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ORIGINAL SUBMISSION

GRAS ASSOCIATES, LLC
Generally Recognized As Safe
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01-24-08 A11:35 OUT

January 18, 2008

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Robert L. Martin

Dear Dr. Martin:

On behalf of Neptune Technologies & Bioressources of Laval (Quebec), Canada, we are submitting for FDA review a GRAS notification for High Phospholipid Krill Oil. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

As part of the deliberations by our Expert Panel on the safety of the subject krill oil as a human food ingredient, two unpublished scientific studies were considered. They have been included as Appendix G in the submission. Even though each of the reports is identified as being confidential, we recognize that the content submitted cannot be held as confidential, and we hereby waive the designation of confidentiality.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
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Enclosure: GRAS Notification – High Phospholipid Krill Oil (in triplicate)

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I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

High phospholipid krill oil, meeting the specifications described below, has been determined to be Generally Recognized As Safe (GRAS), in accordance with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination was made by experts qualified by scientific training and experience; it is based on scientific procedures as described in the following sections; and the evaluation accurately reflects the conditions of the ingredient's intended use in foods.

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Signed:

January 18, 2008

Date

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

B. Name and Address of Notifier

Neptune Technologies and Bioressources
2740 Pierre Peladeau Avenue, Suite H200
Laval (Quebec)
Canada H7T 3B3

As the notifier, Neptune Technologies and Bioressources ("Neptune") accepts responsibility for the GRAS determination that has been made for high phospholipid krill oil as described in the subject notification; consequently, high phospholipid krill oil meeting the conditions described herein is exempt from pre-market approval requirements for food ingredients.

C. Common Name and Identity of the Notified Substance

High phospholipid krill oil; also see Sections II.A and II.B.

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[1] See 62 FR 18938 (17 April 1997).

D. Conditions of Intended Use in Food

High phospholipid krill oil is intended to be added as a substitute or alternative to fish oils to the following food categories at per serving levels ranging from 150 – 500 mg: non-alcoholic beverages; breakfast cereals; cheeses; frozen dairy desserts; milk products; processed fruits/fruit juices; and medical foods. The maximum daily consumption of the subject krill oil will provide up to 2.2 g/person/day of EPA and DHA.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, high phospholipid krill oil has been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. CHEMISTRY AND MANUFACTURE OF NKO™

NKO™ is refined krill oil which is derived from the marine organism, *Euphasia superba,* which is harvested from Antarctic waters. Krill is recognized as a vital part of the marine food chain for baleen whales, mantas, whale sharks, crabeater seals and other seals, and a few seabird species.

The following description of krill is given on Neptune's website.[2]

> "Krill is a generic term used to designate all deepwater marine planktonic crustacean species. It looks like miniature shrimp, ranging between one and six centimeters in length. These 85 species represent the planet's most abundant animal biomass found in banks that may extend over several square kilometers. Krill fisheries can mainly be found in two ocean regions: the Antarctic Ocean and the North Pacific Ocean, along the Canadian and Japanese coasts. According to a World Health Organization estimate, the total quantity of krill in these two oceans exceeds 500 million tons. Approximately 110,000 tons of krill are harvested every year, or about less than one-half of 0.1% of the existing resources."

NKO™ is a whole lipid extract and is an opaque reddish oil with a seafood odor. The major components of NKO™ are triglycerides and phospholipids which are rich in eicosapentaenoic acid (EPA, C20:5 n-3 fatty acid) and docosahexaenoic acid (DHA, C22:6 n-3 fatty acid). NKO™ is an oil intended for use as a dietary ingredient as a source of omega-3 fatty acids, which as noted are found in NKO™ in their phospholipid form. No processing aids or additives are included in the final NKO™ product due to naturally occurring antioxidants that aid in NKO™'s preservation. Additionally, no proprietary or coloring ingredients are added to the oil from Antarctic krill to produce NKO™.

A. Common or Usual Name of the Subject Material

High phospholipid krill oil is the common or usual name of the material that is the subject of the GRAS evaluation. The specific substance evaluated is Neptune Krill Oil™, which is commonly referred to throughout this evaluation as NKO™. As noted in the preceding paragraph, NKO™ is a mixture of triglycerides and phospholipids that contain numerous constituent fatty acids. The compositional character of NKO™ is described in detail in this Section.

B. Manufacturing and Specifications for NKO™

1. Krill Source and Handling

NKO™ is extracted from *Euphasia superba*. The current taxonomic placement of *Euphasia superba* is summarized below:

[2] See www.neptunebiotech.com/whatiskrill.html.

KINGDOM	Animalia
PHYLUM	Arthropoda
SUBPHYLUM	Crustacea
CLASS	Malacostraca
SUPERORDER	Eucarida
ORDER	Euphausiacea
FAMILY	Euphausiidae
GENUS	Euphausia
SPECIES	*Euphausia superba*

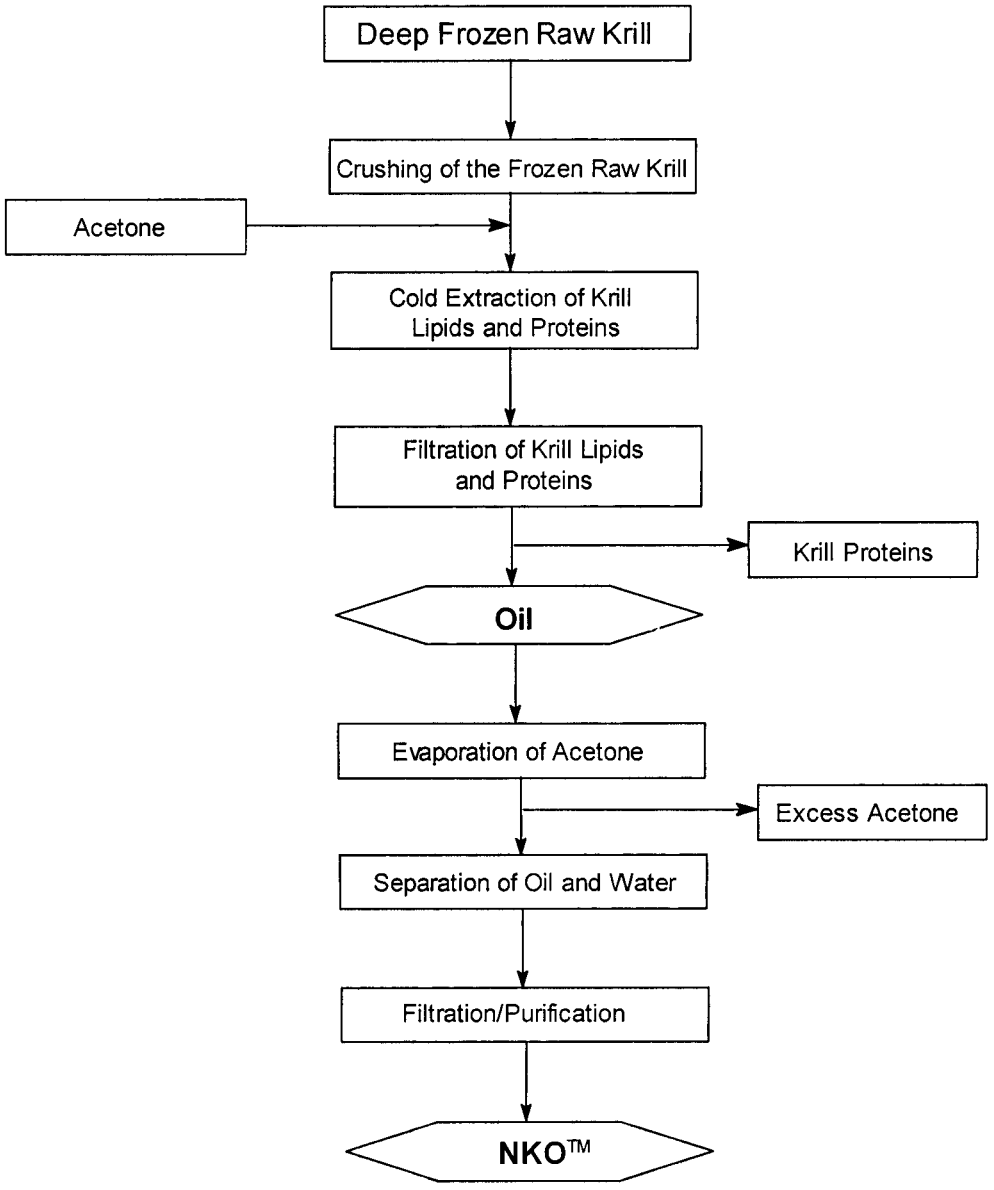
NKO™ is produced from Antarctic krill, which are native to the Atlantic section of the Austral-Antarctic Circumpolar Ocean. In terms of their phylogeny, Antarctic krill are closely related to shrimp and are consumed as human food in a similar fashion. The Antarctic krill used in the production of NKO™ are a non-genetically modified source, are fished from the wild, and are not the result of breeding. No toxic constituents are known to be present in NKO™.

The Antarctic krill used to extract NKO™ are fished in the Atlantic section of the Austral-Antarctic Circumpolar Ocean, originating specifically from Statistical Fishing Area 48, a fishing area designated by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR). The Antarctic krill processed by Neptune are supplied by a Japanese firm that has been fishing Antarctic krill for more than two decades. The Antarctic krill are drained and placed in plastic molds for quick-freezing at about -30°C on board factory ships within a few hours of harvest. The resulting 12 kg blocks of Antarctic krill are individually wrapped in plastic film and are packaged two per carton prior to export to Canada; the packaged Antarctic krill are maintained at temperatures of -30 to -20°C. Upon arrival in Canada, they are inspected and approved by the Canadian Food Inspection Agency (CFIA). Source manipulations on board the fishing vessels are made in compliance with regulations as they apply to the handling of krill intended for human consumption.

2. NKO™ Manufacturing Process

The manufacturing of NKO™ begins with the crushing of deep frozen Antarctic krill at a temperature of 4°C until the Antarctic krill particles are <5 mm in size. The lipids and proteins are separated and extracted from the crushed Antarctic krill *via* the addition of acetone under cold extractions conditions for a period of 2 hours. This step also takes place at a temperature of 4°C, and requires the addition of enough acetone to ensure a ratio of crushed Antarctic krill to acetone of 1:6 (w/v). Filtration occurs through an organic solvent-resistant filter under reduced pressure to enable physical separation of the Antarctic krill lipids and proteins. The remaining crushed Antarctic krill is then subjected to a second round of extraction to ensure that all possible lipids and proteins are extracted. Following extraction, the Antarctic krill proteins and lipids are filtered and separated from each other with this process yielding an oil. Excess acetone is then evaporated and separated from any water remaining in the product. The oil undergoes filtration and purification to remove impurities and is then packaged in a modified N_2-containing atmosphere and stored as NKO™. Figure 1 summarizing the overall NKO™ manufacturing process.

Figure 1. Manufacturing Process for the Production of NKO™



3. Manufacturing Controls

The process by which Neptune produces NKO™ occurs under Canadian Good Manufacturing Practices (GMP) requirements, as certified by the Natural Health Products Directorate (NHPD) of Canada. For GMP Certificate of Compliance (Certification Number 0001349) and Site License (License Number 300191), see Appendix A.

In addition to conforming to Canadian GMP standards, the Neptune production method is internally controlled under the Hazard Analysis Critical Control Points (HACCP) system. Under this system, Neptune has identified potential points for potential contamination or corruption of NKO™. To ensure the quality of the final NKO™ product, production lots are consistently tested to ensure compliance with appropriate international standards addressing residual solvents, microorganisms, heavy metals, arsenic, and pesticides as discussed further.

