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**ORIGINAL SUBMISSION**

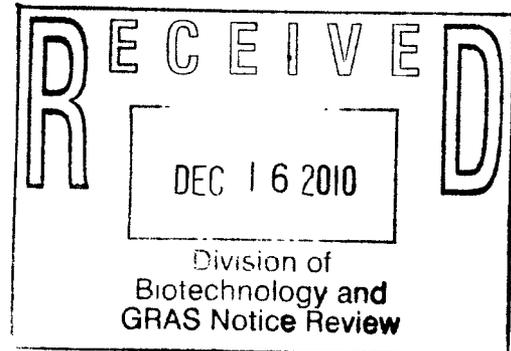
**000001**

# Soni & Associates Inc.

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December 14, 2010

Office of Food Additive Safety (HFS-255)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740-3835



## **Subject: Notification of GRAS Determination for Krill Oil**

Dear Sir/Madam:

In accordance with proposed 21 CFR 170.36 (Notice of a claim for exemption based on a GRAS determination) published in Federal Register (62 FR 18938-18964; April 17, 1997), I am submitting in triplicate, as the agent of the notifier, Aker Biomarine Antarctic AS, Norway, a Generally Recognized As Safe (GRAS) notification for Superba® Krill Oil.

Superba™ Krill Oil extracted from Antarctic krill, *Euphausia superba* is intended for use as a food ingredient in non-alcoholic beverages; breakfast cereals; cheeses; frozen dairy desserts; milk whole and skim; processed fruit and fruit juices; and medical foods, at use levels ranging from 0.05 to 0.50 g per serving (reference amounts customarily consumed, 21 CFR 101.12). The intended use of Superba® Krill Oil is estimated to result in a maximum daily intake of 8.28 g/person.

If you have any questions or require additional information, please feel free to contact me by phone at 772-299-0746 or by email at sonim@bellsouth.net.

Sincerely,  
(b) (6)

Madhu G. Soni, Ph.D.

Enclosures:

# Soni & Associates Inc.

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## GRAS NOTIFICATION

### I. Claim of GRAS Status

#### A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Aker Biomarine Antarctic AS, Norway, has determined that high phospholipid krill oil is Generally Recognized As Safe, and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use. This determination is based on scientific procedures as described in the following sections, under the conditions of krill oil's intended use in food, among experts qualified by scientific training and expertise.

Sig(b) (6)



Date 12/14/10

\_\_\_\_\_  
Madhu G. Soni, Ph.D., FACN

Agent for:

Aker Biomarine Antarctic AS  
Fjordalléen 16, 0115 Oslo  
Norway

**B. Name and Address of Notifier:**

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Aker Biomarine Antarctic AS  
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**C. Common or usual name of the notified substance:**

The common name of the substance of this notification is high phospholipid krill oil. The specific substance of this GRAS determination is Superba™ Krill Oil extracted from Antarctic krill, *Euphausia superba*. Superba™ Krill Oil is rich in omega-3 fatty acids, most of which are attached to phospholipids. Superba™ Krill Oil also contains astaxanthin ester.

**D. Conditions of use:**

High phospholipid krill oil is intended for use as a substitute or alternative to fish oils in the following food categories: non-alcoholic beverages; breakfast cereals; cheeses; frozen dairy desserts; milk whole and skim; processed fruit and fruit juices; and medical foods<sup>1</sup>, at use levels ranging from 0.05 to 0.50 g per serving (reference amounts customarily consumed, 21 CFR 101.12). The intended use of Superba™ Krill Oil, in the above mentioned food categories, is estimated to result in a maximum daily intake of 8.28 g/person. The proposed use of Superba™ Krill Oil will provide a maximum daily consumption of up to 2.20 g/person/day of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

**E. Basis for GRAS Determination:**

In accordance with 21 CFR 170.30, high phospholipid krill oil has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. A comprehensive search of the scientific literature was also utilized for this determination. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for Superba™ Krill Oil. Recently, high phospholipid krill oil (GRN 000242) has been the subject of a GRAS notification, while two of its important component fatty acids, EPA and DHA as part of fish or algal oil, have been the subject of multiple GRAS notifications. In response to these notices, FDA did not question the conclusions that the use of high phospholipid krill oil or sources of fatty acids (EPA and DHA) is GRAS under the conditions described in the notices. The safety

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<sup>1</sup> Under Section 5(b) of the Orphan Drug Act (ODA), a Medical Food is defined as a food that is formulated to be consumed or administered enterally under the supervision of a physician and that is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation. The intended use of krill oil in medical foods will be as per these and other applicable regulations.

determination of high phospholipid krill oil is based on the totality of available scientific evidence that includes human observations and a variety of preclinical and clinical studies. Based on the available safety-related information, the estimated daily intake, if ingested daily over a lifetime, is safe.

**F. Availability of Information:**

The data and information that forms the basis for this GRAS determination will be provided to the Food and Drug Administration upon request and are located at the offices of:

Madhu G. Soni, Ph.D., FACN,  
 Soni & Associates Inc.,  
 749 46<sup>th</sup> Square,  
 Vero Beach FL, 32968  
 Phone: (772) 299-0746; E-mail: sonim@bellsouth.net

**II. Detailed Information About the Identity of the Notified Substance:**

**A. Trade Name:**

The subject of this notification will be marketed as Superba™ Krill Oil

**B. Physical Characteristics**

Superba™ Krill Oil is dark red colored viscous oil

**C. Chemical Abstract Registry Number:**

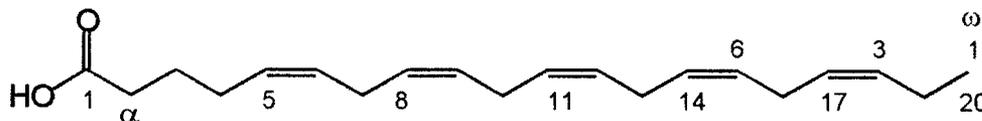
Not available

**D. Chemical Formula:**

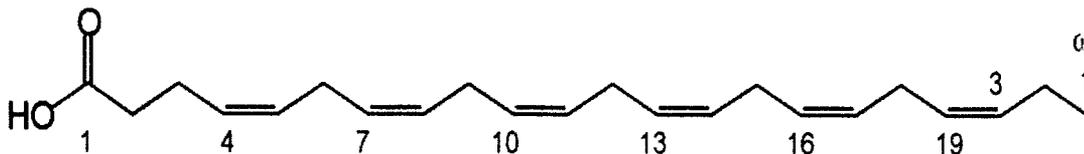
Not applicable

**E. Structure:**

The important constituents of high phospholipid krill oil are the fatty acids, EPA and DHA. The structures of these two fatty acids presented in Figure 1.



Eicosapentaenoic acid (EPA)



Docosahexaenoic acid (DHA)

Figure 1. Chemical structures of EPA and DHA

000005

## F. Typical Composition and Specifications

Typical compositional analysis and specifications of Superba™ Krill Oil are presented in Table 1. Analytical results of five lots from non-consecutive batches (Appendix I) indicate that the product consistently meets these specifications. The major components of Superba™ Krill Oil are triglycerides and phospholipids high in omega-3 fatty acids such as EPA (C 20:5 n-3 fatty acid) and DHA (C 22:6 n-3 fatty acid). The maximum amount of EPA + DHA present in Superba™ Krill Oil will be  $23.5 \pm 2$  g/100 g of the oil. No processing aids or additives, with the exception of residual amounts of ethanol solvent, are included in the final Superba™ Krill Oil product. Likewise due to naturally occurring astaxanthin esters that aid in its preservation, addition of an exogenous antioxidant is not required. Based on an 18 month stability test at different storage temperatures, the shelf life of Superba Krill Oil is set to 18 months when stored at 2-8°C. The results of pesticides and other environmental contaminants including PCBs, dioxins, furans and dioxin like PCBs, organochlorine pesticides, PBDEs, PAHs, and elements and heavy metal analyses from multiple batches of the product are presented in Appendix II.

**Table 1. Typical compositional analysis and specifications of Superba™ Krill Oil**

Parameter	Limits	Assay method
Appearance	Dark red viscous oil	Visual
<b>Lipid composition</b>		
Total phospholipids (g/100 g)	43 ± 3	N A88 <sup>1</sup> /AM-AKMB-012
- Omega-3 phospholipids of total PL <sup>2</sup> % (w/w)	>70	Calculation
Triglycerides (g/100 g)	<50	N A88 <sup>1</sup> /AM-AKMB-012
<b>Fatty acid profile</b>		
Total omega-3 (expressed as g/100 g)	23.5 ± 2	AOCS Ce 1b-89/AM-ABM-013
-C 20:5 n-3 (EPA)(expressed as g/100 g)	14 ± 2	AOCS Ce 1b-89/AM-ABM-013
-C 22:6 n-3 (DHA)(expressed as g/100 g)	6.5 ± 1	AOCS Ce 1b-89/AM-ABM-013
Total omega-6	<3.0	AOCS Ce 1b-89/AM-ABM-013
<b>Stability index</b>		
Peroxide value (mEq peroxide/kg)	<2	AOCS Cd 8b-90/AM-058
<b>Antioxidants</b>		
Astaxanthin <sup>4</sup> (mg/kg)	100 ± 20 (minimum)	N A23 <sup>3</sup> /AM-ABM-011
<b>Water and Ethanol</b>		
Water activity at 25°C	<0.5	AOAC 978.18
Ethanol content (% w/w)	<3.0	GC
<b>Microbiology</b>		
Total plate count (cfu/g)	<2500	NF EN ISO 4833/CQ-MO-231
<i>E. coli</i> (1 sample at 10 g)	Negative	Petrifilm Select EC
Coliform bacteria, 37°C (cfu/g)	<10	NordVal Ref. No. 014
<i>Salmonella</i> negative (PCR) (1 sample at 10 g)	Negative	AES 10/4-025/04
Mold and Yeast (cfu/g)	<10	NordVal Ref. No. 016

<sup>1</sup>Based on Homan and Anderson (1998) and Moreau (2006)

<sup>2</sup>Omega-3 phospholipid: defined as phospholipid where on average one out of two possible positions is occupied by an omega-3 fatty acid.

<sup>3</sup>Based on Schierle J. & Härdi W. (1994); <sup>4</sup>Expressed as astaxanthin diols.

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As available research highlights the potential for seafood to contain substantial amounts of arsenic, an extensive chemical analysis of both organic and inorganic arsenic was undertaken from multiple batches (see Appendix II). These results show that while the total arsenic levels in krill oil ranged from 4 to 6 ppm, the vast majority of this arsenic was in organic form. The inorganic arsenic as measured in the form of arsenite and arsenate was below the level of quantification at 0.05 ppm.

### G. Lipid and Fatty Acid Profile:

The lipid profile composition and fatty acid profile of krill oil is presented in Table 2 and 3, respectively. Analysis of *trans*-fatty acids from four different batches revealed the presence of total *trans*-fatty acids of <0.2% (Appendix III).

**Table 2. Lipid profile, including phospholipids**

Lipids	Percent Oil
Triacylglycerol	38
Diacylglycerol	0.8
Monoacylglycerol	<1
Free fatty acids	5.4
Cholesterol	1.1
Cholesterol ester	<0.5
Phosphatidylethanolamine	1.6
Phosphatidylinositol	<1
Phosphatidylserine	<1
Phosphatidylcholine	39
Lysophosphatidylserine	3.7
Total polar lipids	44.7
Total neutral lipids	45.6

**Table 3. Details of representative fatty acid profile**

Fatty acid	Percent*	Fatty acid	Percent*
C14:0	7.7	C20:4 n-6	0.4
C16:0	15.4	C22:0	<0.1
C18:0	0.9	C22:4 n-6	0.5
C20:0	<0.1	C18:3 n-3	1.4
C22:0	0.1	C18:4 n-3	<0.1
C16:1 n-7	4.9	C20:4 n-3	0.5
C18:1 (n-9) + (n-7) + (n-5)	12.1	C20:5 n-3	14.7
C20:1 (n-9) + (n-7)	0.9	C21:5 n-3	0.4
C22:1 (n-11) + (n-9) + (n-7)	0.7	C22:5 n-3	0.3
C24:1 n-9	0.1	C22:6 n-3	6.2
C16:2 n-4	0.5		
C16:3 n-4	0.2	SFA	24.1
C18:2 n-6	1.2	MEFA	18.7
C18:3 n-6	0.2	PUFA (n-6)	1.9
C20:2 n-6	<0.1	PUFA (n-3)	24.0
C20:3 n-6	0.1	Total PUFA	26.6
		<b>Total Fatty Acids</b>	<b>68.2</b>

\*Percent of total oil; Data from representative batch (A)-U301/006/A10

000007

## H. Manufacturing process

Superba™ Krill Oil is derived from shrimp-like, marine crustaceans of the order *Euphausiacea*, *Euphausia superba*. These organisms have a circumpolar distribution with the highest concentrations found in the Atlantic sector. Antarctic krill exist in large numbers in the open sea and are consumed as food by humans. The Antarctic krill used in the production of Superba™ Krill Oil are naturally occurring organisms fished from the wild. The harvested Antarctic krill is cooked and dried on the vessel to prepare krill meal. The steps involved in the manufacturing are summarized in Figure 1. The raw material that is extracted, krill meal, is a biomass composed of lipids, carbohydrates, and proteins. By using a solvent extraction process, the proteins and free carbohydrates are removed. Thus the oil is produced by subjecting the krill meal to ethanol extraction. The solvent used is food-grade quality and is removed from the product in accordance with current good manufacturing practice.

