and DHA levels in the blood were significantly higher in mice fed diets supplemented with Calanus® Oil compared to animals fed both the control diet and diets containing EPA pus DHA. Although this was not a toxicity study *per se*, ingestion of Calanus® Oil by mice did not produce any adverse effects in relevant endpoints. No differences in tissue weights or macroscopic appearances (*i.e.,* white adipose tissue, gastrointestinal tract, liver, spleen, kidney, and heart) were reported. In addition, no adverse effects were reported with respect to plasma concentrations of total cholesterol, triglycerides, cytokines, or vascular growth factors. Treatment with Calanus® Oil reduced aorta atherogenesis by 35%, and reduced gene expression of ICAM-1, IL-1β, MCP-1, and NFkB1, as well as increased expression of PPARγ in the liver.

XIII.3.2 Fatty Acids

In 2009, EFSA delivered a Scientific Opinion on the safety of Neptune Krill Oil (NKO™) as a novel food ingredient (EFSA, 2009). EFSA concluded: "*The toxicological and clinical data provided for NKO™ are limited. However, in combination with the data available for its main constituents, they support the safety of the novel food ingredient under the proposed conditions of use*". The fatty acid profiles of Krill and Calanus® Oils may be considered comparable for the purposes of this assessment, containing the same major components and in particular, significant levels of EPA and DHA (Krill Oil: min. 15% and 7% EPA and DHA, respectively; Calanus® Oil: min. 5% and 4% EPA and DHA, respectively). Both oils are intended for use in food supplement products at similar levels (no more than 200 mg and 250 mg EPA plus DHA, respectively) and consumption of fatty acids (total fatty acid content in both oils *ca*. 50% of the oil in the form of phospholipids and wax esters, respectively) from these 2 products therefore, may be expected to be equivalent. Since the safety of EPA and DHA from marine sources at intake levels of 200 mg per day are well established, and fish oil products are commonly marketed at levels of at least 250 mg per day (see Section IX.2.3), individual studies relating to these constituents are not discussed in this application. Additionally, several national regulatory bodies have established that the consumption of fish oils providing up to 3,000 mg of EPA + DHA is not associated with any safety concerns (FDA, 1997).

Additionally, the consumption of SDA at levels of up to 500 mg from food supplements containing echium (*Echium plantagineum*) oil as an ingredient has previously been reviewed by the UK Competent Authority as part of the novel foods approval for the substance under Commission Decision 2008/558/EC (Commission of the European Communities, 2008b). Intakes of SDA from the use of Calanus® Oil under the proposed use in food supplement products will not exceed 161 mg (*i.e.*, from 2.3 g of oil; see Section IX.2.2) per day far below the 500 mg limit for SDA imposed for echium oil. Safety was established on the basis that a number of animal and human studies have demonstrated that consumption of SDA was well tolerated and without adverse effects. The 2 clinical studies considered to be the most clinically relevant from a human nutritional safety perspective, administered echium oil at levels producing daily intakes of up to 1.9 g of SDA from echium oil for periods of up to 12 weeks (Miles *et al*., 2006; Surette *et al*., 2004). Although, the studies were primarily for research purposes, safety and tolerability were monitored and no adverse effects were attributable to consumption of echium oil. Similar results were observed in a human clinical study where SDA esters were administered at a dose of 0.75 g/day for 3 weeks and subsequently increased to 1.5 g/day for an additional 3 weeks (James *et al*., 2003). Accordingly, since the safety of SDA from a plant source is well established at far higher levels than from the proposed use of Calanus® Oil there should be no concerns associated with the novel ingredient.

XIII.3.3 Fatty Alcohols

Studies pertaining to the safety of ingesting fatty alcohols derived from zooplankton have not been conducted explicitly, but it may be presumed that these are substances that are used by the body for established metabolic processes (see Section XIII.2.3). As fatty alcohols are known to undergo interconversions with fatty acids, exposure to fatty alcohols through consumption of wax esters in Calanus® Oil is expected to be without adverse effects. Additionally, as demonstrated in Section XI.3, long chain fatty alcohols are natural components of the diet at levels comparable or exceeding the anticipated intakes from Calanus® Oil as an ingredient in food supplement products (*i.e.*, up to 301 and 449 mg of C20:1 n-9 and C22:1 n-11 – see Table IX.2.2-1).