Additionally, Certificate of Registration for a Fish Processing Establishment (Certification Number 5111) was granted to Neptune on February 17, 2005 and was renewed on February 17, 2006 by the CFIA following the implementation of a "Quality Management Program - Fish" (QMP) and is attached in Appendix A.

4. Specifications for NKO™

The compositional specifications for NKO™ as established by Neptune are presented in Table 1.

The compositional specifications established by Neptune address all relevant issues concerning marine oils. They address the quality of the oil by providing minimum requirements for fatty acids and phospholipids content, as well as markers of stability and purity. The specifications provide information concerning the fat, cholesterol, and protein contents of NKO™, as well as common contaminants, such as pesticides, dioxins, and heavy metals, which may be present in marine oils. Finally, Neptune's compositional specifications are similar to those for other marine-derived oils considering phospholipid or omega-3 fatty acid-rich oils and are consistent with the Codex Alimentarius standard for Edible Fats And Oils Not Covered By Individual Standards (CODEX STAN 19-1981 (Rev. 2-1999); see Appendix B).

The analytical procedures employed in the analysis have been validated by a variety of sources, the most prominent of which are the American Oil Chemist's Society (A.O.C.S) and the Association of Official Agricultural Chemists (A.O.A.C) International. Both of these organizations produce validated microbiological and chemical analytical methods. In addition, several methods were developed and validated by the Canadian government, and additional information on these methods appears in Appendix C.

Results from the analyses for the detailed composition of three different batches of NKO™ are presented in Table 2. As can be seen, overall and detailed compositions are consistent among the three batches.

TABLE 1. SPECIFICATIONS AND COMPOSITIONAL PROFILE FOR NKO™

Requirement	Method(s)	Specification
	Description	
Appearance	Visual - Reddish opaque oil	Complies
Odor	Olfactive – Light shrimp odor	Complies
Viscosity	Brookfield 021, 1.5 rpm, 25°C	< 1,500 cP
Humidity	A.O.A.C.984.20	< 0.9%
	Identification	
Peroxide value	A.O.A.C.965.33	< 0.2 mEq peroxide/kg
P-Anisidine Index	A.O.C.S Cd 18-90 (modified)	< 1.2
Saponification value	A.O.A.C.920.160	> 165.0 mg KOH/g
Iodine Value	A.O.C.S Ja 14-91	>130.0 g I_2/100 g
	Assay	
Total Protein	BCA Colorimetric method	0.5 – 3.5 g/100g
Total Cholesterol	A.O.A.C. 976.26 (modified)	0.8 – 1.2 g/100g
Total Phospholipids	A.O.C.S. Ja7-86	> 40.0 g/100g
Total Lipids as Fatty Acids	A.O.A.C.996.06	> 73.0 g/100 g
Saturated fatty acids	A.O.A.C.996.06	< 30.0 g/100 g
Monounsaturated fatty acids	A.O.A.C.996.06	> 12.0 g/100 g
Polyunsaturated fatty acids	A.O.A.C.996.06	> 32.0 g/100 g
Omega-3 Fatty Acids	A.O.A.C.991.39, 963.22, 996.06	> 30.0 g/100g
EPA	A.O.A.C.991.39, 963.22, 996.06	> 15.0 g/100g
DHA	A.O.A.C.991.39, 963.22, 996.06	> 9.0 g/100g
DPA[a]	A.O.A.C.991.39, 963.22, 996.06	> 0.4 g/100 g
Omega –6 Fatty Acids	A.O.A.C.991.39, 963.22, 996.06	1.5 – 2.5 g/100g
Linoleic acid	A.O.A.C.991.39, 963.22, 996.06	> 1.3 g/100 g
Omega-9 Fatty Acids	A.O.A.C.991.39, 963.22, 996.06	> 6.0 g/100g
Oleic acid	A.O.A.C.991.39, 963.22, 996.06	> 5.0 g/100 g
Total *Trans* Fat	A.O.A.C. 996.06	< 0.1 g/100 g
Vitamin A	J. Sci. Agri. Vol 29 p697-702	> 100.0 IU/g
Vitamin E	J. Sci. Agri. Vol 29 p697-702	> 0.5 IU/g
Esterified Astaxanthin	HPLC	> 150.0 mg/100 g

[a] Docosapentaenoic acid.

TABLE 2. BATCH ANALYSES OF THREE LOTS OF NKO™

Assessing the Compositional Guidelines for NKO™

Requirement	Specifications	Lot Number		
		060116	060519	060224
Description				
Appearance	Red opaque oil	Complies	Complies	Complies
Odor	Light shrimp odor	Complies	Complies	Complies
Viscosity	< 1,500 cP	564.0	476.5	585.8
Humidity	<0.9%	0.1%	0.2%	0.1%
Identification				
Peroxide value	< 0.2 mEq peroxide/kg	< 0.1	< 0.1	< 0.1
P-Anisidine	< 1.2	1.0	0.9	0.9
Saponification value	≥ 165.0 mg KOH/g	174.6	170.8	177.6
Iodine Value	>130.0 gI_2/100 g	137.9	135.6	134.3
Assay				
Total Protein	0.5 – 3.5 g/100g	3.0 g/100 g	0.7 g/100 g	0.8 g/100 g
Total Cholesterol	0.8 – 1.2 g/100g	1.1 g/100 g	1.0 g/100 g	0.9 g/100 g
Total Phospholipids	≥ 40.0 g/100g	47.3 g/100 g	44.8 g/100 g	43.8 g/100 g
Total Lipids as Fatty Acids	≥ 73.0 g/100 g	88.5 g/100 g	81.2 g/100 g	88.2 g/100 g
Saturated fatty acids	< 30.0 g/100 g	29.8 g/100 g	29.2 g/100 g	28.9 g/100 g
Monounsaturated fatty acids	≥ 12.0 g/100 g	22.1 g/100 g	21.6 g/100 g	21.9 g/100 g
Polyunsaturated fatty acids	≥ 32.0 g/100 g	36.7 g/100 g	34.2 g/100 g	37.4 g/100 g
Omega-3 Fatty Acids	≥ 30.0 g/100g	33.6 g/100 g	31.5 g/100 g	34.3 g/100 g
EPA	>15.0 g/100g	17.2 g/100 g	16.6 g/100 g	18.0 g/100 g
DHA	> 9.0 g/100g	11.3 g/100 g	10.3 g/100 g	11.4 g/100 g
DPA	>0.4 g/100 g	0.6 g/100 g	0.5 g/100 g	0.6 g/100 g
Omega –6 Fatty Acids	1.5 – 2.5 g/100g	1.6 g/100 g	1.4 g/100 g	1.6 g/100 g
Linoleic acid	> 1.3 g/100 g	1.4 g/100 g	1.2 g/100 g	1.3 g/100 g
Omega-9 Fatty Acids	≥ 6.0 g/100g	9.6 g/100 g	9.3 g/100 g	9.4 g/100 g
Oleic acid	≥ 5.0 g/100 g	8.6 g/100 g	8.5 g/100 g	8.2 g/100 g
Total *Trans* Fat	< 0.1 g/100 g	< 0.01 g/100 g	0.02 g/100 g	Not detected
Vitamin A	≥ 100.0 IU/g	259.1 IU/g	336.6 IU/g	269.9 IU/g
Vitamin E	≥ 0.5 IU/g	0.65 IU/g	0.67 IU/g	0.70 IU/g
Esterified Astaxanthin	≥ 150.0 mg/100 g	162.6 mg/100 g	160.7 mg/100 g	154.0 mg/100 g

5. Purity of NKO™

a. Chemical Characterization and Batch Analyses of NKO™

The potential impurities and incidental constituents present in NKO™ arise largely from environmental exposure of the Antarctic Krill. Neptune routinely analyzes production lots of NKO™ for the presence of 17 pesticides, as well as dioxins, PCBs, heavy metals, fluorine, and various microorganisms. The incidental constituents for which NKO™ are analyzed, as well as the methods of analysis and associated specifications are presented in Table 3. Results from the batch analyses of NKO™ for solvent residues and incidental metal and pesticide constituents are presented in Tables 4 and 5, respectively.

C. Microbiological Characterization of NKO™

The Neptune production method is internally controlled under the Hazard Analysis Critical Control Points (HACCP) system. Under this system, Neptune has identified potential points for contamination or corruption of NKO™. To ensure the quality of the final NKO™ product, production lots are consistently tested to ensure compliance with international standards addressing residual solvents, microorganisms, heavy metals, inorganic arsenic, and pesticides. The microorganisms for which NKO™ is analyzed, as well as the methods of analysis and associated specifications, are presented in Table 6. Results of the batch analyses of NKO™ for the presence of microorganisms are presented in Table 7.

D. Stability of NKO™

Three tests have been conducted to ensure the stability of NKO™ over time. The peroxide values of NKO™ were measured, and the results indicate the extent of oxidation with oils, the Oil Stability Index (OSI), and the Oxygen Radical Absorption Capacity (ORAC).

In the first test, the peroxide value of NKO™ was determined by the A.O.C.S Cd.8b.90 method to be 0.05 mEq peroxide/kg, indicating that it does not have a high rancidity potential.

The second test conducted on NKO™ by Neptune examined the OSI which is a measure of the resistance to oxidation and high temperatures over time. In this test, air heated to 97.8°C was passed through a sample of NKO™ for a period of 50 hours, during which the peroxide values of NKO™ were measured at regular intervals. The results indicate that NKO™ maintains a peroxide value of <0.1 mEq peroxide/kg for over 50 hours at a temperature of 97.8°C, thereby confirming lack of appreciable rancidity potential.

TABLE 3. INCIDENTAL CONSTITUENTS OF NKO™

Requirement	Methods	Specification
Solvent Residues		
Acetone	GC	< 10 mg/kg
Incidental Metals and Non-metals		
Arsenic (inorganic)	Hydride Generation Atomic Absorption	< 0.1 mg/kg
Cadmium	A.O.A.C.986.15, 971.21, 999.11[a]	< 0.1 mg/kg
Mercury	A.O.A.C.986.15, 971.21, 999.11	< 0.1 mg/kg
Lead	A.O.A.C.986.15, 971.21, 999.11	< 0.1 mg/kg
Copper	ICP[b]	< 5 mg/kg
Tin	ICP	< 10 mg/kg
Antimony	ICP	< 1 mg/kg
Pesticide Residues (including agricultural and veterinary substances)		
Aldrin	A.O.A.C.970.52, US EPA 8081	< 0.1 mg/kg
Dieldrin	A.O.A.C.970.52, US EPA 8081	< 0.1 mg/kg
Chlordane (alpha and gamma)	A.O.A.C.970.52, US EPA 8081	< 0.05 mg/kg
Sum of DDT	A.O.A.C.970.52, US EPA 8081	< 0.5 mg/kg
Endrin	A.O.A.C.970.52, US EPA 8081	< 0.05 mg/kg
Heptachlor	A.O.A.C.970.52, US EPA 8081	< 0.1 mg/kg
Toxaphene	A.O.A.C.970.52, US EPA 8081	< 0.01 mg/kg
Hexachlorobenzene	A.O.A.C.970.52, US EPA 8081	<0.1 mg/kg
Alpha-HCH	A.O.A.C.970.52, US EPA 8081	< 0.1 mg/kg
Beta-HCH	A.O.A.C.970.52, US EPA 8081	< 0.05 mg/kg
Lindane	A.O.A.C.970.52, US EPA 8081	< 1 mg/kg
Other Organic or Inorganic Impurities or Toxins		
Benzo(a)pyrene	US EPA 610, 8310, and 8100	< 2.0 ppb
Sum of PCDD/PCDF	US EPA Method 1613 revision B	< 2.0 pg/g fat WHO-PCDD/F-TEQ/ g fat
Sum of PCDF/F/PCBs	US EPA Method 1613 revision B	< 10.0 pg/g fat WHO-PCDD/F-TEQ/ g fat
Microbial Specifications		
Total aerobic bacteria	MFHPB-33	10 CFU/g
Total coliforms and *E. coli*	MFHPB-34	10 CFU/g
Yeasts and Moulds	MFHPB-32	10 CFU/g
Staphylococcus aureus	MFLP-21	10 CFU/g
Listeria monocytogenes	MFHPB-30	Not detected/25 g
Pseudomonas aeruginosa	CFC agar[c]	Not detected/25 g
Salmonella spp.	MFHPB-20-MFO-11	Not detected/25 g

[a] A.O.A.C. Official Methods of Analysis, 18th Ed. Revision 1, 2006.
[b] Inductively coupled plasma mass spectrometry.
[c] A pseudomonas selective agar.