Following extraction, the defatted krill meal and the ethanol oil solution are separated. The ethanol-oil solution is then concentrated by evaporation and stored. The ethanol-oil solution is analyzed for ethanol, neutral and polar lipids, and astaxanthin content. Several batches are blended and the ethanol-oil solution is clarified by centrifugation. The ethanol is then evaporated from the oil solution and the final product is analyzed to verify the conformity with product specifications. The final product is filled into suitable containers and stored at 2-8°C and can be shipped by land, air, or boat. Processing aids, including solvents (which is removed by evaporation) used in the manufacturing process are food-grade quality as specified in the 5<sup>th</sup> Edition of Food Chemicals Codex. The Superba™ Krill Oil production process is controlled under the Hazard Analysis Critical Control Points (HACCP) system and points for likely contamination of the oil are strictly monitored. Additionally, the quality of the final product and production lots are routinely tested for specifications including solvent residue, microorganisms, heavy metals, and pesticides.

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## I. Manufacturing of Superba™ Krill Oil Process Diagram

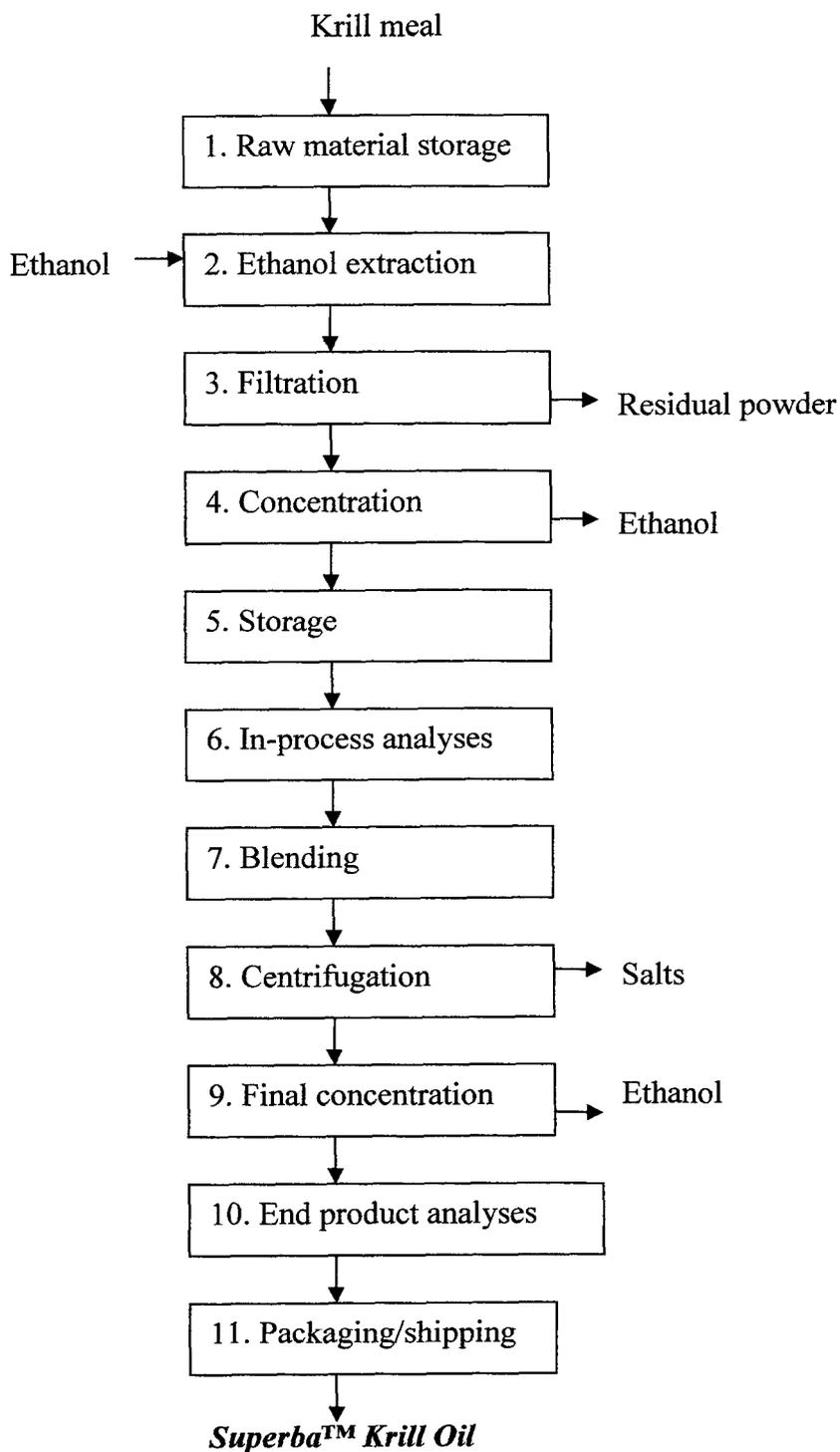


Figure 2. Manufacturing process of Superba™ Krill Oil

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## **J. Intended Technical Effects**

Superba™ Krill Oil is intended for use as a nutrient supplement as defined in 21 CFR 170.3(o)(20). It is intended for use by the general population at levels ranging from 0.05 to 0.50 g/serving for addition to the following food categories: non-alcoholic beverages; breakfast cereals; cheeses; frozen dairy desserts; milk products; processed fruit and fruit juices; and in medical foods. It is recognized that there are Standard of Identity requirements for some of these foods, located in Title 21 of the Code of Federal Regulations. If used in such foods, the name will be changed so as not to be confused with the standardized food. Available information indicates that use levels are self-limiting because of their strong taste that can be detected, depending on food type, at levels greater than 0.30-0.50 g/serving. It is intended to be used as a replacement for fish oil. The intended use of Superba™ Krill Oil is in the same foods and at the same levels of addition as those described in GRN 242 for krill oil. The use of Superba™ Krill Oil in foods is not intended to function as a color additive as defined in 21 CFR 70.3(f).

## **III. Summary of the Basis for the Notifier's Determination that Krill Oil is GRAS**

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Aker Biomarine Antarctic AS to determine the Generally Recognized As Safe (GRAS) status of high phospholipid krill oil. A comprehensive search of the scientific databases for safety and toxicity information on krill oil and its component omega-3 fatty acids (EPA and DHA) was conducted through August 2010 and was utilized for this assessment. Based on a critical evaluation of the pertinent data and information summarized here and employing scientific procedures, the Expert Panel members have individually and collectively determined by scientific procedures that the addition of high phospholipid krill oil to the foods (non-alcoholic beverages; breakfast cereals; cheeses; frozen dairy desserts; milk; processed fruit and fruit juices) containing no other ingredients that are good sources of EPA or DHA, when not otherwise precluded by a Standard of Identity, and to Medical Foods, meeting the specification cited above and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use, as specified herein.

In coming to this decision that krill oil is GRAS, the Expert Panelists relied upon the conclusions that neither high phospholipid krill oil nor any of its constituents pose any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicology studies and other articles relating to the safety of the product. It is also the opinion of the Expert Panelists that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

**IV. Basis for a Conclusion that Superba™ Krill Oil is GRAS for its Intended Use.**

**TABLE OF CONTENT**

**1. INTRODUCTION..... 10**

**1.1. Background ..... 10**

**1.2. Chemistry and Biological Activity..... 11**

**1.3. Description, Manufacturing Process and Specifications..... 11**

**1.4. Similarity with Fish oils..... 12**

**1.5. Technical effects..... 12**

**1.6. Current Uses..... 13**

**1.7. Intended Use Levels and Food Categories..... 13**

**1.7.1. Estimated Daily Intake from the Intended Uses ..... 14**

**2. DATA PERTAINING TO SAFETY ..... 15**

**2.1. Absorption and Metabolism..... 16**

**2.2. Human Studies ..... 17**

**2.3. Animal Studies ..... 19**

**2.4. Safety of Omega-3 fatty acids- EPA and DHA..... 21**

**2.5. Astaxanthin..... 22**

**2.6. *Trans*-Fatty acids..... 22**

**2.7. Other Safety Considerations..... 23**

**2.8. Allergenicity and Other Related Concerns..... 24**

**3. COMMON KNOWLEDGE ELEMENT ..... 24**

**4. SUMMARY ..... 24**

**5. CONCLUSION ..... 27**

**6. REFERENCES..... 28**

**7. APPENDIX I ..... 31**

**8. APPENDIX II..... 33**

**9. APPENDIX III ..... 36**

**10. APPENDIX IV ..... 38**

# DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF KRILL OIL AS A NUTRIENT

## 1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)<sup>2</sup>, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by Soni & Associates Inc., at the request of Aker Biomarine Antarctic AS, Norway, to determine the Generally Recognized As Safe (GRAS) status of high phospholipid krill oil as a nutrient [21 CFR 170.3(o)(20)]<sup>3</sup> in non-alcoholic beverages; breakfast cereals; cheeses; frozen dairy desserts; milk whole and skim; processed fruit and fruit juices; and in medical foods at use levels ranging from 0.05 to 0.50 g/serving resulting in maximum estimated daily intake of 8.3 g/person/day. A comprehensive search of the scientific literature for safety and toxicity information on krill oil and omega-3 fatty acids was conducted through August 2010 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Aker Biomarine Antarctic AS and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

### 1.1. Background

Krill is the common name given to the order Euphausiacea of shrimp-like marine crustaceans. The current taxonomic placement of *E. superba* is summarized in Table 4. These small invertebrates, also known as euphausiids, are found in oceans around the world. The name krill is a Norwegian word that means "young fry of fish", which is also often attributed to other species of fish. Krill is a vital component of the marine food chain for baleen whales, whale sharks, seals, and a few seabird species. In Japan and Russia, krill is also used for human consumption. Since the 19<sup>th</sup> century or may be even earlier, krill has been harvested as a food source for humans (*okiami*) in Japan. Antarctic krill is closely related to shrimp and are consumed as human food in a similar way. Commercially, krill is used for aquaculture and aquarium feeds, as bait in sport fishing, or in the pharmaceutical industry. In the Southern Ocean one species, *Euphausia superba* is abundant. Commercial fishing of krill is done primarily in the Southern Ocean and in the waters around Japan. Approximately 40% of the Japanese Antarctic krill catch is processed for human consumption, and Antarctic krill has been sold as a food for human consumption since the mid-1970s.

In recent years, krill has received considerable attention because it is a rich source of high-quality protein, with the advantage over other animal proteins of being low in fat and rich in omega-3 fatty acids (Tou *et al.*, 2007). Antioxidant levels in krill are higher than in fish, suggesting benefits against oxidative damage. Antarctic krill oil has been reported to contain high levels (30%) of EPA and DHA as well as astaxanthin esters in concentrations of 200 to 400 ppm (Zhu *et al.*, 2008; Kidd, 2007). Additionally, krill oil is also a rich source of phospholipids, vitamin A, and other nutrients (Ruben *et al.*, 2003).

000012

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<sup>2</sup> See also attachments (curriculum vitae) documenting the expertise of the Panel members.

<sup>3</sup> "Nutrient supplements": Substances which are necessary for the body's nutritional and metabolic processes.

**Table 4. Classification of *Euphausia superba***

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustcia
Class	Malacostrasa
Superorder	Eucarida
Order	Euphausiacea
Family	Euphausidae
Genus	Euphausia
Species	<i>Euphausia superba</i>

## 1.2. Chemistry and Biological Activity

The important constituents of krill oil, omega-3 fatty acids, also known as n-3 polyunsaturated fatty acids (PUFA) belong to an essential fatty acid family characterized by their first double bond at carbon atom number 3 counted from the methyl or omega end of the carbon chain constituting the backbone of fatty acids. Omega-3 fatty acids are chemically and biologically distinct from omega-6 fatty acids, where the first double bond is at carbon atom number 6. There are two subgroups of omega-3 fatty acids. One,  $\alpha$ -linolenic acid (ALA), derived from plant oils such as canola oil, rapeseed oil and linseed oil, is composed of 18 carbon atoms with three double bonds (nomenclature; 18:3). The other group is derived from seafood, and the major marine omega-3 fatty acids are EPA (20:5) and DHA (22:6) (Figure 1). In humans, ALA can, to a limited extent, be elongated and desaturated to EPA and DHA. Otherwise, EPA and DHA are only acquired from seafood.