XIII.3.4 Other Minor Components: Astaxanthin

The Advisory Committee on Novel Food Products has published several opinions regarding the use of astaxanthin supplement products, and the intended uses of these products were suggested to provide 4 mg astaxanthin per day (ACNFP, 2004, 2007). Moreover, in a review of eight human clinical studies designed to evaluate the effects of astaxanthin on various health-related biomarkers, no adverse effects were reported following the consumption of up to 100 mg astaxanthin as a single dose, or doses up to 21.6 mg astaxanthin for up to 1 year (Fassett and Coombes, 2011). These levels of intake far exceed the estimated exposure from Calanus® Oil (<1 mg per 1 g of oil) and overall, the intake of astaxanthin through use of the novel ingredient is not expected to pose a safety concern.

XIII.4 Human Clinical Studies

A clinical study was performed to evaluate the safety and tolerability of Calanus® Oil in healthy human subjects (Jorde, 2010). Fifteen healthy subjects (11 males, 4 females) were divided into three groups and administered Calanus® Oil in gelatine capsules twice daily for 4 weeks (5/group). One group received a single 500 mg capsule twice daily (1 g total dose), the second group received two 500 mg capsules twice daily (2 g total dose), and the third group received four 500 mg capsules twice daily (4 g total dose). Adverse effects were evaluated at Week 2 and Week 4. In addition, blood samples were taken at baseline and after 2 and 4 weeks for analyses of haematology and clinical chemistry parameters.

All subjects completed the study. Although there was no placebo control group in this study, no serious adverse effects were reported from consumption of Calanus® Oil. Two subjects reported transient nausea and a single subject reported occasional abdominal gas. Two subjects administered the highest dose of Calanus® Oil reported more frequent bowel habits while one subject in the intermediate dose group reported less frequent bowel habits. Five subjects reported positive effects including increased energy, ease of defecation, less hunger, and less joint/muscle after exercise or in extremities. An increase in serum creatinine in 1 subject and alanine aminotransferase/*gamma* glutamyltransferase in another were considered to be transient and returned to baseline levels by Week 4. As such, it was not considered to be clinically significant. Together, these results suggest Calanus® Oil administered at doses up to 4 g/day was safe and well tolerated, approximately 2-fold greater than the maximum intended use of the novel ingredient in food supplement products (*i.e.*, up to 2.3 g/day).

XIII.5 Allergenicity and Intolerance/Adverse Effects

XIII.5.1 Allergenicity and Labelling Considerations

"Crustaceans and products thereof" are currently included in the list of ingredient under Annex IIIa of Directive 2000/13/EC (European Parliament and Council of the European Union, 2000) as amended by Commission Directive 2007/68/EC (Commission of the European Communities, 2007) which must be indicated on the label of all foodstuffs. Consequently, all food supplement products containing Calanus® Oil will be labelled as "contains oil derived from crustaceans (marine zooplankton)".

In terms of the potential for allergenicity, consistent with its lipid nature, Calanus® Oil will not contain significant levels of protein or peptide fragments. The absence of shellfish allergens in Calanus® Oil has been confirmed by Enzyme Linked Immunosorbent Assay (ELISA) methods for 3 batches of material. No shellfish allergens were detected in the 3 batches tested and the Certificates of Analysis are provided in Appendix A-7, respectively.

XIII.5.2 Intolerance/Adverse Effects to Dietary Wax Esters

Oilfish (*Ruvettus pretiosus*) and Escolar (*Lepidocybioum flavobrunneum*) deep-sea-dwelling fish rich in wax esters, of the family *Gempylidae* are consumed in a number of Member States albeit to a lesser extent than many other fish species (Ling *et al*., 2009). The muscle meat of the 2 fish species comprise approximately 18 to 21% lipids primarily in the wax ester form (>90%). The consumption of these 2 fish species has been associated with adverse gastrointestinal effects such as nausea, vomiting, cramping, and most notably, waxy diarrhoea (*i.e.,* keriorrhea). Despite the high levels of histidine in the flesh of Oilfish and Escolar[3], it has been proposed that these gastrointestinal effects were attributed primarily to the accumulation of undigested wax esters (Sargent, 1981; Ashbolt *et al*., 2002; Gregory, 2002; Shadbolt *et a*l., 2002; Yohannes *et al*., 2002; EFSA, 2004; Ling *et al*., 2009; Dolan *et al*., 2010; Hashimoto and Fusetani, 1978). Most people who experience keriorrhea following consumption of Oilfish or Escolar do not report abdominal pain, suggesting that the keriorrhea is possibly the result of a lubricant effect of the undigested wax esters, rather than an irritant effect induced by other components present (Ling *et al*., 2009). As described in Section XIII.2, mammals have limited capacity to hydrolyse wax esters in the gastrointestinal tract, and consumption of large amounts of wax esters results in an involuntary oily discharge, presumably due to the pooling of undigested wax esters in the rectum (Ling *et al*., 2009). Because of these effects, wax esters are thought to be effective purgatives in