TABLE 4. BATCH ANALYSES OF 4 LOTS OF NKO™ FOR THE PRESENCE OF SOLVENT RESIDUES

Requirement	Specification	Lot Number			
		050201	050223	050610	050909
Solvent Residues					
Acetone	< 10 mg/kg	7.0	9.1	1.7	<1

TABLE 5. BATCH ANALYSES OF 3 LOTS OF NKO™ FOR THE PRESENCE OF INCIDENTAL METAL AND NON-METAL CONSTITUENTS, PESTICIDE RESIDUES AND ORGANIC OR NON-ORGANIC IMPURITIES

Requirement	Specification	Lot Number		
		060116	060519	060224
Incidental Metals and Non-metals				
Arsenic (inorganic)	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Cadmium	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Mercury	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Lead	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Copper	< 5 mg/kg	< 0.3 mg/kg	< 0.3 mg/kg	< 0.3 mg/kg
Tin	< 10 mg/kg	< 0.7 mg/kg	< 0.7 mg/kg	< 0.7 mg/kg
Antimony	< 1 mg/kg	< 0.3 mg/kg	< 0.3 mg/kg	< 0.3 mg/kg
Pesticide Residues (including agricultural and veterinary substances)				
Aldrin	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Dieldrin	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Chlordane (alpha and gamma)	< 0.05 mg/kg	< 0.05 mg/kg	< 0.05 mg/kg	< 0.05 mg/kg
Sum of DDT	< 0.5 mg/kg	< 0.5 mg/kg	< 0.5 mg/kg	< 0.5 mg/kg
Endrin	< 0.05 mg/kg	< 0.05 mg/kg	< 0.05 mg/kg	< 0.05 mg/kg
Heptachlor	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Toxaphene	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg
Hexachlorobenzene	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Alpha-HCH	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Beta-HCH	< 0.05 mg/kg	< 0.05 mg/kg	< 0.05 mg/kg	< 0.05 mg/kg
Lindane	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg
Other Organic or Inorganic Impurities or Toxins				
Benzo(a)pyrene	< 2.0 ppb	< 2.0 ppb	< 2.0 ppb	< 2.0 ppb
Sum of PCDD/PCDF	< 2.0 I-TEQ pg/g	< 2.0 I-TEQ pg/g	< 2.0 I-TEQ pg/g	< 2.0 I-TEQ pg/g
Sum of PCDF/F/PCBs	< 10.0 I-TEQ pg/g	< 10.0 I-TEQ pg/g	< 10.0 I-TEQ pg/g	< 10.0 I-TEQ pg/g

TABLE 6. MICROBIOLOGICAL SPECIFICATIONS FOR NKO™

Requirement	Methods	Specification
Total aerobic bacteria	MFHPB-33	< 1000 CFU/g
Total coliforms	MFHPB-34	< 10 CFU/g
Escherichia coli	USP 27[a]	Negative/25 g
Yeasts and Molds	MFHPB-32	< 100 CFU/g
Staphylococcus aureus	MFLP-21	< 10 CFU/g
Listeria monocytogenes	MFHPB-30	Negative/25 g
Pseudomonas aeruginosa	Oxoid-CFC agar	Negative/25 g
Salmonella spp.	MFHPB-20-MFO-11	Negative/25 g

[a] United States Pharmacopeia, 27th Edition.

TABLE 7. BATCH ANALYSIS OF 3 LOTS OF NKO™ FOR COMPLIANCE WITH MICROBIOLOGICAL SPECIFICATIONS

Requirement	Specification	Lot Number		
		050827	050503	050729
Total aerobic bacteria	< 1000 CFU/g	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g
Total coliforms	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g
Escherichia coli	Negative/25 g	Not Detected	Not Detected	Not Detected
Yeasts and Molds	< 100 CFU/g	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g
Staphylococcus aureus	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g
Listeria monocytogenes	Negative/25 g	Not Detected	Not Detected	Not Detected
Pseudomonas aeruginosa	Negative/25 g	Not Detected	Not Detected	Not Detected
Salmonella spp.	Negative/25 g	Not Detected	Not Detected	Not Detected

The ORAC value for an oil is an indicator of the oil's antioxidant capacity against the formation of peroxyl and hydroxyl radicals. The presence of peroxyl and hydroxyl radicals increases the likelihood of lipid oxidation and associated oxidative rancidity. ORAC analyses involve the addition of a fluorescent probe, which measures the scavenging capacity of the antioxidants contained in the oil. The results reported were compared to those of Trolox®, a water-soluble vitamin E analog which serves as a calibration standard. The ORAC of NKO™ was reported to be 378 µmol Trolox® equivalent/g, an ORAC value that is more than 300-fold greater than that of commercially availably vitamin A and/or vitamin E preparations while also exhibiting more than 48-fold greater capacity than a commercial omega-3 18:12 fish oil that is available in North America. These results indicate that NKO™ is a potent free radical scavenger indicating the antioxidant potential of the oil.

Additionally, long-term stability testing revealed that there is no significant change in the composition of NKO™ following storage at 25°C over a period of 28 months, with an ambient relative humidity of 60%. The results of this stability test are presented in Table 8.

TABLE 8. STABILITY TESTING CONDUCTED WITH NKO™

STABILITY TESTING CONDUCTED WITH NKO™		
Parameters	**Specification**	**Results after 28 Months**
		Bulk Oil (030327)
Omega-3	>30.0 g/100g	33.4 g/100g
EPA	>15.0 g/100g	17.5 g/100g
DHA	>9.0 g/100g	12.7 g/100g
Phospholipids	42.4 mmol/ 100 g	53.5 mmol/ 100 g
Peroxide Value	< 0.2 mEq/kg	0.0 mEq/kg

E. Fatty Acid Composition of NKO™

As an oil extracted directly from Antarctic krill, NKO™ is composed primarily of triglycerides and phospholipids. These lipids contain a wide variety of naturally occurring fatty acids as their side chains, the most prominent of which are EPA and DHA. The fatty acid compositions of multiple production batches of NKO™ were examined by Neptune and the mean values, with standard deviations, are presented in Table 9.

F. Similarity of Krill Oil to Other Fish Oils

Several forms of fish oil have GRAS status as acknowledged by FDA. Menhaden oil has been affirmed as GRAS.[3] In addition, FDA has not objected to GRAS notifications submitted on tuna oil (FDA, 2002b), 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, 2002b). Table 10 captures the values used by FDA and compares them to those of krill oil.

Additional information from the scientific literature on the distribution of fatty acids in several fish oils is shown in Table 11 and is compared to the Neptune data on krill oil. Krill oil contains a high level of the desirable n-3 unsaturated fatty acids that is comparable to that of salmon oil. Its ratio of n-3 to n-6 fatty acids is comparable to the other fish oils in this group.

G. Natural Occurrence of Vaccenic Acid in Food Sources

Vaccenic acid is one of the major fatty acids present in krill oil. The vaccenic acid in krill oil is in the cis- isomeric form as indicated by the low value of trans-fatty acids found in the oil (see Table 2). Vaccenic acid constitutes the majority of the monounsaturated fatty acids present in krill oil. Vaccenic acid is found in various fish oils in both the cis- and trans- forms at levels of

[3] See 21 CFR 184.1472.

2-4% and can also be found in a number of other fats and oils. Table 12 lists the vaccenic acid concentrations in several widely used vegetable oils as found in two references, although it is noted that neither author indicated whether the vaccenic acid was in the cis- or trans-form. These oils are reported to be used in a wide variety of food applications.

TABLE 9. FATTY ACID CONTENT OF NKO™

Fatty Acid	Fatty Acid Content Of NKO™ Lots (%of total lipid)				Mean Fatty Acid Content (Standard Deviation)
	050117	050201	050223	050909	
C12 :0 (lauric)	0.19	0.18	0.20	0.25	0.20 (0.03)
C14 :0 (myristic)	10.51	12.07	11.29	11.12	11.37 (0.57)
C14 :1 (myristoleic)	0.19	0.27	0.17	0.37	0.22 (0.05)
C15 :0 (pentadecanoic)	0.51	0.46	0.51	0.45	0.43 (0.06)
C16 :0 (palmitic)	21.58	23.4	22.14	20.34	22.17 (1.04)
C16 :1 (palmitoleic)	6.58	8.94	7.37	5.34	7.46 (1.40)
C18 :0 (stearic)	1.25	1.31	1.35	0.83	1.27 (0.32)
C18 :1 n-7 (vaccenic)	10.34	10.61	11.14	7.34	10.61 (1.18)
C18 :1 n-9 (oleic)	8.14	9.07	7.33	9.39	8.54 (0.69)
C18 :2 n-6 (linoleic)	2.04	1.57	1.94	2.22	1.73 (0.22)
C18 :3 n-3 (gamma-linoleic)	0.17	0.21	0.2	0.17	0.18 (0.03)
C18 :3 n-6 (alpha-linoleic)	1.59	1.1	1.43	2.47	1.31 (0.60)
C18 :4 n-3 (stearidonic)	4.27	2.39	3.72	6.53	3.57 (1.65)
C20 :1 (eicosenic)	0.63	0.82	0.75	0.47	0.77 (0.12)
C20 :4 n-3 (eicosatetranoic)	0.15	0.04	0.08	0.08	0.08 (0.05)
C20 :4 n-6 (arachidonic)	0.37	0.24	0.37	0.43	0.33 (0.10)
C20 :5 n-3 (eicosapentaenoic acid, EPA)	17.12	16.03	16.43	17.35	16.71 (0.65)
C22 :1 n-9 (erucic)	0.42	0.24	0.54	0.38	0.47 (0.10)
C21 :5 n-3 (heneicosapentaenoic)	0.47	0.41	0.48	0.53	0.47 (0.06)
C22 :5 n-3 (docosapentaenoic acid, DPA)	0.46	0.39	0.46	0.45	0.42 (0.08)
C22 :6 n-3 (docosahexaenoic acid, DHA)	10.53	7.94	9.70	11.97	9.50 (1.54)

TABLE 10. FATTY ACID COMPOSITION OF KRILL OIL[a] COMPARED TO TUNA OIL AND MENHADEN OIL[b] (g/100 g)

Fatty Acid	Krill Oil	Tuna Oil	Menhaden Oil
14:0	11.4	3.0	9.0
16:0	20.3	20.0	19.0
18:0	1.3	6.0	3.0
16:1	5.3	4.5	12.0
18:1	8.5	15.0	13.0
22:1	0.4	1.0	-
18:2	1.7	1.5	1.0
18:3	2.6	1.0	1.0
20:5 (EPA)	16.7	6.0	14.0
22:6 (DHA)	9.5	26.5	8.0

[a] Values for krill oil taken from Table 9.
[b] Values for tuna and menhaden oils taken from FDA response to GRN 000109 (FDA, 2002b).