In a recent review article, Calder (2006) discussed the biological role and mechanism of action of long-chain omega-3 fatty acids. It is well known that the omega-6 fatty acid, arachidonic acid, gives rise to the eicosanoid family of mediators (prostaglandins, thromboxanes, leukotrienes, and related metabolites). These mediators have inflammatory actions in their own right and also regulate the production of other mediators including inflammatory cytokines. Consumption of long chain omega-3 fatty acids decreases the amount of arachidonic acid in cell membranes and the availability for eicosanoid production. Additionally, these fatty acids also decrease the production of the classic inflammatory cytokines, such as tumor necrosis factor, interleukin-1 and interleukin-6, and the expression of adhesion molecules involved in inflammatory interactions between leukocytes and endothelial cells. These latter effects may occur by eicosanoid-independent mechanisms including modulation of the activation of transcription factors involved in inflammatory processes. Because of their potential health benefits, omega-3 fatty acids have been commonly consumed and extensively studied for their physiological effects.

## 1.3. Description, Manufacturing Process and Specifications

Superba™ Krill Oil is a dark red colored viscous oil with a seafood odor. Typical food grade specification and composition of Superba™ Krill Oil produced by Aker Biomarine Antarctic AS are summarized in Tables 1, 2, and 3. The primary constituents of Superba™ Krill Oil are triglycerides and phospholipids which are rich in EPA and DHA fatty acid. Detailed information about the identity of krill oil along with specifications, composition, and manufacturing are described earlier in Section II. Analytical results of five different batches indicate that the product consistently meets the specifications (Appendix I). The results of

000013

pesticide, PCBs and dioxins, and furans analyses are presented in Appendix II. The *trans*-fatty acid profile from four batches of Superba™ Krill is presented in Appendix III.

In an extensive study, Winther *et al.* (2010) used high performance liquid chromatography-electrospray tandem mass spectrometry to elucidate the phospholipids in Superba™ Krill Oil extracted from *Euphausia superba*. The study was carried out in order to map the species of the choline-containing phospholipid classes: phosphatidylcholine and lysophosphatidylcholine. A total of 69 choline-containing phospholipids were detected, whereof 60 phosphatidylcholine substances, among others seven with probable omega-3 fatty acids in both sn-1 and sn-2. The phosphatidylcholine concentration was estimated to be  $34 \pm 5$  g/100 g oil (n = 5). The results of this study reveal the composition of phospholipids of Superba™ Krill Oil and the presence of long chained, heavily unsaturated fatty acids. This study also verifies previous findings and offer new insights into the composition of krill oil. In addition to EPA and DHA, the other major fatty acids present in krill oil are palmitic acid, myristic acid, oleic acid, and palmitoleic acid.

#### 1.4. Similarity with Fish oils

The available information suggests a considerable similarity, particularly omega-3 fatty acids, between krill oil and fish oil from different fish sources. In response to a number of GRAS notices, the FDA has acknowledged the GRAS status of different forms of fish oil. As per 21 CFR 184.1472, menhaden oil has been affirmed as GRAS. Additionally, the FDA has not questioned GRAS notifications submitted on tuna oil (FDA, 2002), salmon oil (FDA, 2004a), and anchovy oil (FDA, 2004b). In FDA's review of tuna oil, the fatty acid content of tuna oil was compared to menhaden oil (FDA, 2002). The fatty acid composition of krill oil is compared with those of FDA's comparison of tuna and menhaden oil in Table 5. Krill oil contains a high level of the desirable n-3 unsaturated fatty acids that is comparable to other oils.

**Table 5. Comparison of fatty acid profile of Superba™ Krill Oil with tuna oil and menhaden oil\* (g/100g)**

Fatty acid	Tuna oil	Menhaden oil	Krill oil
14:0	20.3	9.0	7.7
16:0	20.0	19.0	15.4
18:0	6.0	3.0	0.9
16:1	4.5	12.0	4.9
18:1	15.0	13.0	12.1
22:1	1.0	-	0.6
18:2	1.5	1.0	1.2
18:3	1.0	1.0	0.2
20:5 (EPA)	6.0	14.0	14.7
22:6 (DHA)	26.5	8.0	6.2

\*Values for tuna and menhaden oils adapted from FDA response to GRN 109 (FDA, 2002)

#### 1.5. Technical effects

Superba™ Krill Oil is intended for addition to a limited number of conventional foods as a nutritional ingredient. It is intended for use as a dietary ingredient as a source of omega-3 fatty acids, which are found in their phospholipid form. Supplementation with the omega-3-fatty acids EPA and DHA has been shown to have a wide variety of biological effects. The intended use is for the general population at levels ranging from 0.05 to 0.50 g/serving for addition to the

000014

following food categories: non-alcoholic beverages; breakfast cereals; cheeses; frozen dairy desserts; milk products; processed fruit and fruit juices; and medical foods. It is recognized that there are Standard of Identity requirements for some of these foods, and as such, Aker Biomarine Antarctic AS does not intend to refer to them by the commonly recognized names such as milk, or yogurt.

The use of Superba™ Krill Oil in foods may impart a color to food products. However, the intended use of Superba™ Krill Oil would fall outside the definition of “color additive” because: the intended use levels are low enough to impart a significant color to food products, consistent with the “non-apparent color” Exemption [21 CFR 70.3(f)]; the intended use of Superba™ Krill Oil as a nutrient would contribute a color in a manner consistent with the “unimportant color” exemption addressed in 21 CFR 70.3(g); and the intended use of Superba™ Krill Oil is to provide consumers with an additional source of a nutrient in the diet and does not relate to any use of the ingredient as a color additive [21 CFR 70.3(f)].

## **1.6. Current Uses**

Krill oil has been reportedly used in human food in Japan, Russia, Ukraine, and France since the 1970s. Based on information described in FDA dockets, in 2003 a New Dietary Ingredient Notification was submitted on the use of krill oil as a dietary supplement (FDA, 2003). The FDA filed the notice without any objections. The supplement is sold in 300 and 500 mg capsules with a recommended dose of 1 to 2 capsules/day. Krill oil has been available as a dietary supplement in North America for several years, European Union, Norway, and Taiwan. In the GRN 242 (FDA, 2008), it is stated that a total of 120,000 kg of krill oil has been consumed by customers as a dietary supplement without any reports of serious adverse effects.

Based on information from FDA’s GRAS Notice Inventory<sup>4</sup> website, in February 2008 Neptune Technologies submitted a GRAS notification to the FDA on krill oil (FDA, 2008). The notice indicated that krill oil obtained from krill is intended to be added to a limited number of different food categories. The notice informed the FDA that krill oil is GRAS, through scientific procedures, for use as a food ingredient in non-alcoholic beverages, breakfast cereals, cheeses, frozen dairy desserts, milk products, processed fruit and fruit juices, and medical foods at a use level to provide 150 to 500 mg of the oil per serving. On October 14, 2008,<sup>5</sup> the FDA issued a “No Questions” letter for the GRAS notice.

Recently, on October 12, 2009, the use of krill oil received an approval as a novel food ingredient in Europe, under Commission Regulation (EC) No 258/97 related to novel foods and novel food ingredients. On December 22, 2009, in response to a notification on behalf of Aker Biomarine Antarctic AS, the Novel Food Board found that Superba™ Krill Oil is substantially equivalent to the krill oil authorized by the commission with respect to composition, nutritional value, metabolism, intended use, and the levels of undesirable substances contained therein (Appendix IV).

## **1.7. Intended Use Levels and Food Categories**

Aker Biomarine Antarctic AS intends to offer Superba™ Krill Oil for incorporation into a limited number of human food categories where krill oil would function as a nutrient

<sup>4</sup>Accessible at: [www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true](http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true).

<sup>5</sup>Accessible at: [http://www.accessdata.fda.gov/scripts/fcn/gras\\_notices/grn000242.pdf](http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn000242.pdf)

supplement as defined under 21 CFR 170.3(o)(20). Superba™ Krill Oil is intended for use in the same foods and at the same or lower use levels of addition as described in GRN 242 for krill oil. The proposed food uses as a dietary source of krill oil in foods include addition to: non-alcoholic beverages, breakfast cereals, cheeses, frozen dairy desserts, milk products, and processed fruit and fruit juices. In addition to these categories, it is also intended for use in Medical Food at levels not to exceed 0.50 g/person/day.

#### **1.7.1. Estimated Daily Intake from the Intended Uses**

As Aker Biomarine Antarctic AS intends to use its Superba™ Krill Oil in the same foods and at the same use levels of addition as described in GRN 242, estimates of possible daily intake from the proposed use levels were adapted from GRN 242 (FDA, 2008). In the GRN 242, the use of krill oil was proposed at use levels of 0.15 to 0.50 g of the oil/serving (reference amounts customarily consumed, 21 CFR 101.12) of food. The specific food categories, the intended use levels of krill oil, and the resulting intake of krill oil are summarized in Table 5. In the GRN 242, the estimates of possible daily intake of krill oil were calculated using the FDA guidelines using serving size data and the mean consumption (50%) of each type of food of interest from the CSFII 1994-96 database (USDA, 2005). According to the FDA guidelines, a level twice the mean consumption was calculated to estimate use at the 90<sup>th</sup> percentile consumption level. A summary of dietary intake calculations from the intended food categories is also presented in Table 6.

The intended use levels of krill oil will result in an estimated daily intake at average (50<sup>th</sup> percentile) and high (90<sup>th</sup> percentile) consumption of 4.14 and 8.28 g/person, respectively. The resulting intake of total EPA and DHA from the exaggerated estimated daily intake of krill oil (8.30 g/person/day) would be 2.20 g/person/day. Thus the intended food uses for Superba™ Krill Oil are within the allowances FDA has accepted for the GRAS status use of menhaden oil. The acceptable menhaden oil food use does not exceed safe levels of consumption for total EPA and DHA. The maximum estimated consumption of astaxanthin ester, which is present in krill oil at 100 ppm would be 0.83 mg/person/day. The application of krill oil to the same foods and at the same use levels as those described in GRN 242 are unlikely to affect the dietary intake of krill oil from introduction into the market by another supplier who will have to compete in essentially the same market with the same foods. Hence, there is no need for a cumulative intake analysis.

000016

**Table 6. Intended Food Uses and Use Levels of Superba™ Krill Oil**

Food category	Food subcategory	Use level per serving	Approximate serving size	Food intake (g/p/d) 50%-tile	Krill oil intake <sup>a</sup> (g/p/d) 50%-tile	Krill oil intake (g/p/d) 50%-tile X 2
Breakfast cereals	Cooked cereal	0.05-0.30 g	½ cup of cooked Oatmeal = 117 g	233	0.60	1.19
	Ready-to-eat cereal	0.05-0.30 g	1 cup of corn flakes = 25 g	48	0.60	1.15
Cheeses	Total cheese other than cream or cottage	0.05-0.30 g	1/2 oz. of cheese = 43 g	26	0.18	0.36
	Total cottage cheese	0.05-0.30 g	1/2 cup of cottage cheese = 105 g	50	0.14	0.29
Beverages, Nonalcoholic	Fruit drinks	0.05-0.25 g	8 oz. = 248 g	360	0.22-0.36	0.44-0.73
Milk, whole & skim	Total milk	0.05-0.50 g	1 cup of fluid whole milk = 244 g	216	0.27-0.45	0.53-0.89
Milk products	Sour cream	0.05-0.50 g	1 tablespoon of sour cream = 14 g	6	0.13-0.21	0.26-0.43
	Creams	0.05-0.50 g	1 tablespoon of cream = 15 g	3	0.06-0.10	0.12-0.20
	Yogurt <sup>b</sup>	0.05-0.50 g	No data in USDA survey	0.17 servings	0.05-0.085	0.10-0.17
Frozen dairy desserts	Ice cream, Ice milk	0.05-0.50 g	1/2 cup of hard ice cream = 67 g	132	0.59-0.98	1.18-1.97
Processed fruits/fruit juices	Total orange juice	0.05-0.25 g	6 fl. oz. of orange juice = 187 g	186	0.15-0.25	0.30-0.50
	Total lemon juice	0.05-0.25 g	1 fl. oz. of lemon juice = 30 g	<0.05	0.00	0.00
	Total apple juice	0.05-0.25 g	6 fl. oz. of apple juice = 186 g	150	0.12-0.20	0.24-0.41
Medical foods		0.05-0.50 g <sup>c</sup>	No data in USDA survey			
<b>Sum of all categories</b>					<b>3.08-4.14</b>	<b>6.16-8.28</b>

<sup>a</sup> Dietary intake of krill oil for each food type is calculated by multiplying ,g/serving by grams of food consumed divided by grams of food per serving;

<sup>b</sup> Yogurt consumption in the US has been estimated by Neptune to average 60 servings per year or 0.17 servings per day, with a high consumer exposure at 250 servings per year. This estimate is based on sales data with a per capita consumption of 5-6 kg/person;

<sup>c</sup> It is envisioned that these foods would be meal replacements for patients whose diets would consist of these foods entirely for 3 meals per data and therefore, total krill oil consumption in these patients would be 0.90-1.50 g/day.