[3] Oilfish and Escolar have been reported to contain 8 to 11 mg histidine/g muscle tissue – the potential for formation of histamine from improper storage of fish and the resulting toxicity is well established.

mammals, including humans (Ling *et al*., 2009). Certain individuals with intestinal conditions and pregnant women may be at higher risk of developing keriorrhea (Shadbolt *et al*., 2002).

As a consequence of these concerns, under Commission Regulation (EU) No 1021/2008 (Commission of the European Communities, 2008c) food use of these fish species are subject to restrictions regarding their packaging and labelling in order that the consumer is aware of the preparation/cooking methods and potential adverse effects. Worldwide, while in a limited number of countries the import and sale of Oilfish and Escolar is banned, generally, countries have adopted specific guidelines and warnings regarding their consumption. Advice on the consumption of the fish species by various countries includes the selection of smaller portion sizes (Canada), and separation of the majority of the fat during cooking (Sweden, Denmark). Notably, in many of the reported outbreaks of adverse effects (*i.e.*, keriorrhea) the fish have been identified to be mislabelled as other species such as cod, white tuna or sea bass and the necessary precautions regarding their use were consequently not followed (Ling *et al*., 2009). In 2004, EFSA evaluated the adverse gastrointestinal effects associated with the consumption of Oilfish and Escolar (EFSA, 2004). The Panel noted that these effects "are likely to be caused by wax esters and/or other oily compounds naturally present in the meat of these fishes". However, it was concluded that an intake level of fish which would not lead to adverse effects could not be established due to the limited human study data available. As part of this assessment, it was estimated that a 100 g serving of smoked Oilfish or Escolar would contain around 20 g of wax esters. These levels are at least 10-fold higher than the anticipated intake of wax esters from the proposed use of Calanus® Oil as an ingredient in food supplement products in the EU. Moreover, as outlined in Section XI, there are numerous examples of wax esters in the diet that are not associated with adverse gastrointestinal effects. While not all the wax esters in the diet are chemically identical to Calanus® Oil, many such as those from fish roe products bear many similarities in the key components and their relative proportions. In all cases, the dietary wax esters identified from marine and other sources are expected to undergo similar digestive and absorption processes in the gastrointestinal tract as Calanus® Oil. Combined with the fact that Calanus® Oil was well tolerated in a clinical trial testing doses up to 4 g/day (see Section XIII.4), adverse gastrointestinal effects due to the ingestion of wax esters from Calanus® Oil are unlikely at the proposed maximum use level of *ca*. 2.3 g per daily serving from food supplement products (*i.e.*, 250 mg EPA plus DHA/day).

OVERALL CONCLUSIONS

The safety of Calanus® Oil as an ingredient for use in food supplement products, delivering up to 250 mg EPA plus DHA per day (*ca*. 2.3 g of oil) is based on the following:

- Compositionally, the ingredient consists primarily of wax esters (>85%) with minor amounts of other neutral and polar lipids (*e.g*., triglycerides). The individual fatty acid and fatty acid profiles are typical of products of marine origin (*e.g.*, fish oils).
- The intended use of the Calanus® is as a direct replacement for other sources of EPA and DHA in food supplement products. The maximum proposed use level is typical of fish oil products currently on the market and will not lead to an increased exposure by the general population to any of the individual components.
- *C. finmarchicus* represents a major source of secondary energy to many marine organisms (*e.g.*, Atlantic herring, squid and salmon) which are commonly consumed by the European population, and from which marine oils are traditionally derived. In addition, historically, *C. finmarchicus* was a recognised food source for sailors on long expeditions or that were shipwrecked.
- Nutritionally, the wax esters and individual constituents that comprise Calanus® Oil are normal constituents of the diet of the EU population at levels far greater than those of the intended use of the novel ingredient. Specifically, fish roe products frequently contain significant levels of wax esters with similar compositional profiles to Calanus® Oil where a typical 85 g serving may contain in the region of 13 to 71 g of wax or steryl esters.
- On ingestion, the components of Calanus® Oil are expected to undergo the normal metabolic fate of wax esters, fatty acids and fatty alcohols
- While there are no studies specific to the bioavailability of Calanus® Oil in humans, a study in rats has indicated that polyunsaturated fatty acids from wax esters were incorporated into plasma phospholipid to a similar extent as when ingested in the form of fish oils or ethyl esters
- There were no observed adverse effects in unpublished studies in rats and mice in which animals were fed levels of up to 1.5 and 1%, respectively Calanus® Oil in the diet. In the 112 day rat study, an intake level of 1.5% of the diet was determined to be equivalent to approximately 770 mg/kg body weight/day
- Calanus® Oil was well tolerated by humans with no reports of significant adverse effects in a study in which participants' ingested doses of 1 g, 2 g and 4 g of oil daily over a 4-week period. The highest dose tested is approximately 2-fold greater than the proposed maximum use level of Calanus® Oil as an ingredient in food supplement products.