TABLE 11. COMPARISON OF FATTY ACID CONTENTS OF VARIOUS FISH OILS (PERCENTAGES)

Fatty Acid	Anchovy[a] Oil	Menhaden[a] Oil	Salmon[a] Oil	Krill Oil
C14:0	7.4	7.3	3.7	11.4
C16:0	17.4	19.0	10.2	22.2
C18:0	4.0	4.2	4.7	1.3
C16:1	10.5	9.0	8.7	7.5
C18:1	11.6	13.2	18.6	19.1
C18:2 n-6	1.2	1.3	1.2	1.7
C18:3 n-3	0.8	0.3	0.6	0.2
C18:4 n-3	3.0	2.8	2.1	6.5
C20:1	1.6	2.0	8.4	0.8
C20:4 n-6	0.1	0.2	0.9	0.4
C20:5 n-3	1.6	11.0	12.0	16.7
C22:1 n-9	1.2	0.6	5.5	0.5
C22:5 n-3	1.6	1.9	2.9	0.5
C22:6 n-3	8.8	9.1	13.8	9.5
Σ n-6	1.3	1.5	2.1	2.1
Σ n-3	15.8	25.1	30.9	33.4
n-3:n-6	12.2	16.7	14.7	15.9

[a] From Lucas et al., 2005.

TABLE 12. VACCENIC ACID CONCENTRATION (% FATTY ACIDS) IN VARIOUS FATS AND OILS

Fat or Oil	% Vaccenic Acid[a]	% Vaccenic Acid[b]
Rapeseed (HEAR)	Not Determined	3.1
Canola	2.6	3.1
Lard	Not Determined	3.1
Olive	1.9	3.1
Soybean	1.4	1.5
Sesame	Not Determined	1.1
Corn	Not Determined	0.8
Palm	Not Determined	0.8
Peanut	Not Determined	0.6
Beef Tallow	Not Determined	0.6
Coconut	Not Determined	0.3
Sunflower	Not Determined	0.3

[a] From Wasowicz et., al., 1976
[b] From Sauer et al., 1997

Vaccenic acid and other isomers of 18:1 fatty acids occur naturally in milk fats and have been found to be protective against cancers in animal and *in vitro* models, with some supporting epidemiological evidence in humans (Doyle et al., 2005). The vaccenic acid present in dairy products is in the trans isomeric form. Corl et al. (2003) showed that the level of vaccenic acid and other trans-18:1 isomers in butter can be increased by varying the diets of the producing cows. The levels of trans-18:1 and other C18 fatty acids in butter from cows on a conventional corn ration versus a corn ration supplemented with 2% sunflower oil and 1% fish oil is shown in Table 13.

Table 13. DISTRIBUTION OF C18 FATTY ACIDS IN BUTTER[c]

Fatty Acid	Corn Ration	Supplemented Ration
18:0	11.85	5.09
18:1, *trans*-6-8	0.46	1.21
18:1, *trans*-9	0.36	0.84
18:1, *trans*-10	0.67	2.68
18:1, *trans*-11	1.30	16.28
18:1, *trans*-12	0.77	2.48
18:1, *cis*-9	25.35	14.23
18:2, *cis*-9, *cis*-12	3.33	2.93
18:2 *cis*-9, *trans*-11	0.51	3.76
18:3 *cis*-9, *cis*-12, *cis*-15	0.41	0.46

[c] From Corl et al., 2003

III. INTENDED DIETARY USE

A. Current Usage of NKO™ in the Diet

Krill oil is not presently added to foods and is not naturally present in foods consumed in the US. Neptune reported that krill has been used in human food since the 1970s, principally in Japan, Russia, Ukraine, and, more recently, France.

Neptune submitted a notification to FDA in 2002 for use of krill oil in dietary supplements. FDA did not object to this use (FDA, 2002a). The supplement is sold in 300 and 500 mg capsules with a recommended dose of 1 - 2 capsules per day.

Softgels containing the NKO™ have been available as dietary supplements in North America for several years; in Canada and the US, NKO™ has been commercially available since October of 2002, and in Japan, Korea, Singapore, and Hong Kong, NKO™ has been available since December of 2004. A total of 120,000 kg of Neptune krill oil, which is equivalent to 60,000,000 individual 500 mg softgel capsules, has been ingested by consumers. Throughout this time no serious adverse effects have been reported.

B. Intended Uses

Neptune intends to incorporate krill oil into the various food categories as listed in Table 14. The krill oil would function as a nutrient supplement as defined in 21 CFR 170.3(o)(20). Neptune research has indicated that use levels are self-limiting due to the strong taste which begins to be detected at levels of 300 - 500 mg per serving, depending on the food type. It should be noted that krill oil is intended to be used as a substitute or alternative to fish oil.

TABLE 14. PROPOSED FOOD USES FOR NKO™

Food Category	Use Levels Per Serving
Breakfast cereals	300 mg
Cheeses	300 mg
Fruit juices	150-250 mg
Milk products	300-500 mg
Medical foods	300-500 mg

C. Estimated Daily Intake

The estimated daily intake of NKO™ from proposed food uses above was calculated according to FDA guidelines (FDA, 1995) using serving size data and the mean (50th percentile) consumption of each food type of interest from the CSFII 1994-96 Survey (USDA,

2005). According to FDA guidelines, a level twice the mean consumption was calculated to estimate use by a high consumer, i. e., at the 90th percentile consumption level. This analysis is summarized in Table 15.

TABLE 15. DIETARY INTAKE CALCULATIONS

Food Category (Use Level per serving)	Food Subcategory	Approximate Serving Size	Food Intake (g/p/d) 50%-tile	NKO Intake [a] (mg/p/d) 50%-tile	NKO Intake (mg/p/d) 50%-tile X2
Beverages, Nonalcoholic (150-250 mg)	Fruit Drinks	Eight ounces = 248 g	360	218-363	436-726
Breakfast Cereals (300 mg)	Cooked Cereal	½ cup of cooked Oatmeal = 117 g	233	597	1195
	Ready-to-Eat Cereal	1 cup of corn flakes = 25 g	48	576	1152
Cheeses (300 mg)	Total Cheese Other than Cream or Cottage	One and a half ounces of cheese = 43 g	26	180	360
	Total Cottage Cheese	One-half cup of cottage cheese = 105 g	50	144	288
Frozen Dairy Desserts (300 - 500 mg)	Ice Cream, Ice Milk	One-half cup of hard ice cream = 67 g	132	591-985	1182-1970
Milk, Whole & Skim (300 - 500 mg)	Total Milk	One cup of fluid whole milk = 244 g	216	267-445	534-890
Milk Products (300 - 500 mg)	Sour Cream	One tablespoon of sour cream = 14 g	6	129-214	258-428
	Creams	One tablespoon of cream = 15 g	3	60-100	120-200
	Yogurt[b]	No data in USDA Survey	0.17 servings	50-85	100-170
Processed Fruits/Fruit Juices (150 - 250 mg)	Total Orange Juice	Six fluid ounces of orange juice = 187 g	186	148-248	297-496
	Total Lemon Juice	One fluid ounce of lemon juice = 30 g	<0.05	0.00	0.00
	Total Apple Juice	Six fluid ounces of apple juice = 186 g	150	122-203	244-406
Medical Foods (300 - 500 mg)[b]		No data in USDA survey			
Sum of entire column				**3082-4140**	**6164-8280**

[a] Dietary intake of NKO™ for each food type is calculated by multiplying mg/serving by grams of food consumed divided by grams of food per serving.
[b] 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.
[c] 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 900-1500 mg/day.

The analysis above is a point estimate as described in FDA guidelines and is recognized as overestimating consumption. With the exaggerated daily exposure of 8.3 g/person/day it should be noted that at a combined level of 26% of total EPA and DHA, the maximum daily consumption of EPA and DHA would be 2.2 g/person/day.

In addition, the maximum estimated consumption of esterified astaxanthin, which is present in krill oil at 150 mg %, would be 12 mg/person/day.

The intended food uses for NKO™ are also within the allowances FDA has accepted for the GRAS status use of menhaden oil. As discussed in Section IV.A, the acceptable menhaden oil food use does not exceed safe levels of consumption for total EPA and DHA.[4]

[4] See 21 CFR 184.1472 (a)(3).

IV. Safety Data for NKO™

A. Review of Safety Data

The composition of krill oil is very similar to several fish oils that are considered to be GRAS for use in human foods. The safety reviews conducted by FDA on various fish oils yield the conclusion that these oils can be safely used in human foods if the levels of the fatty acids, DHA and EPA, in the total diet are less than 3 g/person/day. This compositional comparison constitutes the fundamental basis on which the safety of krill oil is established. More details on FDA's safety review of fish oils can be found in FDA's GRAS affirmation of menhaden oil as discussed in the June 5, 1997 final rule (62 FR 30751) and the GRAS notification letters of no objection issued by FDA on tuna oil (FDA, 2002b), salmon oil (FDA 2004a), and anchovy oil (FDA, 2004b). The safety of krill oil is considered further in Section V.B.1 in light of these FDA actions.

The compositional analyses of NKO™ compared with similar fish oils are supported by three animal toxicology studies and four clinical studies on krill oil, which warrant consideration, and these investigations are summarized in Sections IV.B and IV.C.

NKO™ contains approximately 150 mg % of astaxanthin esters. The data on astaxanthin and astaxanthin esters used to support safety for its use in color additives and in dietary supplements have been reviewed as part of the evaluation of NKO™'s safety profile. The astaxanthin studies included subchronic and reproductive studies in animals and mutagenicity tests. More details on the astaxanthin investigations found in the scientific literature, most notably two clinical studies with human volunteers, are summarized in Appendix D.

B. Animal Toxicology Studies on Krill Oil

Ruggiero-Lopez et al. (1994) conducted a rat study to assess the nutritional efficacy of krill oil in (unspecified) rats compared to fish oil and corn oil. Krill oil, as well as fish oil and corn oil, was well tolerated by pups at a level of 10% of the diet over a 3-day period immediately following weaning. The authors noted the absence of adverse effects.

Neptune conducted a repeat-dose toxicity study to examine the safety of dietary consumption of NKO™ in mice over a period of 6 months (Neptune, 2002a). In the study, 96 C57BL6 Nude Congenic mice (B6NU-T heterozygotes) were kept in a controlled environment and administered diets in which NKO™ represented 16.6% of their daily dietary intake, equivalent to approximately 28.3 g NKO™/kg body weight/day (Blackburn, 1988). This strain of mice was selected as a prelude to the study described next which examined the ability of krill oil to prevent skin cancer. All of the animals were examined weekly by a certified veterinarian, and, at the conclusion of the experimental period, all animals were euthanized by gas exposure and subject to histopathological examinations. No adverse effects were noted in the clinical observations over the course of the experimental period. No histopathological abnormalities were observed in any of the organs examined, which included the brain, lungs, heart, stomach, pancreas, liver, kidneys, uterus or prostate, intestines, and the skin.