Adapted from GRN 000242 (FDA, 2008); note that values for low proposed intake are not calculated but the low values from GRN 000242 were considered.

## 2. DATA PERTAINING TO SAFETY

000017

The safety of krill oil and its biologically important constituents such as omega-3 fatty acids is supported by human observations and clinical trials as well as animal experimental

studies. Because of the physiological role of omega-3 fatty acids in human health, there have been considerable efforts to elucidate the mechanism and biological role of these fatty acids in human nutrition. As a result, the literature is full of information on omega-3 fatty acids. Relevant biological and toxicological studies on krill oil and its constituents (omega-3 fatty acids) are included in the following section in support of the safety conclusions determined in this assessment.

## 2.1. Absorption and Metabolism

Krill oil consists primarily of phospholipids that are commonly consumed via diet. It is well established and recognized that dietary phospholipids and fatty acids from either plant or animal sources are handled the same metabolically. The composition of Superba™ Krill Oil is well characterized and from this perspective there is nothing unusual that is not found in a commonly consumed diet. The components of krill oil have been extensively studied for their biological and physiological properties. Despite krill oil's complex composition, available information suggest that the major phospholipids and fatty acids are consistent with other lipid sources with differences noted in proportions of phospholipids, minor constituents, and fatty acid content. Given the metabolic sequelae of different dietary lipids, there is no reason to believe that the Superba™ Krill Oil would pose any different health hazards.

In two separate unpublished pharmacokinetics studies, bioavailability of EPA and DHA was investigated from different oils (Meyer, 2009a, 2009b). The first study was a single centre, open-label, randomized four-way crossover study designed to evaluate the 24 hour pharmacokinetic profiles of EPA, DHA, and astaxanthin after single doses of A: Superba™ Krill Oil (8 g), B: Neptune krill oil (8 g), C: Omega-3 enriched fish oil (8 g), and D: Krill powder (8 g). The doses were separated by 72 hours wash-out periods. In this study, 36 healthy male subjects (age 25 - 45 years) were randomized (1:1:1:1) to one of four treatment sequences. Blood samples were collected pre-dose, and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, and 24 hours after the dosing. A general trend to increases in levels of EPA, DHA, and astaxanthin across the four study periods was observed in the majority of subjects. This trend to continuous increase was confirmed by regression analysis for EPA and DHA in plasma and in phospholipid fractions. The median  $t_{max}$  for EPA in plasma was 12 hours for all products. With regards to DHA in plasma, the median absolute  $t_{max}$  was longest after Superba™ krill oil (10 hours), shortest after omega-3 enriched fish oil (6 hours), and in between after Neptune krill oil (7 hours) and krill powder (8 hours). All study products were safe and well tolerated (Meyer, 2009a).

In another unpublished open-label, randomized two-way crossover study, changes in EPA and DHA in phospholipid membranes were determined following eight weeks of daily intake of 2 g Superba™ Krill Oil or 2 g omega-3 enriched fish oil in healthy male and female subjects (Meyer, 2009b). A total of 28 healthy male and female subjects (14/sex; aged 25-45 years) took part in this study. Blood for the pharmacokinetic analysis was collected on Day 1 (pre-dose) and on Days 14, 28, 42 and 56 ( $\pm$  2 days) of each treatment period for the analysis of EPA and DHA in phospholipid fractions and of omega-3 index in RBCs. In addition to daily enquiry of adverse events, a 12-lead ECG, and a standard clinical laboratory assessment (urinalysis, hematology, clinical chemistry) at screening and on Day 56 of period 2 was performed. Steady state in EPA levels and omega-3 index was attained earlier after Superba™ Krill Oil (Day 14) as compared to omega-3 enriched fish oil (Day 28). Steady state in DHA levels was attained later after Superba™ Krill Oil (Day 42) than after omega-3 enriched fish oil (Day 28).

000018

In female subjects, the bioavailability of EPA in plasma (after dose adjustment) in krill oil administered subject was higher compared to fish oil (Meyer, 2009b). Similarly, across males and females, DHA in plasma (after dose adjustment) was higher in subjects receiving krill oil. Statistically significant differences between the treatments could not be demonstrated with respect to omega-3 index in RBCs (after dose adjustment). In subjects receiving krill oil, overall AUC(0-56D) of EPA and DHA in plasma and omega-3 index in RBCs was determined as 97908, 98261, 4208 ng\*h/(mg\*ml), respectively. Overall, there were no trends related to the study products in the adverse event reports, in clinical laboratory, ECG, and physical examinations. There were no withdrawals due to adverse effects. Krill oil ingestion decreased the mean serum insulin level, whereas the mean adiponectin level increased. Following omega-3 enriched fish oil administration, both the mean serum insulin level and the mean adiponectin level decreased. No statistically significant treatment effects were seen in the analysis of platelet aggregation, lipid parameters and the other selected clinical chemistry parameters (glucose, CRP, insulin TNF alpha, and adiponectin). The investigator concluded that both krill oil and fish oil were safe and well-tolerated (Meyer, 2009b).

## 2.2. Human Studies

In a randomized, double-blind parallel arm trial, overweight and obese subjects (n=76; 13 men, 63 women) were randomly assigned to receive double-blind capsules containing 2 g/day of krill oil (n=25), menhaden oil (n=26), or control (olive) oil (n=25) for four weeks (Maki *et al.*, 2009). The objective of this study was to examine the effects of krill oil supplementation on plasma EPA and DHA concentrations, indicators of safety, tolerability, and selected metabolic parameters. The krill oil used in this study was Superba™ Krill Oil, the subject of this GRAS determination. In addition to physical examination, clinical laboratory measurements (plasma chemistry, hematology, urine, and lipids) were performed. At baseline and at the end of week 4, subjects completed a gastrointestinal (GI) tolerability questionnaire, which assessed the presence and severity (on a scale of 0 to 5) of GI symptoms such as gas, bloating, nausea, flatulence, diarrhea, constipation, and cramping over the period of seven days. Subjects also completed a symptom checklist at the end of week 4, which assessed the incidence of or changes in a variety of symptoms (e.g., irritability, nervousness, mood, blurred vision, drowsiness, mental sharpness, and hair and skin changes) in the previous four weeks on a scale of 1 (a lot less) to 5 (a lot more). Adverse events were assessed from the time subjects signed the informed consent form at screening (week -1) and continued through the end of the study.

The changes from baseline to week 4 did not differ significantly among the treatment groups for hematology values or for plasma concentrations of albumin, electrolytes, creatinine, or liver enzymes. Responses for measures of glucose homeostasis, lipoprotein lipids, hs-CRP (high-sensitivity C-reactive protein), and F2-isoprostanes did not vary significantly by treatment group. The results revealed that compared to the control group, plasma EPA and DHA concentrations increased in the krill oil and menhaden oil groups. Blood urea nitrogen declined in the krill oil group as compared with the menhaden oil group. The frequencies of adverse events were similar in the three treatment groups. At week 4, significant differences were observed among the treatment groups in the number of subjects with scores of 4 or higher for gas or bloating ( $P = 0.05$ ) and flatulence ( $P = 0.034$ ). The number of subjects with gas or bloating increased from 2 (8%) at baseline to 5 (20%) at week 4 in the krill oil group and from 1 (4%) at baseline to 5 (20%) in the control group. No significant differences were observed among the treatment groups in the frequencies of any symptoms assessed with the symptom checklist. The

investigators concluded that compared with both menhaden oil and olive oil, krill oil was generally well tolerated and showed no indication of adverse effects on safety parameters (Maki *et al.*, 2009).

Ulven *et al.* (2010) investigated the effects of krill oil (Superba™ Krill Oil) and fish oil on serum lipids and markers of oxidative stress and inflammation. The objective of this study was to evaluate if different molecular forms, triacylglycerol and phospholipids, of omega-3 polyunsaturated fatty acids (PUFAs) influence the plasma level of EPA and DHA differently. In this study, 113 subjects with normal or slightly elevated total blood cholesterol and/or triglyceride levels were randomized into three groups and given either six capsules of krill oil (n = 36; 3.0 g/day, EPA + DHA = 543 mg) or three capsules of fish oil (n = 40; 1.8 g/day, EPA + DHA = 864 mg) daily for 7 weeks. The third group did not receive any supplementation and served as controls (n = 37). Safety was evaluated by assessment of hematology and biochemistry parameters, and by reported adverse events.

Compared to control group, a significant increase in plasma EPA, DHA, and DPA was noted in the subjects supplemented with n-3 PUFAs. However, there were no significant differences in the changes in any of these fatty acids between the fish oil and the krill oil groups. The serum lipids or the markers of oxidative stress and inflammation did not reveal any statistically significant differences between the study groups. The safety assessment did not reveal any patterns in the changes in any of the hematological or serum biochemical variables, vital signs or weight that might indicate a relation with administration of any of the studied products. Clinical symptoms registered during the study included mainly symptoms of common cold or gastrointestinal symptoms. One subject in the fish oil group experienced moderate bruises, and one subject in the krill oil group withdrew from the study because of an outbreak of rash that was possibly related to intake of the study products. There were no apparent differences in the rate of adverse events or blood safety parameters between the krill oil, fish oil or control groups. These observations indicate that krill oil was well tolerated. The results of this study show that krill oil and fish oil are comparable dietary sources of n-3 PUFAs, even if the EPA + DHA dose in the krill oil was 62.8% of that in the fish oil (Ulven *et al.*, 2010).

Sampalis *et al.* (2003) investigated the effects of krill oil on premenstrual syndrome (PMS) and dysmenorrhoea in 70 female adults of reproductive age. The females were randomized to receive either krill oil or fish oil. The subjects consumed two 1 g capsules once per day with meals during the first month. Subsequently, the subjects consumed same dose during the second and third months but for eight days prior to menstruation and for two days during menstruation. During the course of study, no serious adverse effects were reported. Three subjects reported a reduction in the duration of the menstrual cycle during the first month of treatment. In subjects receiving krill oil, a slight increase in the oiliness of the facial skin was noted. No subjects reported gastrointestinal disturbances. However, in fish oil group 64% of the participants reported “unpleasant” reflux following consumption. The results of this study suggest that krill oil softgels were well tolerated.

In another study, Deutsch (2007) investigated the effects of krill oil on markers of chronic inflammation in 90 subjects (age 50 to 68 years) recruited from primary care physicians. The subjects recruited had been diagnosed with cardiovascular disease, rheumatoid arthritis, or osteoarthritis, and were reported to have C-reactive protein levels greater than 1.0 mg/dL. Except for acetaminophen, the subjects were asked not to consume any other pain medication. The

000020

subjects were administered either 100 mg of placebo or 300 mg krill oil/day and were followed for 30 days. C-reactive protein levels and pain and functional impairment scores were assessed during the experimental period on a weekly basis. Compared to baseline, a significant decrease in C-reactive protein levels was observed in subjects consuming krill oil at the end of 7, 14, and 30 days. No adverse effects were associated with the consumption of krill oil.

Bunea *et al.* (2004) evaluated the effects of krill oil on the clinical course of hyperlipidemia in 120 patients (mean age 51 years). The patients were randomized into four groups which were further subdivided according to their body mass index (BMI) (Bunea *et al.*, 2004; FDA 2008). Group 1 was administered either 2 g krill oil/day (BMI<30) or 3 g krill oil/day (BMI>30). Group 2 was administered either 1 or 1.5 g krill oil/day (BMI< or >30, respectively). Group 3 was administered a fish oil capsule that provided 180 mg EPA and 120 mg DHA, and Group 4 was the placebo group. The experimental period was 12 weeks while Group 2 consumed 500 mg krill oil/day for an additional 90 days. No adverse effects were noted in any of the groups.