- A shellfish allergens test indicates that Calanus® Oil should not be associated with any allergenicity, although, products will be labelled in accordance with Annex IIIa of Directive 2000/13/EC to indicate the crustacean source.

REFERENCES

Ackman RG. Fish oils. In: Shahidi F, editor. *Bailey's Industrial Oil and Fat Products, 6th ed*. John Wiley & Sons, Inc., Hoboken, NJ; 2005, pp. 279-317.

ACNFP. *Request for an Article 5 Opinion on the Substantial Equivalence of Astaxanthin-Rich Carotenoid Oleoresin Extracted from Haematococcus pluvialis*. Advisory Committee on Novel Foods and Processes (ACNFP), London, UK; 2004. Available from: http://www.food.gov.uk/multimedia/pdfs/astaxanthinfinal.PDF.

ACNFP. *Opinion on Substantial Equivalence of Astaxanthin-Rich Oleoresin Extracted from Haematococcus pluvialis Considered Under Article 5 of the Novel Foods Regulation*. Advisory Committee on Novel Foods and Processes (ACNFP), London, UK; 2007. Available from: http://www.food.gov.uk/multimedia/pdfs/cyanotechastaxopinionfeb07.pdf.

AFSSA. *Apports Nutritionnels Conseillés Pour la Population Française.* ANC. 3e éd. Agence Française de Sécurité Sanitaire des Aliments (AFFSA). CNERNA-CNRS, coordonnateur A Martin, Editions Tec&Doc, Paris; 2001, 605 pp. Cited In: EFSA, 2010.

Ashbolt R, Givney R, Gregory JE, Hall G, Hundy R, Kirk M, McKay I, Meuleners L, Millard G, Raupach J, Roche P, Prasopa-Plaizier N, Sarna MK, Stafford R, Tomaska N, Unicomb L, Williams. Enhancing foodborne disease surveillance across Australia in 2001: the OzFoodNet Working Group. *Communicable Diseases Intelligence* 2002, 26: 375-406.

ASTER. *ASTER (Assessment Tools for the Evaluation of Risk) Ecotoxicity Profile*. U.S. Environmental Protection Agency, Washington, DC; 1994. Cited In: EFSA, 2009.

Beitz R, Mensing GMB, Rams S, Döring A. Vitamin- und Mineralstoffsupplementierung in Deutschland. *Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz* 2004, 47: 1057-1065.

Bernasconi R, Bolzacchini E, Galliani G, Gugliersi F, Rindone B, Rindone M, Tacconi, M.T.; & Terraeno, A. Determination of the content of wax esters in some sea foods and their molecular compositon. a comparison with ω-3 enriched wax esters. *Lebensmittel-Wissenschaft und -Technologie* 2007, 40: 569-573.

Bézard J, Blond JP, Bernard A, Clouet P. The metabolism and availability of essential fatty acids in animal and human tissues. *Reproduction, Nutrition, Development* 1994, 34:539-568.

Bledsoe GE, Bledsoe CD, Rasco B. Caviars and fish roe products. *Critical Reviews in Food Science and Nutrition* 2003, 43: 317-356.

Buisson DH, Brody DR, Dougherty GJ, Eyres L, Vlieg P. Oil from deep water fish species as a substitute for sperm whaile and jojoba oils. *Journal of the American Oil Chemists Society* 1982, 59: 390-395.

Carey MC, Small DM, Bliss CM. Lipid digestion and absorption. *Annual Review of Physiology* 1983, 45:651-677.