No traditional carcinogenicity studies employing NKO™ were identified; however, no evidence of carcinogenicity was observed in a trial conducted to examine the effect of NKO™ on the development of UVB-Radiation Induced Skin Cancer (Neptune, 2002b). In the trial C57BL6 Nude Congenic mice (B6NU-T heterozygotes), a species known to be more susceptible to skin cancer, were randomized into 2 groups, each containing 48 male and female mice. The first group was administered either oral, topical, or oral and topical treatments or NKO™, while the second group was administered Soya oil by the same dosing regimes. In the oral dosing regime, mice were administered diets in which 10% of the daily dietary intake consisted of NKO™ or Soya oil, equivalent to 17.1 g/kg body weight/day (Blackburn, 1988). In the topical treatment regime, NKO™ or Soya oil was applied to the skin of the mice. The mice were observed for a period of 20 weeks, during which time they were exposed to 30 minutes of UVB radiation, at a distance of 30 cm, daily. After 20 weeks, all animals were euthanized and subjected to histological examinations.

In mice consuming diets containing NKO™ alone, the incidence of cancers and pre-malignant tumors was 18.8% (3 of 16 animals) as compared to 37.5% (6 of 16 animals) and 18.8% (3 of 16 animals), respectively, in the soy oil group. In mice administered topical treatments of NKO™ alone, the incidence of cancers and pre-malignant tumors was 12.5% (2 of 16 animals) and 31.3% (5 of 16 animals), respectively, as compared to 37.5% (6 of 16 animals) and 31.3% (5 of 16 animals), respectively, in the soy oil group. In mice receiving both oral and topical treatment, the incidence of cancers and pre-malignant tumors was reported to be 18.8% (3 of 16 animals) and 31.3% (5 of 16 animals), respectively, in the NKO™ group and 37.5% (6 of 16 animals) and 12.5% (2 of 16 animals), respectively, in the soy oil group. Overall, a statistically significant reduction in the incidence of cancer was observed in the mice receiving NKO™ as compared to those in the soy oil group.

C. Clinical Studies on Krill Oil

Neptune has conducted several clinical trials employing NKO™ including one trial designed to examine the safety of NKO™ consumption. In the trial examining the safety of NKO™, 25 healthy male and female volunteers between the ages of 25 and 53 years were recruited (Neptune, 2002a). All subjects were advised to consume 2 NKO™ gelcaps, 3 times daily for a period of 2 months. Each gelcap contained 1 g of NKO™ and provided 386 mg of omega-3 fatty acids, 416 mg of phospholipids, and 0.16 mg of astaxanthin. Complete blood counts and biochemical blood tests, medical histories and vital signs were collected at baseline, 1 month, and 2 months; the subjects were questioned about any occurrence of adverse effects, and particularly effects related to regurgitation of the capsules. The 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. Additionally, subjects were advised to stop consuming NKO™ if they observed any of the following symptoms: low blood pressure, high blood pressure, difficulty breathing, bleeding, loss of consciousness, unusual migraines or body pain, weight gain, or significant alterations in blood test results.

No serious side effects were observed in the subjects consuming 6 g NKO™/day throughout the experimental period. None of the subjects reported any regurgitation or unpleasant aftertaste associated with the consumption of the NKO™ capsules. Of the 25 subjects, 3 withdrew from the trial for reasons associated with the consumption of NKO™. One female patient withdrew from the study due to a known salt intolerance, for whom the consumption of

NKO™ resulted in a moderate increase in water retention. Two female patients withdrew from the study and stated that they observed a rapidly increasing greasiness of their facial skin, which was attributed to NKO™ consumption. Neither of these reasons for withdrawal was considered to constitute a serious adverse effect. In the remaining 22 subjects, no noticeable physical or biochemical changes were observed over the course of the 2-month experimental period. Significant decreases in serum total cholesterol, triglycerides, LDL cholesterol, the ratio of total cholesterol to HDL cholesterol, albumin, and amylase were observed, as was a significant increase in HDL cholesterol. These treatment-related effects were viewed as beneficial changes in blood lipids and pancreatic function and were not considered to be adverse effects. While decreased albumin levels may be indicative of underlying disease processes, their occurrence in the absence of other biochemical abnormalities suggested that they were not indicative of any adverse effects.

In a trial designed to examine the effect of NKO™ on the clinical course of hyperlipidemia, 120 patients with a mean age of 51 years were randomized into 4 groups; these groups were further subdivided according to their body mass indices (BMI) (Bunea et al., 2004). Individuals in Group A were administered either 2 g NKO™/day (BMI<30) or 3 g NKO™/day (BMI> 30) and individuals in Group B were administered either 1 or 1.5 g/day (BMI of <30, and >30, respectively). In Group C, the subjects were administered a fish oil capsule providing 180 mg EPA and 120 g DHA, while Group D was the placebo group. All individuals consumed their treatments for a period of 12 weeks, with Group B followed for an additional 90 days while consuming 500 mg NKO™/day. No adverse effects resulting from the consumption of NKO™, fish oil, or the placebo were reported. The authors did observe that the consumption of NKO™ was associated with beneficial changes in blood lipids.

The effects of NKO™ on the management of premenstrual syndrome (PMS) and dysmenorrhoea were examined in a clinical trial conducted by Sampalis et al. (2003). In this trial, 70 female adults of reproductive age were randomized to receive either NKO™ or fish oil. During the first month of the trial, the subjects consumed two 1 g capsules once per day with meals. During the second and third months of the trial, the subjects consumed two 1 g capsules once daily for 8 days prior to menstruation and 2 days during menstruation. No serious adverse effects were reported by the subjects during the duration of the trial. A reduction in the duration of the menstrual cycle was reported by 3 subjects during the first month of treatment; however, this was not reported during the second and third months when the dose regime decreased to 10 days/month. Minor increases in the oiliness of the facial skin were reported by individuals in the NKO™ treatment group. None of the subjects consuming NKO™ reported gastrointestinal disturbances, such as regurgitation; however, 64% of the women in the fish oil group reported "unpleasant reflux" following consumption of the fish oil capsules. Overall, the authors concluded that the NKO™ softgels were well tolerated and beneficially affected symptoms of PMS and dysmenorrhoea.

The effect of NKO™ on markers on chronic inflammation was examined in a clinical trial in which 90 subjects were recruited from primary care physicians and administered either 100 mg of a placebo or 300 mg NKO™/day (Deutsch, 2007). The subjects ranged in age from 50 to 68 years and were diagnosed with cardiovascular disease, rheumatoid arthritis, or osteoarthritis, and were reported to have C-reactive protein levels of >1.0 mg/dL. The patients were asked to refrain from consuming any pain medication, with the exception of acetaminophen. The patients were followed for a period of 30 days, during which time their C-reactive protein levels and pain and functional impairment scores were assessed on a weekly basis. No adverse effects associated with the consumption of NKO™ were reported.

A significant decrease in C-reactive protein levels was observed in subjects taking NKO™ after 7, 14, and 30 days as compared to baseline, while no decrease was observed in the placebo group. The scores for pain decreased significantly in the NKO™ group, and the decrease was significantly lower than that observed in the placebo group. Additionally, significantly larger reductions in stiffness and functional impairment scores were observed in the NKO™ group compared to the placebo group.

D. Toxicology of Vaccenic Acid

A standard literature search was conducted on the safety/toxicity of vaccenic acid. No evidence of any unusual nutritional problem or special toxicological issues were uncovered, nor were any anticipated since vaccenic acid is an isomer of oleic acid---a monounsaturated eighteen carbon fatty acid (18:1). Interestingly, vaccenic acid was specifically ruled out as a cause of the cardiopathology seen with rapeseed oil (Wasowicz et. al., 1976).

E. Potential Allergenicity

While NKO™ contains Antarctic krill lipids separated from Antarctic krill protein, its consumption by individuals with allergies to shellfish may trigger allergic reactions. NKO™ is contraindicated for individuals who are allergic to crustaceans, and NKO™ should be consumed with caution by individuals suffering from coagulopathy and those who take anticoagulants or other related medications.

Neptune contracted for ELISA Shellfish assays on 3 samples of NKO™. None of the samples contained any measurable shellfish protein at a limit of detection of 1 ppm. The report for this assay is attached as Appendix E.

V. DISCUSSION OF REVIEWED INFORMATION AND GRAS CRITERIA

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[5]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance, and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."[6]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following components:[7]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and

- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as the National Academy of Sciences.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu 1988; Renwick 1990).

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[5] See 21 CFR 170.3(i).
[6] See 21 CFR 170.30(a).
[7] See Footnote 1.

B. Analysis of Scientific Data on Krill Oil

The safety of NKO™ is predicated on multiple factors which include:

- The similarity of the composition of krill oil to other fish oils,
- The expected levels in the diet of EPA and DHA fatty acids, vaccenic acid, and astaxanthin forms from krill oil; and
- The safety of NKO™ itself as demonstrated in pre-clinical and clinical trials.

In addition, Antarctic krill has a history of human consumption as food, albeit somewhat limited, in Japan, Russia, the Ukraine, and France. However, dietary consumption of EPA and DHA has a substantial history, with several widely consumed foods, including fish and seafood, cod liver oil, other omega-3 PUFA-rich oils, and human milk, reported to be rich in these fatty acids.

1. Composition of Krill Oil and Similarity to Fish Oil

The Panel has reviewed the manufacturing procedure, food grade specifications and batch analyses for NKO™ and agrees that Neptune's manufacturing and analytical procedures provide ample documentation that the product is food grade with one caveat. The specifications provided by the supplier of acetone are inadequate to determine whether this solvent meets Food Chemicals Codex specifications. A GRAS conclusion for NKO™ is contingent on Neptune's confirming that its acetone supply used during its production of NKO™ complies with Food Chemicals Codex specifications.

The Panel reviewed the composition of four fish oils that have attained GRAS status: menhaden oil, salmon oil, sardine oil, and tuna oil as summarized in Tables 10 and 11. The predominant saturated fatty acids (myristic, palmitic and stearic acids) found in krill oil are identical to those found in these designated fish oils that have otherwise attained GRAS status. Krill oil is similar also with regard to total content of PUFA. The sum of the relative percentages of n-3 and n-6 PUFA in krill oil is close to that of salmon oil. The basis of the safety determination for fish oils was to limit uses so that combined total consumption of the fatty acids, EPA and DHA, does not exceed 3 g/p/d (FDA, 2002b, 2004a and 2004b). The Panel considers the fatty acid compositions of krill oil to be sufficiently similar to these fish oils, and agrees that the same consumption limitations placed on the related fish oils with their DHA and EPA levels should apply to krill oil.

2. Presence of DHA, EPA, and Other Dominant Components of Krill Oil

The Panel concurs that the maximum expected daily consumption of DHA and EPA from krill oil is 2.2 g/person, and this dietary exposure is within the ADI that was found to be acceptable by FDA for related fish oils. The Panel also agrees that consumers of high amounts of krill oil will likely preferentially consume it instead of fish oil and, therefore, consumption of DHA and EPA is not likely to exceed 3 g/person/day. Based on an ADI of 3 g/person/d for total consumption of DHA and EPA, and further recognizing at krill oil consists of 26% combined DHA and EPA content, the ADI for krill oil is 11.5 g/person/day.

The Panel also considered the presence of vaccenic acid and astaxanthin esters which are present in krill oil at higher levels than are found in other food sources.