In an unpublished study described in GRN 242 (FDA, 2008), the safety of krill oil was examined in 25 healthy male and female subjects between the ages of 25 and 53 years. The volunteers consumed two krill oil gelcaps, three times a day for two months. Each gelcap contained 1 g of krill oil that provided 386 mg of omega-3 fatty acids, 416 mg phospholipids, and 0.16 mg of astaxanthin. As described in GRN 242, complete blood counts and biochemical blood tests, medical histories, and vital signs were collected at baseline, one month, and two months. The volunteers were asked about the occurrence of adverse effects and if there was any regurgitation effects of the capsules. The subjects were also asked to stop consuming the gelcaps if they had the following symptoms: low or high blood pressure, difficulty breathing, bleeding, loss of consciousness, unusual migraines or body pain, weight gain, or significant alterations in blood test results. Biochemical parameters examined included cell counts, PTT, creatinine, glucose, alkaline phosphatase, albumin, amylase, total bilirubin, total cholesterol, HDL and LDL cholesterol, triglycerides, urea, and TSH levels. As described in GRN 242, no serious side effects were reported in volunteers consuming 6 g krill oil throughout the experimental period. No regurgitative effects were reported or any unpleasant aftertaste. Of the 25 volunteers, three withdrew for reasons associated with consuming krill oil. One female withdrew due to a known salt tolerance for which consumption of krill oil resulted in a moderate increase in water retention. Two females withdrew because they felt an increasing greasiness of their facial skin which was attributed to consuming krill oil. In the remaining volunteers, no noticeable physical or biochemical changes were observed. A significant decrease in serum total cholesterol, triglycerides, LDL cholesterol, the ratio of total cholesterol to HDL cholesterol, albumin, and amylase were observed. A significant increase in HDL cholesterol was also observed. These effects were not considered adverse effects but beneficial changes in blood lipids and pancreatic function. While a decrease in albumin levels might be indicative of underlying disease processes, their occurrence in the absence of other biochemical abnormalities suggested they were not adverse effects (FDA, 2008).

### 2.3. Animal Studies

Batetta *et al.* (2009) compared the effects of dietary (n-3) LC-PUFA, in the form of either fish oil or krill oil (Superba™ Krill Oil) balanced for EPA and DHA content, with a control diet containing no EPA and DHA and similar contents of oleic, linoleic, and  $\alpha$ -linolenic acids, on ectopic fat and inflammation in Zucker rats, a model of obesity and related metabolic

000021

dysfunction. In this study, male Zucker rats (Harlan) four weeks of age, with an initial weight of 250±30 g, were equally divided into three groups and were fed either a control diet or diets containing krill oil or fish oil for four weeks. The amount of 0.5 g of EPA + DHA per 100 g of diet, equivalent to 0.8% by energy in the rat diet, was chosen. Effects on lipid metabolism, ectopic fat deposition, and susceptibility to inflammation was measured. The investigators concluded that diets rich in (n-3) LCPUFA, and a krill oil-based diet in particular, exert beneficial effects on several metabolic dysfunctions in Zucker rats, which was associated with lower endocannabinoid concentrations in several peripheral tissues. Although the objective of the study was to investigate the efficacy of krill oil, growth and food intake was not affected by krill oil diet. Additionally, the investigators also reported that none of the rats exhibited adverse effects.

In another study, Di Marzo *et al.* (2010) investigated whether in Zucker rats, under the same conditions as described above by Batetta *et al.* (2009), fish and krill oil are also able to influence LC-PUFA and endocannabinoid profiles in the brain. The study design and protocol of this study was identical to the above described study. In this study, only krill oil was able to significantly increase DHA levels in brain phospholipids, with no changes in arachidonic acid. Based on the results of this study, the investigators claimed the beneficial effect of krill oil on the metabolic syndrome is mostly exerted by modifying endocannabinoid levels in peripheral tissues. Similar to the above described study, feeding krill oil in the diet for four weeks did not affect growth and food intake. No differences in growth and food intake among groups, nor any adverse effects of the diets, were observed.

Ruggiero-Lopez *et al.* (1994) investigated the effect of krill oil, as compared to fish and corn oil, on the rat intestinal fucosylation process at weaning, a very sensitive model of the influence of nutritional factors. In this study, the effects of oil were studied over a three-day period immediately after weaning. All the oils were well-tolerated by pups at a level of 10% of the diet. The use of krill oil was not reflected in the enzymatic activities involved in the fucosylation pathway. The investigators concluded that the results of their study confirm the harmlessness of krill derived products and their possible use in human nutrition.

A repeat-dose toxicity study described in GRN 242 (FDA, 2008) was conducted to examine the safety of krill oil in mice for six months. In this study, 96 C57BL6 nude congenic mice (B6NU-T heterozygotes) were fed a diet containing 16.6% krill oil (equivalent to 28.3 g krill oil/kg body weight/day). The animals were examined weekly by a certified veterinarian. At the end of the experiment, all the animals were euthanized by gas exposure and subjected to histopathological examinations. No adverse effects were noted over the experimental period and no histopathological abnormalities were observed in the brain, lungs, heart, stomach, pancreas, liver, kidneys, uterus or prostate, intestines, or skin.

In a follow up investigation to the above described study, also described in GRN 242, the development of UVB-Radiation Induced Skin Cancer in mice was investigated (FDA, 2008). In this study, C57BL6 Nude Congenic mice (B6NU-T heterozygotes) were randomized into two groups (48/sex/group). One group was administered oral, topical, or oral and topical treatments of krill oil. The second group was administered soya oil. In the oral dosing regime, mice were administered diets where 10% of the daily dietary intake consisted of either krill oil or soya oil (equivalent to 17.1 g/kg body weigh/day). In the topical treatment regime, krill oil or soya oil was applied to the skin. The mice were exposed for 30 minutes to UVB radiation, at a distance of 30 cm, daily for 20 weeks. After 20 weeks, the animals were euthanized and subjected to

histological examinations. The occurrence of cancers and pre-malignant tumors in mice administered topical treatments was 12.5% and 31.3%, respectively, as compared to 37.5% and 31.3%, respectively, in the soya oil group. In mice administered both oral and topical treatments, the occurrence of cancers and pre-malignant tumors was reported to be 18.8% and 31.3%, respectively in the krill oil group and 37.5% and 12.5% respectively, in the soya oil group. As compared to the soya oil group, a significant reduction in the incidence of cancers was noted in mice administered krill oil.

#### **2.4. Safety of Omega-3 fatty acids- EPA and DHA**

The principal fatty acid constituents of krill oil, EPA, and DHA are typically contained in oily fish, such as salmon, lake trout, tuna, and herring. The composition of EPA and DHA in krill oil, which is the subject of this notification ranges from 14±2 and 6.5 ±1% w/w, respectively. The total of EPA+DHA in krill oil is 23.5 ± 2%. In the 1997 final rule on the GRAS affirmed use of menhaden oil as a direct food ingredient (FDA, 1997) and also regarding the use of omega-3 fatty acids as a dietary supplement in 2005 (FDA, 2005), FDA has critically evaluated the safety of DHA and EPA. The FDA (1997) has affirmed menhaden oil as GRAS in 1997, as a direct human food ingredient with specific limitations of use to ensure that the total daily intake of EPA and DHA would not exceed 3 g/person/day (62 FR 30751; June 5, 1997; 21 CFR 184.1472). In these regulations, the FDA established maximum use levels of menhaden oil in certain foods (62 FR 30751 at 30757; June 5, 1997; amended March 23, 2005) because of concerns over possible adverse effects of consumption of fish oil on bleeding coagulation time, glycemic control, and LDL cholesterol,. The FDA reaffirmed the maximum intake of DHA and EPA to 3.0 g/day from all fish oil sources. To ensure the consumption remains below 3.0 g/day, the agency placed specific limitations, including the category of foods, the functional use of the ingredient, and the level of use.

Besides the menhaden oil GRAS affirmation, the FDA has not questioned multiple GRAS notices for additional sources of EPA and DHA as food ingredients. These notices include GRN 000102, GRN 000105, GRN 000109, GRN 000138; GRN 000146; GRN 000193; GRN 000200; GRN 000217<sup>6</sup>. In these GRAS Notifications, the intended maximum use levels were consistent with those specified in the final rule affirming GRAS status of menhaden oil as a direct human food ingredient with specific limitations of use. Furthermore, the FDA did not object to a GRAS notification for high DHA algal oil (GRAS Notice No. GRN 000137). In this case the notifier estimated that the use of algal oil in a number of food categories at the maximum proposed use levels would result in a mean exposure of no more than 1.5 g DHA/day.

In order to support the safety in use of DHA and EPA, the composition of principal krill oil fatty acids was compared with menhaden oil and tuna oil (Table 5). As noted in Table 5, menhaden oil contains 8% DHA and 14% EPA. The total of DHA+EPA (22%) in menhaden oil is essentially similar to that in krill oil (23%). Similarly, the individual levels of DHA (8% vs 6.5%) and EPA (14% vs 14%) are also essentially similar between menhaden and krill oil. In different FDA GRAS Notifications, the total amount of DHA+EPA ranged from 20 to 41% and was reported as follows: GRN 000105 = 38%, GRN 000109 = 28%, GRN 000138 = 29%, GRN 000146 = 20%, GRN 000200 = 41%, and GRN 000279 = 22%. In all of these notices, the

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<sup>6</sup> The FDA response to all these and other GRAS notices is assessable at GRAS Notice Inventory: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>

maximum levels of use in food categories were adjusted such that the resulting intake of DHA+EPA was similar to or lower than what is currently permitted for menhaden oil under 21 CFR 184.1472. As krill oil is proposed for use as a substitute or alternative to fish oils, the intended use of krill oil will not add to the existing intake of DHA and EPA.

## 2.5. Astaxanthin

In addition to lipids, one of the minor components of biological importance of the oil is astaxanthin. In Krill, either one or both of the alcoholic hydroxyl functional groups of astaxanthin may be esterified to fatty acids. Thus astaxanthin from krill are found almost exclusively in esterified form. Takaichi *et al.* (2003) determined that only five kinds of fatty acids, dodecanoate, tetradecanoate, hexadecanoate, hexadecenoate, and octadecenoate were esterified to astaxanthin in krill. Assuming one C16 fatty acids in each position gives a molecular weight of the esterified molecule of 1110 or approximately twice as much as astaxanthin alone. Hence to specify the astaxanthin content of krill oil, one can consider the molar concentration or the amount of astaxanthin diol. Because of the general unfamiliarity with molar concentrations, Aker Biomarine declares its product on the basis of astaxanthin diol. Thus the levels presented in Table 1 for astaxanthin of 100 ppm means the product contains 100 µg/g of the diols, regardless of fatty acids that may be esterified.

As mentioned earlier, the intended use of the krill oil will result in a maximum estimated consumption of 0.83 mg astaxanthin/person/day. Although there is no recommended daily allowance (RDA) for astaxanthin, available safety-related information suggests that the estimated daily intake of astaxanthin (0.83 mg) from the intended uses of Superba™ Krill Oil is lower than the generally considered safe levels of 6 mg/day. It has been reported that in consumers with a high intake of fish and seafood, the estimated daily intake of astaxanthin ranges from 1.6 to 4.1 mg/day. Recently, in response to a GRAS notice on *Haematococcus pluvialis* extract containing astaxanthin esters (GRN 000294)<sup>7</sup>, the FDA did not question the safety of astaxanthin intake at levels of 1.08 mg/person/day.

## 2.6. Trans-Fatty acids

As shown in Appendix III, high phospholipid krill oil contains only small amount of *trans*-fatty acids (<0.3%). Accordingly, one of the fatty acids vaccenic acid (C18:1, n-7) in Superba™ Krill Oil is almost exclusively present in the *cis*-isomeric form. The vaccenic acid content of high phospholipid krill oil in GRN 243 was reported as about 10% (FDA, 2008). From more common sources such as fat from ruminants and in dairy products, vaccenic acid is present naturally as *trans*-fatty acid in the fat of ruminants and in dairy products such as milk and yogurt. In krill oil, the vaccenic acid (C18:1, n-7) primarily occurs in the *cis*-isomeric form. The fatty acid profile presented in Table 3 provides values for C18:1 that includes n-5, n-7, n-9 and n-11. Among these, n-7 represents vaccenic acid, while n-9 represents oleic acid. Additional analysis of C18:1 fatty acids revealed that Superba™ Krill Oil primarily contains C18:1 n-9 + n-11 in *cis* configuration at levels of ~11%, while the levels of vaccenic acid are below 1%. As compared to these low levels, the vaccenic acid content (10%) reported in GRN 243 (FDA, 2008) is significantly higher. It is possible that the differences in manufacturing method may affect the levels of vaccenic acid.