Carlier H, Bernard A, Caselli C. Digestion and absorption of polyunsaturated fatty acids. *Reproduction, Nutrition, Development* 1991, 31:475-500.

Commission of the European Communities. Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council (97/618/EC) [L253]. *Official Journal of the European Communities* 1997, 40: 1-36. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31997H0618:EN:HTML.

Commission of the European Communities. Commission Decision of 5 June 2003 authorising the placing on the market of oil rich in DHA (docosahexaenoic acid) from the microalgae Schizochytrium sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2003) 1790) (2003/427/EC) [L144]. *Official Journal of the European Union* 2003, 46: 13-14. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:144:0013:0014:EN:PDF.

Commission of the European Communities. Commission Regulation (EC) No 1881/2006 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance) [L364]. *Official Journal of the European Union* 2006, 49: 5-24. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF.

Commission of the European Communities. Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients [L310]. *Official Journal of the European Union* 2007, 50: 11-14. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:310:0011:0014:EN:PDF.

Commission of the European Communities. Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs [L173]. *Official Journal of the European Union* 2008a, 49: 6-9. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:173:0006:0009:EN:PDF.

Commission of the European Communities. Commission Decision of 27 June 2008 authorising the placing on the market of refined echium oil as novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document number C(2008) 3049) (2008/558/EC) [L180]. *Official Journal of the European Union* 2008b, 49: 17-19. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:180:0017:0019:EN:PDF.

Commission of the European Communities. Commission Regulation (EC) No 1021/2008 of 17 October 2008 amending Annexes I, II and III to Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption and Regulation (EC) No 2076/2005 as regards live bivalve molluscs, certain fishery products and staff assisting with official controls in slaughterhouses [L277]. *Official Journal of the European Union* 2008c, 49: 15-17. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:277:0015:0017:EN:pdf.

Commission of the European Communities. Commission Decision of 12 October 2009 authorising the placing on the market of a lipid extract from Antarctic Krill Euphausia superba as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (2009/752/EC) [L268]. *Official Journal of the European Union* 2009, 52: 33-34. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:268:0033:0034:EN:PDF.

Council of the European Communities. Council Directive of 24 September 1990 on nutrition labelling for foodstuffs (90/496/EEC) [L276]. *Official Journal of the European Communities* 1990*, 33: 40-44*. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1990L0496:20081211:EN:PDF [Consolidated version: 2008-12-11].

D-A-CH (Deutsche Gesellschaft für Ernährung - Österreichische Gesellschaft für Ernährung - Schweizerische Gesellschaft für Ernährungsforschung - Schweizerische Vereinigung für Ernährung). *Referenzwerte für die Nährstoffzufuhr*. Umschau Braus Verlag, Frankfurt am Main, Germany; 2008. Cited In: EFSA, 2010.

Danish Veterinary and Food Administration. Denmark's Trans Fat Law: Executive Order No. 160 of 11 March 2003 on the Content of Trans Fatty Acids in Oils and Fats etc. (English Translation). Danish Veterinary and Food Administration (DVFA), Soborg, Denmark; 2003. Available from: http://www.tfx.org.uk/page116.html [2004-2010©].

Department of Health. *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy*. Department of Health (DoH) / Her Majesty's Stationery Office (HMSO), London, Engl.; 1991.

Dolan LC, Matulka RA, Burdock GA. Naturally occurring food toxins. *Toxins* 2010, 2: 2289-2332.

EFSA. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to the toxicity of fishery products belonging to the family of Gempylidae. (Question no EFSA-Q-2004-016, adopted on 30 August 2004 by European Food Safety Authority). *EFSA Journal* 2004, 92: 1-5. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/92.htm.

EFSA. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies [NDA] related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat. (Question no EFSA-Q-2004-107, Adopted on 6 July 2005 by the European Food Safety Authority). *The EFSA Journal* 2005, 253: 1-29. Available from: http://www.efsa.europa.eu/en/scdocs/scdoc/253.htm [Last updated: 19 May 2006].

EFSA. Beeswax (E 901) as a glazing agent and as carrier for flavours Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC). (Question no EFSA-Q-2006-021, adopted on 27 November 2007 by European Food Safety Authority). *The EFSA Journal* 2007, 615: 1-28. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/615.htm.

EFSA. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. (Question no EFSA-Q-2007-138, adopted on 30 January 2009 by European Food Safety Authority). *The EFSA Journal* 2009, 980: 1-139. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/980.htm.