Vaccenic acid comprises 10% of the fatty acids in krill oil. Vaccenic acid is an 18:1 fatty acid where the double bond occurs at the 7-position as opposed to the 9-position for the more common oleic acid. Lower levels of vaccenic acid are present in several vegetable and animal oils sources. Butter also contains large amounts of various isomers of 18:1 fatty acids in the trans configuration. These 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. Searches of the scientific literature have not identified any studies that indicate that dietary vaccenic acid poses any threat to human health. The Panel maintains that there is no toxicological concern for any monounsaturated fatty acid of 18 carbons (18:1) that exists in the *cis*- conformation.

Astaxanthin and astaxanthin esters occur in various food sources including salmon and marine shell fish. Astaxanthin is used as a dietary supplement in the US and Europe. The Panel also reviewed the safety data on astaxanthin and astaxanthin esters as listed in Appendix D and finds that the dietary levels of astaxanthin esters expected from the use of krill oil are safe.

3. Safety Studies on Krill Oil

In addition to the large amount of available data supporting the safety of DHA and EPA and the similarity of krill oil to fish oil, the safety of NKO™ is also supported by pre-clinical and clinical trials conducted by Neptune. In pre-clinical trials, no adverse effects were associated with the consumption of 28.3 g NKO™/kg body weight/day by C57BL6 Nude Congenic mice (Neptune, 2002a).

Of the four clinical studies reviewed, two were more significant with regard to dose and duration. In a clinical trial conducted to examine the safety of NKO™, no adverse effects were observed following the consumption of 6,000 mg NKO™/day for two months (Neptune, 2002a). In the second clinical study (Bunea et al., 2004) to examine health benefits of NKO™, participants tolerated 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. No adverse effects were observed.

In addition, krill oil is available as a dietary supplement where users can be expected to consume up to 4 g/day. This use has occurred without any reports of adverse effects.

The Panel agrees that the primary evidence for safety of krill oil is its compositional similarity to fish oil with supporting safety documentation on krill oil's primary components for the levels of consumption expected. The Panel notes that krill oil is intended as an alternative or substitute for fish oil. Consequently, dietary intake of total DHA and EPA from krill oil will be substitutional and will not be additive to that which would be ingested from fish oil. Therefore, the total daily exposure to combined DHA and EPA will remain within the ADI of 3 g/person/day as accepted by FDA.

4. Allergenicity

Despite the minimal amount of Antarctic krill protein contained in NKO™ and the lack of allergic responses reported based on the use of NKO™ as a dietary supplement, Neptune voluntarily intends to include a warning of the type noted below on food products containing NKO™:

> WARNING: Persons with seafood allergies, coagulopathy or who are taking anticoagulants or other medications should discuss their situation with their doctor before taking NKO™ as an ingredient in conventional foods or in nutritional supplements.

In addition, allergic responses may develop by other mechanisms once krill oil becomes a more common ingredient in food. While GRAS Associates has not found any reason to suspect any additional causes of allergic reactions to NKO™, Neptune has agreed that it will maintain vigilance to this concern and will remain sensitive to consumer complaints that could reflect allergenicity. Spikes in incidents in reporting these effects can indicate a problem with processing or a higher than normal level of a contaminant.

C. Common Knowledge Elements

An important consideration of GRAS determinations requires that the information on which GRAS conclusions are based must fulfill common knowledge elements---reliance upon generally available safety information and basic consensus within the scientific community on the interpretation of the safety information.

The most critical portion of the safety review of krill oil is its composition, purity, and similarity to fish oils which already enjoy GRAS status. FDA 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. The compositions of krill oil and common fish oils are published and the similarity in compositions is readily ascertainable in the cited public documents (FDA, 2002b, 2004a, b).

The FDA criterion for safety of consumption of fish oil for total daily per person consumption of EPA and DHA of less than 3 g is also publicly available as contained in the documents cited. The Panel has reviewed this criterion and agrees that it is a scientifically reasonable assessment for which there is consensus among experts.

In addition, the Panel has reviewed publicly available safety information on astaxanthin and vaccenic acid and concurs that these constituents are safe to consume in the daily quantities forecast.

With regard to supportive studies, the majority of clinical studies (Bunea et al., 2004; Deutsch, 2007; Sampalis et al., 2003) on krill oil are published, as is one published toxicology study (Ruggiero-Lopez et al., 1994) on krill oil. In addition, the remaining clinical and toxicology studies reviewed are in the process of being submitted to a peer reviewed scientific journal for publication. This remaining to-be-published study has been viewed as corroborative by the Panel.

VI. CONCLUSIONS[8]

The Panel has concluded that there is sufficient evidence to consider high phospholipid krill oil to be GRAS at a dietary level expected when used in foods as a substitute or alternative for fish oil.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

In conclusion, adult human exposure to NKO™ that meets acceptable food grade specifications as represented and documented by Neptune and which has been manufactured in accordance with Good Manufacturing Practices, is considered to be generally recognized as safe (GRAS) when used as a substitute or alternative for use in those foods which are commonly supplemented with fish oil.

Richard C. Kraska, Ph.D., DABT — **Date: January 18, 2008**

Robert S. McQuate, Ph.D. — **Date: January 18, 2008**

Stanley T. Omaye, Ph.D. — **Date: January 18, 2008**

[8] Appendix F contains the educational and professional backgrounds for Richard C. Kraska, Ph.D., DABT, Robert S. McQuate, Ph.D. and Stanley T. Omaye, Ph.D. Each has extensive experience in the evaluation of food ingredient safety. Drs. Kraska and McQuate each worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Omaye is a professor in the Department of Nutrition at the University of Nevada, Reno and has published extensively on the nutritional and toxicological aspects of food ingredients. Dr. Kraska served as Chair of the Panel.

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Appendix A

Manufacturing Controls Certifications

GMP Certificate of Compliance

Health Canada Site License

Certificate of Registration of a Fish Processing Establishment

Health Canada Santé Canada

Natural Health Products Directorate

Direction des produits de santé naturels

Natural Health Product GMP Certificate of Compliance
Certificate No: 0000349

This Good Manufacturing Practice (GMP) Certificate is issued by Natural Health Products Directorate of Health Canada

Exporting (certifying) Country: CANADA

Name of Applicant: NEPTUNE TECHNOLOGIES & BIORESSOURCES INC.

Address: **500, BOULEVARD SAINT-MARTIN OUEST, BUREAU 550**
LAVAL
QUÉBEC
H7M 3Y2
CANADA

SITE INFORMATION:

Authorized Activities	Site Address	Site Licence Number	Date of Expiry
Manufacturing Packaging Labelling	795, rue Pépin Sherbrooke QC J1L 2P8	300191	Jan. 25, 2007

Sites and operations have been assessed to be in compliance with the Good Manufacturing Practice requirements of the Natural Health Products Regulations

Address of certifying authority:

Natural Health Products Directorate
2936 Baseline Road, AL 3300B
Ottawa, Ontario K1A 0K9

Health Canada Santé Canada
Natural Health Products Directorate
Direction des produits de santé naturels
0002134
Canada

For Manager, Submission Management Division

Date: March 1, 2006

Canada

Health Canada / Santé Canada

SITE LICENCE

Site Licence Number:
Numéro de la licence :
300191

LICENCE D'EXPLOITATION

This Licence is issued by the Minster of Health under the Authority of section 22 of the Natural Health Products Regulations
Cette licence est délivrée par le ministre de la Santé conformément à l'article 22 du Règlement sur les produits de santé naturels

Issued to
Délivré à

Name of Licensee:
Nom du titulaire

NEPTUNE TECHNOLOGIES & BIORESSOURCES INC.

Address
Addresse :

795, RUE PÉPIN
SHERBROOKE
QUÉBEC
CANADA
J1L 2P8

to perform the following activities at authorized buildings listed on the Domestic Site Annex and Foreign Site Annex.
pour exécuter les activités suivantes dans les bâtiments autorisés lister sur Annexe des sites Canadiens et Annexe des sites étrangers :

Activities/ *Activités*	**Authorized Activities/ *Activités authorisées***	**Specific Authorization/ *Autorisation spécifique***	
		Sterile Dosage Form/ *Forme posologique stérile*	**Homeopathic Medicine/ *Remède homéopathique***
Manufacturing/*Fabrication*	YES/*OUI*	NO/*NON*	NO/*NON*
Packaging/*Emballage*	YES/*OUI*	NO/*NON*	NO/*NON*
Labelling/*Étiquetage*	NO/*NON*	NO/*NON*	NO/*NON*
Importing/*Importation*	NO/*NON*	NO/*NON*	NO/*NON*

This licence is renewable pursuant to section 36 of the Natural Health Products Regulations. Any changes to the activities authorized by this licence are subject to sections 32 and 33 of the Regulations.
Cette licence est renouvelable annuellement en vertu de l'article 36 du Règlement sur les produits de santé naturels. Tout changement aux activités autorisées par cette licence est régi par les articles 32 et 33 du Règlement.

Issued/ *Délivrée :*	**25 janvier 2007**	Amended/ *Modifiée :*	**N/A**	Expiry/ *Expiration :*	**25 janvier 2008**

Annex Attached/ *Annexes jointes:*

Director General, Natural Health Product Directorate
Directeur général, Direction des produits de santé naturels

BEST ORIGINAL COPY

Health Canada / Santé Canada

SITE LICENCE

Site Licence Number:
Numéro de la licence :
300191

LICENCE D'EXPLOITATION

Canadian Site Annex/*Annexe des sites Canadienne*

The following sites are considered to be in compliance with GMP requirements outlined in PART 3 of the Natural Health Products Regulations

Les sites suivants sont considérés conforme avec les normes des bonnes pratiques de fabrication tel que stipulé dans la partie 3 du Règlement sur les produits de santé naturels.

Building Name / *Nom du bâtiment*	**Neptune Technologies & Bioressources Inc.**	
Address / *Adresse :* **795, rue Pépin**	City / *Ville* **Sherbrooke**	
Province **Québec**	Postal Code / *Code postal :* **J1L 2P8**	Country / Pays **Canada**

Activities/ *Activités*	**Authorized Activities/ *Activités authorisées***	**Specific Authorization/ *Autorisation spécifique***	
		Sterile Dosage Form/ *Forme posologique stérile*	**Homeopathic Medicine/ *Remède homéopathique***
Manufacturing/*Fabrication*	YES/*OUI*	NO/*NON*	NO/*NON*
Packaging/*Emballage*	YES/*OUI*	NO/*NON*	NO/*NON*
Labelling/*Étiquetage*	NO/*NON*	NO/*NON*	NO/*NON*
Importing/*Importation*	NO/*NON*	NO/*NON*	NO/*NON*

Page 2 of 2

BEST ORIGINAL COPY

Certificate of Registration of a Fish Processing Establishment

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

CERTIFICATE OF REGISTRATION OF A FISH PROCESSING ESTABLISHMENT	CERTIFICAT D'AGRÉMENT D'UN ÉTABLISSEMENT DE TRANSFORMATION DU POISSON	Registration No. / N° d'agrément	Region / Région
		5111	MONTREAL WEST REGIONAL OFFICE - QUEBEC AREA
Name of Establishment / Nom de l'établissement		Issue Date / Date d'émission Y/A M D/J	Expiry Date / Date d'expiration Y/A M D/J
NEPTUNE TECHNOLOGIES & BIORESSOURCES INC.		2007/02/17	2008/02/29
Mailing Address / Adresse postale		Type(s) of Process Operation / Type(s) d'opération de transformation	
2740 Ave. Pierre Péladeau LAVAL QUEBEC CANADA H7T 3B3		Other Process	
Location of Establishment / Lieu de l'établissement			
795 RUE PÉPIN SHERBROOKE QUEBEC CANADA J1L2P8			
Signature			
Regional Director Directeur régional ▶			