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<sup>7</sup> The FDA response is assessable at GRAS Notice Inventory:  
<http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>

The presence of vaccenic acid is also reported in edible fats and oils (Wasowicz and Hougen, 1976; Sauer *et al.*, 1997). Several vegetable and animal oils are known to contain lower levels of vaccenic acid, while butter contains higher amounts of various isomers of 18:1 fatty acids in the *trans* configuration. These fatty acids are not believed to exhibit the same clot-forming potential as saturated fatty acids or other *trans*-fatty acids formed by partial hydrogenation of vegetable oils. In a critical review on the health benefits of vaccenic acid, Field *et al.* (2009) noted that epidemiological, clinical, and rodent studies to date have not demonstrated a relationship of vaccenic acid with heart or cardiovascular disease, insulin resistance, or inflammation. Available evidence does not indicate that dietary vaccenic acid poses any safety concerns and levels of this fatty acid in Superba® Krill Oil are very low.

## 2.7. Other Safety Considerations

As krill oil, the subject of this GRAS determination, is derived from marine organism, it is important to characterize the nature and quantity of impurities/contaminants that might be stored in marine lipids that may pose a health hazard. The potential impurities and incidental constituents present in krill oil arise largely from environmental exposure of the Antarctic Krill. As krill oil is derived from the lipid fraction of krill biomass, Aker Biomarine Antarctic AS routinely analyzes production lots of Superba™ Krill Oil for the presence of dioxins, furans, organochlorine pesticides, PBDEs, PAHs, heavy metals and PCBs. Likely contaminants were analyzed from multiple representative batches. These results, presented in Appendix II, demonstrate the levels of contaminants are low and consistent with levels of other food ingredients.

It is well recognized that arsenic especially in seafood is present in an organic form that is less toxic (EFSA, 2009). Hence, there is a need for speciation data for arsenic. As presented in Appendix II, an extensive chemical analysis of both organic and inorganic arsenic was undertaken from multiple batches of krill oil. These results of eleven different forms of arsenic show that the total arsenic levels in krill oil ranged from 4 to 6 ppm, the majority of which was in organic form. The organic arsenic was found to be primarily in the form of dimethylarsinate, arsenobetaine, and trimethylarsine oxide (Appendix II). The inorganic arsenic as measured by the levels of arsenite and arsenate was below the level of quantification at 0.05 ppm. In a critical scientific opinion on arsenic in food, the European Food Safety Authority (EFSA, 2009) panel reported that on the basis of limited data on inorganic arsenic in foods, fixed values for inorganic arsenic of 0.03 mg/kg in fish and 0.1 mg/kg in seafood were considered realistic for calculating human dietary exposure. The levels of inorganic arsenic in krill oil are lower than these assumptions, particularly for seafood. The EFSA panel also stated that the organic forms of arsenic, arsenobetaine, which is the major form in fish and most seafood, is widely assumed to be of no toxicological concern. The available evidence suggests that arsenic levels in krill oil are similar to other sea-foods. Considering that krill oil contains maximum total arsenic levels of 6 ppm, the intended use Superba™ Krill Oil will result in maximum daily intake of 48 µg/person or 0.08 µg/kg body weight/day. The WHO/FAO (1989) has suggested a provisional maximum tolerable weekly adult intake (PTWI) for inorganic arsenic of 0.015 mg/kg of body weight. Thus, the WHO/FAO provisional maximum tolerable intake is about 130 µg inorganic As/day for a 60 kg individual (15 µg/kg/week x 60 kg / 7 days/week = 128.6 µg/day). The above reported total arsenic intake of 0.08 µg/kg body weight/day is negligible compared to the tolerable daily intake of inorganic arsenic. This also suggests that krill oil consumption does not represent a major increase in the expected total daily arsenic exposure, and especially with regards to inorganic

000025

arsenic. Thus the intended use of Superba™ Krill Oil is unlikely to present any safety hazards to human health.

## **2.8. Allergenicity and Other Related Concerns**

As krill oil is prepared by the separation of lipids from protein of krill meal, consumption of krill oil by individuals allergic to shellfish may trigger an allergic response. Generally, krill oil is contraindicated for individuals who are allergic to crustacean. There is a lack of allergic responses based on the use of krill oil as a dietary supplement. While krill is known to contain allergens, its processing in the production of oil results in a reduction of its protein content to typically less than 1% which is an order of magnitude lower than in krill (about 10-15% protein). While this does not eliminate a risk, the risk is certainly no greater and possibly lower than that naturally contained in the starting materials. Aker Biomarine Antarctic AS will market krill oil in full compliance with the Food Allergen Labeling and Consumer Protection Act of 2004 (Title II of Public Law 108-282) (FDA, 2004). Aker Biomarine Antarctic AS intends to include a warning on food products containing Superba™ Krill Oil to suggest that individuals with seafood allergies, coagulopathy or who are taking anticoagulants or other medications should consult their situation with their physician before taking Superba™ Krill Oil as an ingredient in conventional foods or as nutritional supplements.

## **3. COMMON KNOWLEDGE ELEMENT**

The compositional similarity of krill oil with fish oils from multiple sources that already have GRAS status supports the common knowledge element. The composition of krill oil and common fish oils are published and the similarity in compositions is readily ascertainable in the cited public documents (FDA, 2002, 2004a, 2004b, 2008). As described in GRN 242 (FDA, 2008) documentation exists in the Federal Register for the GRAS status of menhaden oil and on the FDA website for tuna oil, salmon oil, and sardine oil. These documents cite and support the consumption of fish oil resulting in total daily consumption of EPA plus DHA of less than 3 g/person. This GRAS determination is based on the totality of the available evidence, particularly from human observations, in concert with animal experimental studies. Majority of this information as described above, particularly in Sections 2.2 and 2.3 is available in public domain. Furthermore, safety documentation for food uses of krill oil is found in GRN 242, which also constitutes information that is generally available for review and evaluation. The composite information noted thereby fulfills the common knowledge element required for GRAS determination.

## **4. SUMMARY**

Krill, a vital component of the marine food chain, is also consumed by humans, particularly in Japan and Russia. Because it is a rich source of high-quality protein as well as omega-3 fatty acids, krill has received considerable attention in recent years. Two fatty acids, EPA and DHA, that have received considerable attention for their potential health benefits have been reported to be present at high levels (30%) in krill oil. Aker Biomarine intends to use standardized krill oil (Superba™ Krill Oil) as a nutrient at levels of 0.05 to 0.50 g of the oil per serving in non-alcoholic beverages, breakfast cereals, cheeses, frozen dairy desserts, milk products, and processed fruit and fruit juices. In addition to the above categories, krill oil is also intended for use in Medical Food at levels not to exceed 0.50 g/person/day. The intended use of krill oil will result in an estimated daily mean and high (90<sup>th</sup> percentile) intake of 4.1 and 8.3

000026

g/person/day. The resulting high intake of EPA+DHA is estimated as 2.2 g/person/day. Krill oil has been the subject of a GRAS Notice submitted to the FDA for use as a nutrient. In this case, the FDA responded that they had no questions on the proposed use and did not object to the GRAS determination. The composition of Superba™ Krill Oil is well characterized and is substantially equivalent to the European Commission approved krill oil.

It is well established and recognized that dietary phospholipids and fatty acids from either plant or animal sources are handled the same metabolically. Given the metabolic sequelae, there is no reason to believe that the minor variations in the levels of lipids including phospholipids or fatty acids between these oils would pose any different health hazards. Similar to other phospholipids from other sources, phospholipids from krill oil will be absorbed, transported, and converted into endogenous constituents. The fatty acids present in krill oil are typical components of the diet and are not anticipated to pose any risk at the levels consumed. Furthermore, the different fatty acid chains are unlikely to affect the overall oral toxicity, as the fatty acid portions of molecules are largely cleaved prior to absorption by mucosal cells.

Among the fatty acids of krill oil, there is a potential safety concern for EPA and DHA at high levels of intake. The safety of these two fatty acids has been extensively evaluated by the US FDA in the final rule on the approved use of menhaden oil as a direct food ingredient and subsequently in 2005, regarding the use of omega-3 fatty acids as a dietary supplement. The FDA affirmed the GRAS status of menhaden oil for use in foods provided daily intakes of DHA and EPA did not exceed 3 g/person/day from all fish oil sources. The FDA also permitted the use of a Qualified Health Claim on dietary supplements containing EPA and DHA as well as for conventional foods. The FDA concluded that the use of EPA and DHA omega-3 fatty acids as dietary supplements is safe, provided that daily intakes of EPA and DHA do not exceed 3 g/person/day from conventional food and dietary supplement sources. For the food uses of menhaden oil, the FDA imposed specific limitations in its use in different food categories to ensure that total intake of EPA and or DHA is safe. Further, the FDA concluded that in order to help ensure that a consumer does not exceed an intake of 3 g/person/day of EPA and DHA omega-3 fatty acids from consumption of a dietary supplement with the qualified claim, an EPA and DHA omega-3 fatty acid dietary supplement bearing a qualified claim should not recommend or suggest in its labeling, or under ordinary conditions of use, an intake exceeding 2 g EPA and DHA/day. Given the substitutional (for substances with DHA and EPA) uses of krill oil, the resulting intake of DHA and EPA is unlikely to exceed 2.2 g/person/day and is considered as safe.

The safety of krill oil has been investigated in human clinical and animal experimental studies. Although the majority of these studies were designed to investigate the potential health benefits of krill oil, no adverse effects were noted. These studies support the safety of krill oil. Of the five clinical studies on krill oil, three were more significant with regard to dose and duration. In one clinical trial conducted to examine the safety, krill oil was well tolerated at a dose of 2 g/day for four weeks. In the second study, no adverse effects were noted following the consumption of 6 g krill oil/day for two months. In the third clinical study, participants tolerated krill oil at doses of up to 3 g/day for a period of 12 weeks, followed by an additional 0.5 g/day by some participants for 90 days. In these studies no significant adverse effects of krill oil consumption were noted.

There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use for krill oil. The safety of krill oil is based on several

factors that include the inherent safety of the fatty acid, phospholipids and other components in the oil, the compositional similarity of the krill oil with fish oils, extensive knowledge of their metabolism, the expected levels in the diet of EPA and DHA fatty acids, and astaxanthin from the intended use of krill oil, the safety of krill oil as demonstrated in pre-clinical and clinical trials, and the absence of reports of toxicity. Additionally, Antarctic krill also has some history of consumption by humans in Japan and Russia. On the basis of scientific procedures<sup>8</sup>, the consumption of krill oil as an added food ingredient is considered safe at levels up to 8.3 g/person/day. The intended uses are compatible with current regulations, *i.e.*, krill oil is used in non-alcoholic beverages, breakfast cereals, cheeses, frozen dairy desserts, milk products, and processed fruit and fruit juices, and Medical Foods.

000028

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<sup>8</sup> 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

## 5. CONCLUSION

Based on a critical evaluation of the publicly available data summarized above, the Expert Panel members whose signatures appear below, have individually and collectively concluded that krill oil (Superba™ Krill Oil), meeting the specifications cited above, and when used as a food ingredient in selected food products (non-alcoholic beverages, breakfast cereals, cheeses, frozen dairy desserts, milk products, and processed fruit and fruit juices, and Medical Foods) at levels of 0.05 to 0.50 g krill oil/serving (reference amounts customarily consumed, 21CFR 101.12) when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the 90<sup>th</sup> percentile (high) estimated intake of 8.3 g krill oil/person/day is Generally Recognized As Safe (GRAS).

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Superba™ Krill Oil, when used as described, is GRAS, based on scientific procedures.

### Signatures

(b) (6)

[Redacted Signature]

John A. Thomas, Ph.D., F.A.C.T., D.A.T.S.

12/8/10

Date

(b) (6)

[Redacted Signature]

Stanley T. Omaye, Ph.D., D.A.T.S.

12/07/10

Date

(b) (6)

[Redacted Signature]

Madhusudan G. Soni, Ph.D., F.A.C.N.