EFSA. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA); Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. (Question no EFSA-Q-2008-466, adopted on 04 December 2009 by European Food Safety Authority). *EFSA Journal* 2010, 8: 1461. [107 pp.]. doi:10.2903/j.efsa.2010.1461. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/1461.htm.

Eilertsen K-E, Mæhre HK, Jensen IJ, Devold H, Olsen JO, Lie RK, Brox J, Berg V, Elvevoll EO, Østerud B. Reduced atherosclerotic lesion development in Apolipoprotein E–deficient mice fed a Western-type diet enriched with oil extracted from the marine copepod *Calanus finmarchicus*. *The Journal of Nutrition* 2011, submitted and in revision (peer-review process).

EUFIC. [Proteases, Aminopeptidases]. In: *Modern Biotechnology in Food: Applications of Food Biotechnology: Enzymes*. European Food Information Council (EUFIC), Brussels, Belgium; 2011. Available from: http://www.eufic.org/article/en/food-technology/gmos/rid/modern-biotechnology-food-enzymes/.

European Parliament and the Council of the European Union. Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners [L61]. *Official Journal of the European Communities* 1995, 38: 1-40. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1995L0002:20060815:EN:PDF [Consolidated version: 2006-08-15].

European Parliament and the Council of the European Union. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients [L43]. *Official Journal of the European Communities* 1997, 40: 1-6. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090807:EN:PDF [Consolidated Version: 2009-08-07].

European Parliament and the Council of the European Union. Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs [L109]. *Official Journal of the European Communities* 2000, 43: 29-42. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:109:0029:0042:EN:PDF.

European Parliament and the Council of the European Union. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to dietary supplements [L43]. *Official Journal of the European Communities* 2002, 45: 51-57. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:183:0051:0057:EN:PDF.

European Parliament and the Council of the European Union. Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. *Official Journal of the European Union* 2008, 51: 7-15. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0007:0015:EN:PDF.

FAO. *The State of World Highly Migratory, Straddling and Other High Seas Fishery Resources and Associated Species* (FAO Fisheries Technical Paper 495). Food and Agriculture Organization of the United Nations (FAO), Rome, Italy; 2006. Available from: http://www.fao.org/docrep/009/a0653e/a0653e00.HTM.

FAO. *Clupea harengus (Linnaeus, 1758)*. Aquatic Species Fact Sheets. Food and Agriculture Organization of the United Nations (FAO), Fisheries and Aquaculture Department, Rome, Italy; 2011. Available from: http://www.fao.org/fishery/species/2886/en.

Fassett RG, Coombes JS. Astaxanthin: a potential therapeutic agent in cardiovascular disease. *Marine Drugs* 2011, 9: 447-465.

Firestone D, ed. Herring oil; Redfish oil; Sardine, pilchard oil. In: *Physical and Chemical Characteristics of Oils, Fats and Waxes*, 2nd ed. AOCS Press, Urbana, Illinois; 2006. [Includes CD - Fatty Acid Composition of Oils], pp. 184-185, 194, 196.

Friedberg SJ. Plasma transport forms of ingested fatty alcohols in the rat. *Lipids* 1976, 11: 587-593.

FSA. *Food Portion Sizes*, 3rd ed. Food Standards Agency (FSA), Her Majesty's Stationery Office (HMSO), London, Engl.; 2002.

Gorreta F, Bernasconi R, Galliani G, Salmona M, Tacconi MT, Bianchi R (2002). Wax esters of *n*-3 polyunsaturated fatty acids: a new stable formulation as a potential food supplement. 1 — digestion and absorption in rats. *Lebensmittel-Wissenschaft und – Technologie* 2002, 35: 458-465.

Gouni-Berthold I, Berthold HK. Policosanol: clinical pharmacology and therapeutic significance of a new lipid-lowering agent. *American Heart Journal* 2002, 143: 356-365.

Gezondheidsraad. *Dietary Reference Intakes: Energy, Proteins, Fats and Digestible Carbohydrates*. (Publication no. 2001/19R). Health Council of the Netherlands, The Hague, Netherl. Available at: http://www.gezondheidsraad.nl/en/publications/dietary-reference-intakes-energy-proteins-fats-and-digestible-carbohydrates.

Gregory J. Outbreaks of diarrhoea associated with butterfish in Victoria. *Communicable Diseases Intelligence* 2002, 26: 439-440.

Hansen IA, Mead JF (1965). The fate of dietary wax esters in the rat. *Proceedings of the Society for Experimental Biology and Medicine* 1965, 120: 527-532.