This certificate is issued in accordance with the *Fish Inspection Regulations.*

Le présent certificat est délivré en vertu du *Règlement sur l'inspection du poisson.*

Canada

CFIA / ACIA 0191 (2003/11)

000037

Appendix B

Codex Alimentarius Standard

Edible Fats And Oils Not Covered By Individual Standards
CODEX STAN 19-1981 (Rev. 2-1999)

Pages 000039 - 000044 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Appendix C

Test Methods
(Cited in Table 1)

LIST OF TEST METHODS CITED IN TABLE 1

METHOD	SOURCE	TITLE	DATE
A.O.A.C.970.52, US EPA 8081	UNITED STATES EPA	ORGANOCHLORINE PESTICIDES BY GAS CHROMATOGRAPHY	DECEMBER 1996
US EPA 610, 8310, and 8100	UNITED STATES EPA	POLYNUCLEAR AROMATIC HYDROCARBONS	SEPTEMBER 1986
US EPA Method 1613 revision B	UNITED STATES EPA	FISH TISSUE ANALYSIS	AUGUST 2007
MFHPB-20-MFO-11	HEALTH PRODUCTS AND FOOD BRANCH OTTAWA, CANADA[a]	ISOLATION AND IDENTIFICATION OF SALMONELLA FROM FOODS MICROBIOLOGICAL EXAMINATION OF COCOA AND CHOCOLATE	JANUARY 2005
MFHPB-30	HEALTH PRODUCTS AND FOOD BRANCH OTTAWA, CANADA	ISOLATION OF LISTERIA MONOCYTOGENES FROM ALL FOOD AND ENVIRONMENTAL SAMPLES	JANUARY 2001
MFHPB-32	HEALTH PRODUCTS AND FOOD BRANCH OTTAWA, CANADA	ENUMERATION OF YEAST AND MOLD IN FOOD PRODUCTS AND FOOD INGREDIENTS USING 3M™ PETRIFILM™ YEAST AND MOLD COUNT PLATES	MARCH 2003
MFHPB-33	HEALTH PRODUCTS AND FOOD BRANCH OTTAWA, CANADA	ENUMERATION OF TOTAL AEROBIC BACTERIA IN FOOD PRODUCTS AND FOOD INGREDIENTS USING 3M™ PETRIFILM™ AEROBIC COUNT PLATES	FEBRUARY 2001
MFHPB-34	HEALTH PRODUCTS AND FOOD BRANCH OTTAWA, CANADA	ENUMERATION OF E. coli AND COLIFORMS IN FOOD PRODUCTS AND FOOD INGREDIENTS USING 3M™ PETRIFILM™ E. coli COUNT PLATES	FEBRUARY 2001
MFLP-21	HEALTH PRODUCTS AND FOOD BRANCH OTTAWA, CANADA	ENUMERATION OF STAPHYLOCOCCUS AUREUS IN FOODS AND ENVIRONMENTAL SAMPLES USING 3MT PETRIFILMT STAPH EXPRESS COUNT (STX) PLATES	JULY 2004, Supplement SEPTEMBER 2005

[a] Canadian Methods Available at http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index_e.html.

Appendix D

Safety of Astaxanthin and Astaxanthin Esters

Safety of Astaxanthin and Astaxanthin Esters

NKO™ contains a minimum of 150 mg% of astaxanthin esters. Astaxanthin is a pigment carotenoid compound, which occurs naturally in a variety of foods including salmon, shrimp, lobster and fish eggs (Hussein et al., 2006). Its presence in foods is indicated by its characteristic pink color. The pink color of flamingos is due to their ability to convert carotenoids in the diet to astaxanthin where it is stored in their skin and feathers. It is FDA listed for use as a color additive[1] for use in fish feed. Astaxanthin has potent antioxidant properties that have been demonstrated in several animal studies and clinical trials. In addition to antioxidative properties, there is evidence that astaxanthin has anti-inflammatory properties, beneficial effects on cancer, diabetes, the immune system and ocular health.

Natural astaxanthin exists in several isomeric forms of the carotenoid structure and predominately exists as mono- and di- esters of various fatty acids (Higuera-Ciapara et al., 2006). Levels in various species of salmon were reported to be in the range of 1-58 ppm (Turujman, et. al, 1997). The richest source noted in the literature is *Haernatococcus pluvialis*, a unicellular green algae that contains from 0.1 to 0.4% astaxanthin (Turujman, et. al, 1997).

FDA did not object to a notice for use of astaxanthin in dietary supplements at 2 mg/day (FDA, 1999). The FDA website lists several safety studies in connection with the dietary supplement notice.

- In an unpublished study submitted with the notice, no adverse effects were noted in a 14 day oral study on *Haernatococcus pluvialis*, a unicellular green algae using a dose of 6 g/kg. The algae sample was rich in astaxanthin, presumably existing largely in an ester form.

The notice referenced studies submitted in the original color additive petition.

- Astaxanthin was not mutagenic in a bacterial assay;
- Astaxanthin did not induce micronuclei in bone marrow cells from mice receiving oral doses of up to 2000 mg/kg bw;
- No teratological or embryotoxic effects were seen in a rabbit study with oral doses up to 400 mg/kg;
- No reproductive effects were seen in a rat study with oral doses up to 400 mg/kg;
- The no effect level in a dietary subchronic study in rats was 6.25% or approximately 310 mg/kg bw; and
- The no effect level in a dietary subchronic study in rats was 10 % or approximately 162 mg/kg bw.

A placebo-controlled clinical study by Spiller and Dewell (2003) showed that 6 mg per day of astaxanthin from the algal extract of *Haematococcus pluvialis* can be safely consumed by human adults. The 8-week study involved 35 healthy adults between the ages of 35-69 years. All participants took three gelcaps per day, one at each meal. Nineteen participants received the algal extract in safflower oil, containing 2 mg of astaxanthin each (treatment), and 16 participants received gelcaps containing safflower oil only (placebo). To determine safety of consumption, blood pressure and blood chemistry tests were conducted at the beginning of

[1] 21 CFR 73.35.

the study and after 4 and 8 weeks of supplementation. No clinically important differences were detected between the treatment and placebo groups after 8 weeks, and no adverse effects of the astaxanthin administration were reported.

A study by Iwamoto et al. (2000) investigated the effects of astaxanthin on LDL oxidation *in vitro* and *ex vivo* on human subjects. Many studies have shown that astaxanthin exhibits protective effects against lipid peroxidation induced by free radicals or oxygen, but no study to date was done on LDL. In the *in vitro* study the oxidation of LDL was measured in a 1 mL reaction system consisting of increasing concentrations of astaxanthin (12.5,25.0,50.0 µg/mL), 400 µM V-70 (2,2'-azobis(4-methoxy-2,4-dimethyl-valeronitrile)), and LDL (70 µg/mL protein). The astaxanthin dose dependently prolonged the oxidation lag time (31.5, 45.4, 65.0 min) compared with the control (19.9 min). For the *ex vivo* study, 24 volunteers (mean age 28.2 years) consumed astaxanthin at doses of 1.8, 3.6, 14.4 and 21.6 mg per day for 14 days. No other changes were made in the diet. Fasting venous blood samples were taken at days 0 and 14. The LDL lag time was longer (5.0, 26.2, 42.3 and 30.7%, respectively) compared with day 0 after consuming astaxanthin at doses of 1.8, 3.6,14.4 and 21.6 mg for 14 days compared with day 0, but there was no difference in oxidation of LDL between day 0 and day 14 with the control group (6 untreated volunteers). No adverse effects were reported and the authors concluded that astaxanthin inhibits LDL oxidation, which may prevent atherosclerosis.

Odeberg et al. (2003) investigated the bioavailability of astaxanthin because it is known that the bioavailability of carotenoids is limited due to the extent of their lipophilicity. The main purpose of the study was to determine if the bioavailability of astaxanthin could be increased in humans by incorporating the compound into different lipid compositions. A total of 32 healthy male volunteers (age 20-46, mean age 26.5 years) received a single dose of 40 mg astaxanthin as lipid based formulations or as a commercially available food supplement, followed by blood sampling at specific times (from 2 hours to 28 days after dose). Analysis of blood for *trans*-astaxanthin was performed using liquid–liquid extraction for sample preparation and high performance liquid chromatography with visible spectrometric detection. The reference formulation was a commercial formulation consisting of algal meal and dextrin in hard gelatin capsules (Napro Pharma, Brattvaag, Norway). The three lipid based formulations included long-chain triglyceride (palm oil) and polysorbate 80 (formulation A), glycerol mono- and dioleate and polysorbate 80 (formulation B), and glycerol mono- and dioleate, polysorbate 80 and sorbitan monooleate (formulation C). All three lipid based formulations showed enhanced bioavailability, with the highest being from formulation B, which increased availability by nearly 4-fold and contained a high content of hydrophilic synthetic polysorbate 80. The authors conclude that the dose of 40 mg showed a half-life of 15.9 ± 5.3 hours, and was well tolerated with no adverse effects.

Several studies in animals have demonstrated the antioxidative effects of astaxanthin. One study by Kang et al. (2001) showed that astaxanthin protects against liver damage that was induced by carbon tetracholoride (CCl_4) in male Sprague-Dawley rats. Animals received 2 mg/kg (body weight) or 100 mg/kg of Astaxanthin for 15 days. Both doses were protective against liver damage induced by CCl_4 by inhibiting lipid peroxidation and stimulating the cellular antioxidant system. There were no adverse effects reported due to either dose of astaxanthin.

Jewell and O'Brien (1999) also studied the antioxidant effects of astaxanthin on enzyme systems in the liver, lung, kidney, and small intestine of male Wistar rats. At a dose of 300

mg/kg diet for 16 days, results showed that astaxanthin is a potent inducer of liver P450 enzymes, but did not have significant effects in other organs. No adverse effects were reported due to the administration of astaxanthin.

Ohgami et al. (2003) conducted a study that demonstrates the potential anti-inflammatory affect of astaxanthin. Male Lewis rats with lipopolysaccharide (LPS)-induced uveitis (inflammation of the middle eye) were injected intravenously with 1, 10, or 100 mg/kg astaxanthin or 10 mg/kg prednisolone 30 minutes before, at the same time as, or at 30 minutes after LPS treatment. The number of infiltrating cells and protein concentration in the aqueous humor collected at 24 hours after LPS treatment was determined. Results showed that astaxanthin dose-dependently suppressed ocular inflammation. No adverse effects due to administration and dose of astaxanthin were reported.

Much attention is given to the anticarcinogenic effects of various carotenoids, especially astaxanthin, because it is a non pro-Vitamin A carotenoid (which eliminates the toxicity that is associated with retinoids) which are also used as a cancer preventative (Bertram and Vine, 2005). Tanaka el al. (1994) studied the chemopreventive effect of astaxanthin on urinary bladder carcinogenesis ICR mice. Mice were given astaxanthin in drinking water at a concentration of 50 ppm for 20 weeks, which resulted in the significant reduction of bladder cancer. There were no toxic effects of astaxanthin administration observed in the mice. In another study by Tanaka et al. (1995), the administration of 100 ppm of astaxanthin in the drinking water of F344 rats for no more than 32 weeks significantly reduced oral carcinogenesis.

Astaxanthin References

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Appendix E

ELISA Assay

Page 1 of 4
Prepared for: M.E.Gauthier
Neptune Technologies and Bioresources
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Sherbrooke
QC
Canada
Report Number: 01-0607b

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REPORT

Sample Appearance

Three test samples were supplied in brown opaque 60mL containers labelled as shown in the table below. The samples were observed as dark red oils.