Dec. 10, 2010

Date

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000030

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000031

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000032

## 7. APPENDIX I

### Analytical data from different manufacturing lots of Superba™ Krill Oil (Aker Biomarine, 2010)

Parameter	Limits	U133 002 A10	U176 004 A10	U141 001 A10	U141 003 A10	U141 002 A10
Appearance	Dark red viscous oil					
<b>Lipid composition</b>						
Total phospholipids (g/100g)	43 ± 3	40.3	44.8	40.8	45.3	42.7
-Omega-3 phospholipids <sup>1</sup> of total PL % (w/w)	>70	>70	>70	>70	>70	>70
Triglycerides (g/100g)	<50	39	36	32	32	32
<b>Fatty acid profile</b>						
Total omega-3 (expressed as g/100g)	23.5 ± 2	22.9	22.4	24.5	26.2	25.5
-C 20:5 n-3 (EPA)(expressed as g/100g)	14 ± 2	13.4	14.3	14.7	16.7	16.3
-C 22:6 n-3 (DHA)(expressed as g/100g)	6.5 ± 1	6.5	5.8	6.7	6.7	6.5
Total omega-6	<3.0	1.9	2.0	2.2	2.4	2.4
<b>Stability index</b>						
Peroxide value (mEq peroxide/kg)	<2	<1	<1	<1	<1	<1
<b>Antioxidants</b>						
Astaxanthin (mg/kg)	100 ± 20 (minimum)	164	125	144	96	92
<b>Water and Ethanol</b>						
Water activity at 25°C	<0.5	0.116	0.149	0.143	0.115	0.139
Ethanol content (% w/w)	<3.0	1.8	1.52	1.58	1.37	1.21
<b>Microbiology</b>						
Total plate count (cfu/g)	<2500	<100	<100	<100	<100	<100
<i>E. coli</i> (1 sample at 10 g)	Negative	Negative	Negative	Negative	Negative	Negative
Coliform bacteria, 37°C (cfu/g)	<10	<10	<10	<10	<10	<10
<i>Salmonella</i> negative (PCR) (1 sample at 10 g)	Negative	Negative	Negative	Negative	Negative	Negative
Mold and Yeast (cfu/g)	<10	<10	<10	<10	<10	<10
<sup>1</sup> Omega-3 phospholipid: defined as phospholipid where on average one out of two possible positions is occupied by an omega-3 fatty acid.						

000033

**Additional Specification and compositional analysis data of  
Superba™ Krill Oil from five different batches  
Adapted from Superba™ Krill oil substantial equivalence notification**

Parameter	Unit	Batch 233/34/A 8	Batch 234/42/A 8	Batch 234/43/A8	Batch 235/24/A 8	Batch 280/42/A 9	Batch 279/22/ A9
1. Saponification value	Mg KOH/g	N.D	N.D	N.D	N.D	149	160
2. Peroxide value*	eEq/kg	<2	<2	<2	<2	<2	<2
3. Moisture**		0.19	0.251	0.27	0.339	N.D	N.D
4. Total phospholipids	g/100g	46.0	44.3	45.7	44.5	N.D	N.D
5. <i>Trans</i> -fatty acids	% of lipids	0.23	0.23	0.23	0.24	N.D	N.D
6. EPA (20:5)		14.8	14.9	14.3	14.9	N.D	N.D
7. DHA (22:6)		8.6	8.7	8.4	8.7	N.D	N.D

Analysis 3-7 was performed by validated methods at an accredited laboratory (NOFIMA). Analysis number 1 was performed at NOFIMA. Adapted from Superba™ Krill Oil substantial equivalence notification.

\* As assayed by the relevant AOCS method.

\*\* Moisture expressed as water activity at 25°C. N.D. = not determined.

000034

**8. APPENDIX II**

**Analytical Results of Dioxins, Furans, Organochlorine Pesticides,  
PBDEs, PAHs, and Heavy Metals from Five Batches, and  
Marker PCBs from Four Batches of Superba™ Krill Oil**

**000035**

000036

Parameter	Unit	Method	233/34/A8 (262/72/A8)	234/42/A8	234/43/A8	235/24/A8	341/70/A9	average
<b><i>Dioxins, furans and dioxine like PCBs</i></b>								
Total PCDDs/PCDFs	ng/kg	EN 1948 modified, HRMS	0.16	0.16	0.17	0.15	0.294	0.187
PCDDs/PCDFs and dioxine like PCBs	ng/kg	EN 1948 modified, HRMS	0.27	0.26	0.26	0.26	0.436	0.297
<b><i>Organochlorine pesticides</i></b>								
DDTs/DDDs/DDEs	ug/kg	Internal method, HRGC-HRMS	<1.7	<1.37	<1.43	<1.45	<1.2	
Aldrin	ug/kg	Internal method, HRGC-HRMS	<0.5	<0.5	<0.5	<0.5	<0.5	
Dieldrin	ug/kg	Internal method, HRGC-HRMS	0.72	0.65	0.64	0.57	0.42	
Toxaphen	ug/kg	Internal method, HRGC-HRMS	<3.3	<2.1	<2.2	<2.1	<1.8	
<b><i>PBDEs</i></b>								
PBDE #17	ng/g	LRMS	<0.02	<0.01	<0.01	<0.01	<0.02	<0.014
PBDE #28	ng/g	LRMS	<0.02	<0.01	<0.01	<0.01	<0.019	<0.0138
PBDE #49	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #71	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #47	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #66	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #77	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #100	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #119	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #99	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #85	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #126	ng/g	LRMS	<0.04	<0.03	<0.03	<0.03	<0.048	<0.036
PBDE #154	ng/g	LRMS	<0.06	<0.04	<0.04	<0.04	<0.077	<0.051

000037

PBDE #153	ng/g	LRMS	<0.06	<0.04	<0.04	<0.04	<0.077	<0.051
PBDE #138	ng/g	LRMS	<0.06	<0.04	<0.04	<0.04	<0.077	<0.051
PBDE #183	ng/g	LRMS	<0.07	<0.06	<0.06	<0.06	<0.096	<0.069
PBDE #190	ng/g	LRMS	<0.07	<0.06	<0.06	<0.06		
PBDE #203	ng/g	LRMS	<0.15	<0.15	<0.15	<0.15		
PBDE #207	ng/g	LRMS	<0.15	<0.12	<0.11	<0.12	<0.479	<0.196
PBDE #209	ng/g	LRMS	<1.48	<1.19	<1.14	<1.16	<1.91	<1.38
<b>PAHs</b>								
Benzo(a)anthracene	ug/kg	GC-MS	<0.5		ND	<0.5	<0.5	<0.5
Chrysene/triphenylene	ug/kg	GC-MS	0.7		ND	0.6	<0.5	<0.6
Benzo(b)fluoranthene	ug/kg	GC-MS	<0.5		ND	<0.5	<0.5	<0.5
Benzo(k/j)fluoranthene	ug/kg	GC-MS	<0.5		ND	<0.5	<0.5	<0.5
Benzo(a)pyrene	ug/kg	GC-MS	<0.5		ND	<0.5	<0.5	<0.5
Indeno(1,2,3-cd)pyrene	ug/kg	GC-MS	<0.5		ND	<0.5	<0.5	<0.5
Dibenzo(a,h)anthracene	ug/kg	GC-MS	<0.5		ND	<0.5	<0.5	<0.5
Benzo(ghi)perylene	ug/kg	GC-MS	<0.5		ND	<0.5	<0.6	<0.53
Dibenzo(a,l)pyrene	ug/kg	GC-MS	<1		ND	<1	<1	<1
Dibenzo(a,i)pyrene	ug/kg	GC-MS	<1		ND	<1	<1	<1
Dibenzo(a,h)pyrene	ug/kg	GC-MS	<1		ND	<1	<1	<1
Dibenzo(a,e)pyrene	ug/kg	GC-MS	<1		ND	<1	<1	<1
Cyclopenta(c,d)pyrene	ug/kg	GC-MS	<1		ND	<1	<1	<1
5-methylchrysene	ug/kg	GC-MS	<1		ND	<1	<1	<1
Benzo-(o)-fluorene	µg/kg	GC-MS					<1	<1
Benzo(a)pyrene	µg/kg	GC-MS					<0.5	<0.5
<b>Arsenic</b>								
Arsenite	mg/kg	Extraction/digestion, HPLC-ICP-MS	<0.005	<0.005	<0.005	<0.005	0.015	0.007
Arsenate	mg/kg	Extraction/digestion, HPLC-ICP-MS	<0.005	<0.005	<0.005	<0.005	<0.005	0.005

000038

Monomethylarsonate	mg/kg	Extraction/digestion, HPLC-ICP-MS	0.075	0.041	0.057	0.062	0.042	
Dimethylarsinate	mg/kg	Extraction/digestion, HPLC-ICP-MS	3.18	3.18	3.3	3.29	3.64	
Arsenobetaine	mg/kg	Extraction/digestion, HPLC-ICP-MS	0.91	0.771	0.886	0.914	0.723	
Arsenocholine	mg/kg	Extraction/digestion, HPLC-ICP-MS	<0.005	<0.005	<0.005	<0.005	<0.005	
Trimethylarsine oxide	mg/kg	Extraction/digestion, HPLC-ICP-MS	0.399	0.42	0.417	0.431	0.519	
Tetramethylarsonium ion	mg/kg	Extraction/digestion, HPLC-ICP-MS	0.063	0.062	0.062	0.064	<0.005	
Arsenosugar a	mg/kg	Extraction/digestion, HPLC-ICP-MS	<0.005	<0.005	<0.005	<0.005	<0.005	
Arsenosugar b	mg/kg	Extraction/digestion, HPLC-ICP-MS	<0.005	<0.005	<0.005	<0.005	<0.005	
Arsenosugar c	mg/kg	Extraction/digestion, HPLC-ICP-MS	0.011	0.008	0.011	0.01	0.02	
Arsenosugar d	mg/kg	Extraction/digestion, HPLC-ICP-MS	0.038	0.036	0.041	0.037	0.022	
Arsenic (As)	mg/kg	Microwave assisted digestion, ICP-MS	5.5	4.9	5.5	5.2	5.6	
<b>Heavy metals</b>								
Pb	mg/kg	§64 LFGB L00.00-19/3, AAS-Gr.	<0.05	<0.1	<0.05	<0.05	<0.04	<0.058
Cd	mg/kg	§64 LFGB L00.00-19/3, AAS-Gr.	<0.01	<0.01	<0.01	<0.01	<0.02	<0.012
Hg	mg/kg	§64 LFGB L00.00-19/4, AAS-cold vapour	<0.005	<0.005	<0.005	<0.005	<0.02	<0.008
Cu	mg/kg	EN ISO 11885, mod., ICP-OES	6.3	7.7	7.2	5.8	10	7.4
Fe	mg/kg	EN ISO 11885, mod., ICP-OES	0.4	0.21	0.18	0.2	<2	0.598
Zn	mg/kg	EN ISO 11885, mod., ICP-OES	2.9	2.5	2.8	2.9	2.5	2.72

Data information provided by Aker Biomarine.

Analytical Results on Marker PCBs from four representative batches of Superba™ Krill Oil are presented separately (see below)

**Levels of Marker PCBs from four representative batches of Superba™ Krill Oil**

<b>Marker PCBS</b>	<b>Unit</b>	<b>341 70 A9</b>	<b>A112/011/A10</b>	<b>U194/001/A10</b>	<b>U232/002/A10</b>
PCB 28	pg/g	<54.6	<89.7	<92.8	<90.7
PCB 52	pg/g	<43.1	<46.2	<47.7	56.8
PCB 101	pg/g	<54.6	<66.7	<69.0	<67.4
PCB 118	pg/g	<21.6	<24.1	62.7	36.2
PCB 138	pg/g	<63.2	<79.5	<82.2	<80.3
PCB 153	pg/g	<66.1	<84.6	<87.5	<85.5
PCB 180	pg/g	<26.4	<61.5	<63.7	<62.2
<b>Total 7 indicator PCBs</b>	pg/g	330	452	506	479

**000039**

## 9. APPENDIX III

### *trans*-Fatty acid profile from four batches of Superba™ Krill Oil

Fatty acids	Batch 235-24-A8	Batch 234-33-A8	Batch 02925-01	Batch 234-43-A8
<i>trans</i> 16:1	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 18:1	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 18:2	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 18:3	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 20:1	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 20:2	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 20:3	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 20:4	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 20:5	0.1	0.1	0.1	0.1
<i>trans</i> 22:1	<0.1	<0.1	<0.1	<0.1
<i>trans</i> 22:6	<0.1	0.1	0.1	0.1
Total <i>trans</i> -fatty acids	0.2	0.2	0.2	0.2

Values are expressed as g/100 g of fatty acids; Method: AOCS Ce 1h-05; Data information provided by Aker Biomarine

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10. APPENDIX IV

Novel Food Ingredient approval for Superba™ Krill Oil



EUROPEAN COMMISSION  
HEALTH AND CONSUMERS DIRECTORATE-GENERAL  
Safety of the Food chain  
Food law, nutrition and labelling

SANCO  
22. 12. 2009

Brussels,  
SANCO/E4/AK/fs (2009) D/540876

Note to the Permanent Representations of

Austria, Belgium, Bulgaria, Czech Republic, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, The Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

**Subject: Regulation (EC) N° 258/97 concerning novel foods and novel food ingredients  
Notification pursuant to Article 5 of the above mentioned Regulation  
Lipid extract from Antarctic Krill**

Pursuant to Article 5 of Regulation (EC) N° 258/97, the Commission has received a notification for the placing of the above-mentioned product on the Community market on 17 December 2009.