Hargrove JL, Greenspan P, Hartle DK. Nutritional significance and metabolism of very long chain fatty alcohols and acids from dietary waxes. *Experimental Biology and Medicine* 2004, 229: 215-226.

Hashimoto K, Fusetani N. Toxins occurring in commercially important marine organisms. In: APFIC. *Eighteenth IPFC Fisheries Symposium*. Asia-Pacific Fishery Commission, Bangkok, Thailand; 1978, pp. 416-424. Available at: www.apfic.org/Archive/symposia/1978/53.pdf.

Henderson L, Gregory J, Swan G. *The National Diet & Nutrition Survey: Adults Aged 19 to 64 Years: Vol. 1: Types and Quantities of Foods Consumed*. Carried out by the Social Survey Division of the Office for National Statistics and Medical Research Council Human Nutrition Research on behalf of the Food Standards Agency (FSA), London, Engl.; 2002. Available from: http://www.food.gov.uk/multimedia/pdfs/ndnsprintedreport.pdf.

Herdman WA. Copepoda as an article of food. *Nature* 1891, 44: 273-274.

IOM. Dietary fats: total fat and fatty acids. In: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Institute of Medicine (IOM) of the National Academies / The National Academy Press (NAP), Washington, DC; 2005, pp. 422-541. Available from: http://fnic.nal.usda.gov/nal_display/index.php?info_center=4&tax_level=4&tax_subject=256&topic_id=1342&level3_id=5141&level4_id=10588.

James MJ, Ursin VM, Cleland LG. Metabolism of stearidonic acid in human subjects: Comparison with the metabolism of other n-3 fatty acids. *American Journal of Clinical Nutrition* 2003, 77:1140-1145.

Jónasdóttir SH. Lipid content of *Calanus finmarchicus* during over wintering In the Faroe-Shetland. *Fisheries Oceanography* 1999, 9(s1): 61-72.

Jorde R. *Safety and tolerability of oil from Calanus finmarchicus in human subjects* [study report]. Submitted to Calanus AS, Tromsø, Norway by Professor Terje Larsen: University of Tromsø, Faculty of Biomedicine, Tromsø, Norway; 2010.

Kalogeropoulos N, Nomikos T, Chiou A, Fragopoulou E, Antonopoulou S. Chemical composition of Greek avgotaracho prepared from mullet (Mugil cephalus): nutritional and health benefits. *Journal of Agricultural and Food Chemistry* 2008, 56: 5916-5925.

Kiely M, Flynn A, Harrington KE, Robson PJ, O´Connor N, Hannon EM, O´Brien MM, Bell S, Strain, JJ. The efficacy and safety of nutritional supplement use in a representative sample of adults in the North/South Ireland Food Consumption Survey. *Public Health Nutrition* 2001, 4: 1089-1097.

Larsen T. *Oil from Calanus finmarchicus (Calanus® Oil): Long Term Safety Assessment/Animal Studies*. Submitted to Calanus AS, Tromsø, Norway by Professor Terje Larsen: University of Tromsø, Faculty of Biomedicine, Tromsø, Norway; 2011.

Lee RF, HagenW, Kattner G. Lipid storage in marine zooplankton. *Marine Ecology (Progress Series)* 2006, 307: 273-306.

Ling KH, Nichols PD, But PP. Fish-induced keriorrhea. *Advances in Food and Nutrition Research* 2009, 57: 1-52.

Miles EA, Banerjee T, Calder PC. Self-reported health problems in young male subjects supplementing their diet with oils rich in eicosapentaenoic, gamma-linolenic and stearidonic acids. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 2006, 75: 57-60.

Ministère de l'économie, des finances et de l'industrie. Arrêté du 19 octobre 2006 relatif à l'emploi d'auxiliaires technologiques dans la fabrication de certaines denrées alimentaires. NOR : ECOC0600115A). *Journal Officiel de la Republique Francaise, Paris, France*, 2006, 279. Available from: http://admi.net/jo/20061202/ECOC0600115A.html.

Nordic Council of Ministers. Fat (chapter 11). In: Nordic Nutrition Recommendations (NNR) 2004: *Integrating Nutrition and Physical Activity*. Nordic Council of Ministers, Copenhagen, Denmark; 2004, pp. 157-172.

Pepping J. Alternative therapies: policosanol. *American Journal of Health-System Pharmacy* 2003, 60: 1112, 1114-1115.

Ph. Eur. Fish oil, rich in omega-3-acids. In: *European Pharmacopoeia, 6th ed.* (European Treaty Series, no. 50, vols. 1 & 2). Directorate for the Quality of Medicines of the Ph. Eur., Strasbourg, France, 2007, pp. 1893-1895.

Place AR (1992). Comparative aspects of lipid digestion and absorption: physiological correlates of wax ester digestion. *The American Journal of Physiology* 1992, 263: R464-R471.

Ratnayake WN, Ackman RG. Fatty alcohols in capelin, herring and mackerel oils and muscle lipids: I. Fatty alcohol details linking dietary copepod fat with certain fish depot fats. *Lipids* 1979a, 14: 795-803.

Ratnayake WN, Ackman RG. Fatty alcohols in capelin, herring and mackerel oils and muscle lipids: II. A comparison of fatty acids from wax esters with those of triglycerides. *Lipids* 1979b, 14: 804-810.

SACN. *Advice on Fish Consumption: Benefits and Risks*. Published for the U.K. Department of Health, Scientific Advisory Committee on Nutrition (SACN) by The Stationary Office (TSO), London, Engl.; 2004. Available from: http://www.food.gov.uk/multimedia/pdfs/fishreport2004full.pdf.

Sargent JR, Gatten RR, Henderson RJ. Marine wax esters. *Pure and Applied Chemistry* 1981, 53: 867-871.

Savary P. The action of pure pig pancreatic lipase upon esters of long-chain fatty acids and short-chain primary alcohols. *Biochimica et Biophysica Acta* 1971, 248: 149-155.

SCF. *Nutrient and Energy Intakes for the European Community (Opinion Expressed on 11 December 1992).* (Food Science—Reports of the Scientific Committe for Food, Thirty-first Series). Commission of the European Communities, Directorate-General Industry, Scientific Committee for Food (SCF), Luxembourg; 1993. Available from: http://ec.europa.eu/food/fs/sc/scf/out89.pdf.

Schwimmer M, Schwimmer D. IV. Plankton [Calanus]. In: *The Role of Algae and Plankton in Medicine*. Grune & Stratton, New York / London; 1955, pp. 22-27.

Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR. Lipids and life strategies of Calanus finmarchicus, Calanus glacialis and Calanus hyperboreus in late autumn, Kongsfjorden, Svalbard. *Polar Biology* 2000, 23: 510-516.

Shadbolt C, Kirk M, Roche P. Diarrhoea associated with consumption of escolar (rudderfish). *Communicable Diseases Intelligence* 2002, 26: 436-438.

Skjoldal HR, Misund OA, Saetre R, Faerno A, Rottingen I, eds. *The Norwegian Sea Ecosystem*. Tapir Academic Press, Trondheim, Norway; 2004.

Smith J, Hong-Shum L, eds. Enzymes. In: *Food Additives Data Book*. Blackwell, Oxford, Engl; 2003, pp. 390-462 [See pp. 436, 448, 460].

Stetten D Jr, Schoenheimer R. The biological relations of the higher aliphatic alcohols to fatty acids. *Journal of Biological Chemistry* 1940, 133: 347-357.

Surette ME, Edens M, Chilton FH, Tramposch KM. Dietary echium oil increases plasma and neutrophil long-chain (n-3) fatty acids and lowers serum triacylglycerols in hypertriglyceridemic humans. *Journal of Nutrition* 2004, 134: 1406-1411.

Touvier M, Dufour A, Gourillon S, Potier de Courcy G, Volatier JL, Martin A. Les forts consommateurs de compléments alimentaires en France. *Cahiers de Nutrition et de Diététique* 2003, 38: 187-194.

WHO/FAO. *Expert Report: Diet, Nutrition and Prevention of Chronic Diseases. Report of a Joint WHO/FAO Expert Consultation*. (WHO Technical Report Series 916). Geneva, Switz., World Health Organization (WHO)/Food and Agriculture Organization (FAO); 2003. Available from: http://whqlibdoc.who.int/trs/WHO_TRS_916.pdf.

Yohannes K, Dalton CB, Halliday L, Unicomb LE, Kirk M. An outbreak of gastrointestinal illness associated with the consumption of escolar fish. *Communicable Diseases Intelligence* 2002, 26: 441-445.

Yusuf AA, Webster L. Seasonal variation in the physical characteristics of the Copepod *Calanus finmarchicus* (Gunnerus) along the North Atlantic. *Journal of Biological Sciences* 2008, 8: 95-100.