**Notifier: Aker BioMarine Antarctic AS**  
Fjordalléen 16  
P.O.Box 1423 Vika  
NO - 0115 Oslo  
Norway.

The Novel Food Board (NFN) has delivered an opinion that the Krill oil to be placed on the market by the company Aker BioMarine Antarctic AS is substantially equivalent to the Krill oil authorised by Commission Decision 2009/752/EC with respect to composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein (Article 3.4 of Regulation (EC) N° 258/97).

Pursuant to Article 5 of Regulation (EC) N° 258/97 you are now receiving a copy of the notification with its enclosures.

(b) (6)

Andreas Klepsch

Enclosures

cc: Competent authorities, EFTA Secretariat, Mr Hogne Vik

Commission européenne, B-1049 Bruxelles / Europese Commissie, B-1049 Brussel - Belgium Telephone (32-2) 299 11 11  
Office: FIC 1 B/22. Telephone: direct line (32-2) 2953210 Fax: (32-2) 2951 735

000041

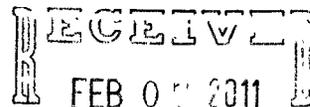
Pages 000042-000129 containing curriculum vitae removed under Freedom of Information exemption 6.

# Soni & Associates Inc.

749 46<sup>th</sup> Square  
Vero Beach, FL 32968, USA  
Telephone: 772-299-0746

E-mail: [msoni@soniassociates.net](mailto:msoni@soniassociates.net)

January 28, 2011



BY: (b) (6)

Dr. Paulette Gaynor  
Office of Food Additive Safety (HFS-255)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740-3835

**Subject: GRAS Notification for Kril**

Dear Dr. Gaynor:

This has reference to our discussion about Superba™ Krill Oil GRAS notification submitted on behalf of Aker Biomarine Antarctic AS, Norway. As discussed, please find attached three copies of the revised Availability of Information statement (page 3).

If you have any questions or require additional information, please feel free to contact me at 772-299-0746 by phone or at [msoni@soniassociates.net](mailto:msoni@soniassociates.net) by email.

Sincerely,

(b) (6)

Madhu G. Soni, Ph.D.

Enclosure:

determination of high phospholipid krill oil is based on the totality of available scientific evidence that includes human observations and a variety of preclinical and clinical studies. Based on the available safety-related information, the estimated daily intake, if ingested daily over a lifetime, is safe.

**F. Availability of Information:**

The data and information that forms the basis of Aker Biomarine's Superba™ Krill Oil GRAS determination will be available for the Food and Drug Administration's review and copying at the following address or will be provided to the FDA upon request:

Madhu G. Soni, Ph.D., FACN,  
 Soni & Associates Inc.,  
 749 46<sup>th</sup> Square,  
 Vero Beach FL, 32968  
 Phone: (772) 299-0746; E-mail: sonim@bellsouth.net

**II. Detailed Information About the Identity of the Notified Substance:**

**A. Trade Name:**

The subject of this notification will be marketed as Superba™ Krill Oil

**B. Physical Characteristics**

Superba™ Krill Oil is dark red colored viscous oil

**C. Chemical Abstract Registry Number:**

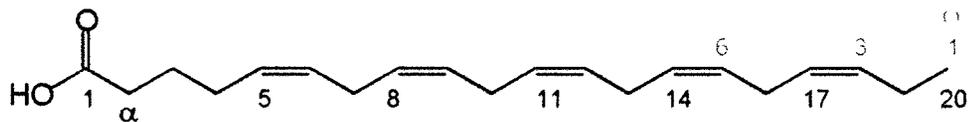
Not available

**D. Chemical Formula:**

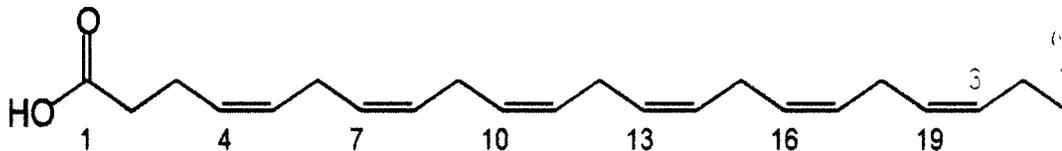
Not applicable

**E. Structure:**

The important constituents of high phospholipid krill oil are the fatty acids, EPA and DHA. The structures of these two fatty acids presented in Figure 1.



**Eicosapentaenoic acid (EPA)**



**Docosahexaenoic acid (DHA)**

**Figure 1. Chemical structures of EPA and DHA**

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1  
SUBMISSION END

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AM

**Fus, Andrea**

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**From:** Madhu Soni [sonim@bellsouth.net]  
**Sent:** Friday, April 08, 2011 9:28 AM  
**To:** Fus, Andrea \*  
**Subject:** RE: FDA Request for Clarification Regarding GRN 371, Krill Oil  
**Attachments:** GRN 371 Krill Oil GRAS FDA Query Response.pdf

Dear Dr. Fus,

Please find attached an electronic file providing a point-by-point response to your queries. I hope the information and clarifications, along with some discussion in the response addresses your queries. If you have any questions or need additional explanation, please let me know. Thank you for the opportunity to provide this explanation.

Best regards

Madhu

---

**From:** Fus, Andrea \* [mailto:Andrea.Fus@fda.hhs.gov]  
**Sent:** Monday, March 21, 2011 2:31 PM  
**To:** sonim@bellsouth.net  
**Subject:** FDA Request for Clarification Regarding GRN 371, Krill Oil

Dear Dr, Soni,

I am glad we were able to speak on the phone today.

As we discussed, an electronic file describing several points of clarification for GRN 371, krill oil by Aker Biomarine Antarctic AS, is attached. I understand that you estimate it may take two or three weeks to finalize a response from the notifier. Please let me know if there are any significant changes in your time line.

Please do not hesitate to contact me should you have any questions or concerns.

Thanks

*Andrea Fus*

Andrea F. Fus, Pharm.D  
ORISE / Regulatory Team B  
U.S. Food and Drug Administration  
Center for Food Safety and Applied Nutrition  
Division of Biotechnology and GRAS Notice Review  
5100 Paint Branch Parkway  
College Park, MD 20740  
(301) 436-1351  
[Andrea.Fus@fda.hhs.gov](mailto:Andrea.Fus@fda.hhs.gov)

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9/21/2011

Dear Dr. Fus,

**RE: Krill oil GRAS Notice (GRN 371)**

This responds to your email of March 21, 2011 regarding additional information and clarifications required for our Krill oil GRAS notice (GRN 000371). We are providing a point-by-point response to your queries along with some relevant discussion.

1. **FDA Query:** Please address methods used by Aker Biomarine Antarctic's to calculate a maximum 2.2 g per person per day total omega-3 (DHA and EPA) exposure for your krill oil and its typical composition (as indicated in Table 1).

**Response:** Thank you for bringing this to our attention. By oversight we forgot to include the correct value for total omega-3 exposure. Based on the data provided in Table 1 of our GRN, the maximum omega-3 content (EPA- 14±2 and DHA- 6.5±1) of the krill oil will be 23.5 (EPA- 16.0 and DHA- = 7.5). As the intended use of krill oil will result in an estimated daily maximum (90<sup>th</sup> percentile) intake of 8.3 g/person/day, the resulting high intake of EPA+DHA is estimated as 1.95 g/person/day. Hence the correct value for total omega-3 (EPA and DHA) exposure should be 1.95 g/person/day.

2. **FDA Query:** Please include specifications for incidental chemicals in Aker Biomarine Antarctic's krill oil, at minimum, for arsenic, mercury, and lead.

**Response:** As desired, we are including specification for incidental chemicals below:

**Specifications for Incidental Chemicals (Superba™ Krill Oil)**

Incidental Chemical	Units	Specifications	Method
<b>Heavy metals</b>			
Arsenic (inorganic)	mg/kg	< 0.05	Extraction/digestion, HPLC-ICP-MS
Mercury	mg/kg	< 0.05	ALC 208:112
Lead	mg/kg	< 0.10	NMKL161 mod;ICP-MS
Cadmium	mg/kg	< 0.10	NMKL161 mod;ICP-MS
Copper	mg/kg	< 10.0	NMKL161 mod;ICP
Iron	mg/kg	< 2.00	NMKL161 mod;ICP
Zinc	mg/kg	< 5.00	NMKL161 mod;ICP
<b>Dioxins, furans and dioxine like PCBs</b>			
PCDDs/PCDFs (WHO98-TEQ)	pg/g	< 0.30	EN 1948 modified, HRGC/HRMS
PCCDs/PCDFs and dioxine like PCBs (WHO98-TEQ)	pg/kg	< 0.50	EN 1948 modified, HRMS/HRMS
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	µg/g	< 6 x 10 <sup>-8</sup>	EN 1948 modified HRGC/HRMS
PCBs (28,52,101,118,138,153,180)	µg/g	< 6 x 10 <sup>-4</sup>	EN 1948 modified HRGC/HRMS
<b>PAHS</b>			
Benzo(a)pyrene	µg/kg	< 2.0	GC-MS
Benzo(a)anthracene	µg/kg	< 2.0	GC-MS

3. **FDA Query:** GRAS notice 243 (D-Ribose) (rather than GRN 242) is referred to twice in section 2.6 in reference to *Trans*-Fatty acids.

**Response:** We apologize for the incorrect citation. The correct reference should be GRN 242.

4. **FDA Query:** The Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional maximum tolerable intake PTWI for inorganic arsenic of 15 µg/kg body weight/week has been withdrawn, and is no longer appropriate.

**Response:** Thank you for bringing to our attention the JECFA withdrawal of inorganic arsenic PTWI. We are sorry that we missed this recent JECFA withdrawal. As discussed in our GRAS notice regarding the safety of arsenic, not all forms of arsenic are associated with health concerns and organic arsenic is considered to be relatively non-toxic. As the specifications for inorganic arsenic for Superba® Krill Oil is set at < 0.05 ppm (below detection limits), the resulting intake of inorganic arsenic from the intended maximum exposure of 8.3 g of krill oil will be 0.415 µg/person/day (0.0069 µg/kg bw/day for an individual weighing 60 kg). The Agency for Toxic Substances and Disease Registry (ATSDR, 2007)<sup>1</sup> has derived Minimal Risk Level (MRL)<sup>2</sup> of 0.0003 mg/kg bw/day (0.3 µg/kg bw/day) for inorganic arsenic for chronic oral exposure. Compared to the MRL, the resulting intake of inorganic arsenic from the intended uses of krill oil is very small and is considered as safe.

In 2008, the Natural Health Products Directorate, Health Canada<sup>3</sup> has suggested a limit of < 0.03 µg/kg bw/day for inorganic arsenic and < 20 µg/kg bw/day for organic arsenic. The batch analysis data of Superba® Krill Oil revealed maximum total arsenic levels of approximately 6 ppm, primarily containing organic arsenic. Based on this, the intended use of Superba™ Krill Oil will result in maximum daily intake of 50 µg/person/day or 0.8 µg/kg bw/day of total arsenic, majority of which is organic arsenic. The total intake of arsenic, including organic and inorganic, from the intended uses of krill oil is 25-fold lower than those set by Health Canada for organic arsenic.

Additionally, in a 1993 Guidance Document for Arsenic in Shellfish<sup>4</sup>, FDA provided guidance on determining permitted levels of contaminant using information on tolerable daily intake of arsenic. In this document the daily tolerable intake of arsenic is considered as 130 µg/person/day. The plausible concentration level of concern for crustacean shellfish at mean and 90<sup>th</sup> percentile was determined as 140 and 76 µg/person/day, respectively. Compared to this, the resulting intake of inorganic arsenic of 0.415 µg/person/day from the intended uses of krill oil is very small and is considered as safe.

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<sup>1</sup> Report available at the website: <http://www.atsdr.cdc.gov/ToxProfiles/tp2.pdf>

<sup>2</sup> An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure.

<sup>3</sup> Report available at the website: [http://standards.nsf.org/apps/group\\_public/download.php/1436/4-addendum%20-%20DS-2008-2%20Arsenic%20HC%20-%20summary.pdf](http://standards.nsf.org/apps/group_public/download.php/1436/4-addendum%20-%20DS-2008-2%20Arsenic%20HC%20-%20summary.pdf)

<sup>4</sup> Food and Drug Administration. 1993. Guidance Document for Arsenic in Shellfish. U.S. Department of Health and Human Services, Public Health Service, Office of Seafood (HFS-416), 200 C Street, SW, Washington, DC 20204. 44 pages.

In conclusion, the intake of Superba® Krill Oil from its intended uses does not represent a major increase in the expected total daily arsenic exposure, and especially with regards to inorganic arsenic. Based on the available information, the resulting intake of arsenic from the proposed uses of Superba® Krill Oil is considered as safe.

We hope the above information and clarification addresses your queries. If you have any questions or need additional explanation, please let me know.

Thank you for the opportunity to provide this explanation.

Best regards

Madhu Soni, PhD