Work Schedule

Work was performed as directed in the test quotation 07060123-MB.
Briefly:
Protein was isolated from the three sample oils with extraction buffer
A protein quantification of the extracts was performed.
An allergen test (Shellfish ELISA) was performed on the protein extract.
In addition to the quotation and in order to confirm that the extraction procedure did not influence the down-stream ELISA analysis, sample oil NEP00267 was spiked with 5µl of 'shellfish spike control' ('oil spike'). This control sample was processed in the same way as the test samples.
A second control sample was prepared by spiking extraction buffer with 5µl 'shellfish spike control' ('extraction buffer spike'). This second control is performed to confirm the success of the ELISA assay. This control sample was tested in the ELISA assay but was not sub ject to the extraction procedure.

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REPORT

Methodologies

Protein extraction procedures

The samples provided contained a complex mixture of phospholipids, polyunsaturated fatty acids and proteins. Due to the novel nature of these samples it was necessary to investigate appropriate protein extraction methods to ensure that the allergen partitioned into the aqueous layer and did not remain in the oil phase. Two different protein extraction procedures were performed on each sample.

The first method involved addition of 0.5x volumes of 100% trichloroacetic acid to the oil, followed by agitation and protein precipitation overnight at 4oC. The precipitated protein was isolated by centrifugation, washed in methanol and re-suspended in a TRIS -NaCl buffer. The protein yield, as assessed using a Bradford (Coomassiebased) assay was very low, therefore this approach was abandoned.

The second method used a high salt extraction procedure. This involved the addition of 10x volumes of 60oC TRIS -NaCl to the oil sample followed b y agitation and centrifugation. The aqueous phase of this extract was assessed using a Bradford (Coomassie-based) assay. Protein yields in agreement with previously reported levels were obtained. These samples were used for further analysis.

Protein assay procedure

Protein assays were carried out using a Coomassie (Bradford) Protein Assay Kit (Manufactured by Pierce Biotechnology, Inc; Product number 23200).

Shellfish ELISA assay

The shellfish ELISA assay was carried out using the Biokits shellfish assay kit produced by Tepnel Life Sciences PLC (product number 902076K). It is a sandwichtype ELISA (Enzyme Linked Immunosorbent assay) which utilises biotin-avidin enhancement. Shellfish protein is detected using a polyclonal antibody to crustacean tropomyosin. The protein has multiple epitopes to which the polyclonal antibodies are directed, enabling detection of both intact and fragmented tropomyosin. The limit of quantification of this method is 1ppm shellfish protein. This assay has been demonstrated to be highly sensitive in the detection of the following: crab, lobster, brown shrimp, tiger prawn langoustine and crayfish. Other crustaceans not listed here may also be detected by the assay. The assay also has a low cross-reactivity to some molluscs including oyster, mussel and squid.

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REPORT

Results

A protein precipitation was performed from 1mL of oil from the three test samples (1-3) and one sample spiked with shellfish spike control. A protein quantification was performed on the resultant extract; the results are shown in the table below.
* The protein quantification was complicated by high levels of insoluble matter in the extracts of all samples. Despite centrifugation at 13000 rpm for 30 minutes, the insoluble fraction could not be completely removed. The consequential increase in opacity in the sample wells resulted in an over-estimation of the concentration of the soluble protein in all samples. Visual observations allow an estimate of the actual soluble protein concentration of between 6.5 and 12.5 mg per mL of oil for all 4 samples (assuming 100% protein extraction from the oil).
The shellfish ELISA assay gave a **negative** result for all three oil samples (see table below). Therefore, under the conditions of this test no indication of shellfish protein to a detection limit of 1ppm was observed. Although every effort has been made to ensure the test was as rigorous as possible, this does not eliminate the possibility of the presence of shellfish protein which is unreactive or below the limits of detection for this method
The 'extraction buffer spike' result was within the acceptable limits outlined by the shellfish assay protocol, indicating the ELISA was successful. The 'oil spike' gave an assay result in accordance with the 'extraction buffer spike' indicating the extraction method did not interfere greatly with the ELISA assay and that the extraction procedure was successful.

Neptune Lot No.	Neptune Sample No.	Genon Labs reference No.	Protein quantification result	Shellfish ELISA assay result
NKO-0060822	1	NEP00367	33.8 mg/mL*	Negative(<1ppm)
NKO-060116	2	NEP00167	32 mg/mL*	Negative(<1ppm)
NKO-060127	3	NEP00267	24.4 mg/mL*	Negative(<1ppm)
n/a	n/a	OIL SPIKE	26.2 mg/mL*	Positive(4.4ppm)
n/a	n/a	Extraction Buffer Spike	n/a	Positive(4.5ppm)

Report prepared by Dr M J Bromley Technical Manager 12-06-2007.

Page 4 of 4
Prepared for: M.E.Gauthier
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REPORT

About Genon Laboratories Limited

We specialise in immunological and genetic testing designed to meet legislative requirements for the food and foodstuff industries. The company is managed by PhD level scientists with over 30 years experience in molecular biology, immunology, diagnostic development and food testing services. Genon Laboratories Limited systems of work operate to the requirements of ISO17025.

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Hebden Bridge
West Yorkshire
HX7 5RU

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Certificate of Analysis

Certificate number **01-0607-1**

Company details
Contact: M.E.Gauthier
Name: Neptune Technologies and Bioresources
Address: 795 Rue Pepin
Sherbrooke, QC
Canada

Sample details
Date of arrival: 08.06.07
Sample code: NEP 001 67
Description: Krill Oil (NEPT#06-32)
Weight: 50g
Batch/lot no.: NKO-060116
Storage conditions: Ambient

Test details:
SHELLFISH PROTEIN ASSAY
L.O.Q. 1ppm

Test results:
Controls: OK
Sample: Less than limit of quantification (1ppm)

Additional Comments:

See report 01-0607

Signed: Name: Louise Melling Date:12.06.07

Title: Laboratory Manager Page:1/1

Results are representative of the sample provided

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Certificate of Analysis

Certificate number **01-0607-2**

Company details
Contact: M.E.Gauthier
Name: Neptune Technologies and Bioresources
Address: 795 Rue Pepin
Sherbrooke, QC
Canada

Sample details
Date of arrival: 08.06.07
Sample code: NEP 002 67
Description: Krill Oil (NEPT#06-65)
Weight: 50g
Batch/lot no.: NKO-060127
Storage conditions: Ambient

Test details:
SHELLFISH PROTEIN ASSAY
L.O.Q. 1ppm

Test results:
Controls: OK
Sample: Less than limit of quantification (1ppm)

Additional Comments:

See report 01-0607

Signed: Name: Louise Melling Date:12.06.07

Title: Laboratory Manager Page:1/1

Results are representative of the sample provided

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Certificate of Analysis

Certificate number **01-0607-3**

Company details
Contact: M.E.Gauthier
Name: Neptune Technologies and Bioresources
Address: 795 Rue Pepin
Sherbrooke, QC
Canada

Sample details
Date of arrival: 08.06.07
Sample code: NEP 003 67
Description: Krill Oil (NEPT#06-668)
Weight: 50g
Batch/lot no.: NKO-0060822
Storage conditions: Ambient

Test details:
SHELLFISH PROTEIN ASSAY
L.O.Q. 1ppm

Test results:
Controls: OK
Sample: Less than limit of quantification (1ppm)

Additional Comments:

See report 01-0607

Signed: Name: Louise Melling Date:12.06.07

Title: Laboratory Manager Page:1/1

Results are representative of the sample provided

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Certificate of Analysis

Marie-Eve Gauthier Report No:
Purchase Order:
Date Received:
Date Started:
P7-04370
To Follow
21st May 2007
22nd May 2007
Page 1 of 3

DNA Testing

Neptune Technologies and Bioressources
Inc.
795 Pepin Street
Sherbrooke
Quebec
J1L 2P8, Canada
Sample Code: **P7-04370-1** Your Refs: NKO (euphasia superba) Lot: NKO-060116, Sample #1
Description: Neptune Krill Oil (Bulk) 50g
Allergen Species Identification by PCR (Method TM114)
Internal PCR Control Satisfactory
Crustacean (Non Specific) DNA Not Detected
Fish (Non Specific) DNA Not Detected
Conclusion
The sample contained no detectable Crustacean and Fish DNA.
Barbara Hirst
Technical Manager (DNA &
Protein)
Approved By: *READING SCIENTIFIC SERVICES LTD*
The Lord Zuckerman Research Centre,
Whiteknights, Pepper Lane, Reading, RG6 6LA
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Marie-Eve Gauthier Report No: P7-04370
Purchase Order: To Follow
Date Received: 21st May 2007
Date Started: 22nd May 2007
Page 2 of 3

DNA Testing

Neptune Technologies and Bioressources
Inc.
795 Pepin Street
Sherbrooke
Quebec
J1L 2P8, Canada

Sample Code: **P7-04370-2** Your Refs: NKO (euphasia superba) Lot: NKO-060127, Sample #2

Description: Neptune Krill Oil (Bulk) 50g

Allergen Species Identification by PCR (Method TM114)
Internal PCR Control Satisfactory
Crustacean (Non Specific) DNA Not Detected
Fish (Non Specific) DNA Not Detected

Conclusion
The sample contained no detectable Crustacean and Fish DNA.

Barbara Hirst
Technical Manager (DNA & Protein)

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Marie-Eve Gauthier Report No: P7-04370
Purchase Order: To Follow
Date Received: 21st May 2007
Date Started: 22nd May 2007
Page 3 of 3

DNA Testing

Neptune Technologies and Bioressources
Inc.
795 Pepin Street
Sherbrooke
Quebec
J1L 2P8, Canada

Sample Code: **P7-04370-3** Your Refs: NKO (euphasia superba) Lot: NKO-0060822, Sample #3
Description: Neptune Krill Oil (Bulk) 50g
Allergen Species Identification by PCR (Method TM114)
Internal PCR Control Satisfactory
Crustacean (Non Specific) DNA Not Detected
Fish (Non Specific) DNA Not Detected
Conclusion
The sample contained no detectable Crustacean and Fish DNA.

These results relate only to the sample(s) tested and do not guarantee the bulk of the material to be of equal quality This report shall not be reproduced, except in full, without the written approval of RSSL RSSL staff were not
responsible for sampling and cannot be held liable in respect of the use to which this information is put All samples will be retained for a period of one month (or ten days, if perishable) from the date of this certificate
2

For meat speciation, the manufacturer's stated limit of detection for the test kit used is ca 1% For fish speciation, the limit of detection is ca 1% The limit of detection for allergens is as follows Brazil Nut, Hazelnut, Peanut
0 0001% (1mg/Kg), Almond, Black Mustard, Macadamia Nut, Pecan Nut, Walnut, Fish DNA, Kiwi 0.001% (10mg/Kg),Crustacean DNA, Pistachio Nut, Cashew Nut, Pine Nut, Chestnut 0 01% (100mg/Kg) and Celery, Celeriac,
Lupin 0 1% (1g/Kg) NB The test for Black Mustard also detects Yellow Mustard and White Mustard DNA
2

Barbara Hirst
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Protein)
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Appendix F

Qualifications of Expert Panel Members

Richard C. Kraska, Ph.D., DABT

Robert S. McQuate, Ph.D.

Stanley T. Omaye, Ph.D.

Richard C. Kraska
Chief Operating Officer and Co Founder
GRAS Associates, LLC

Curriculum Vitae

EDUCATION	B.S., Chemistry; Providence College Ph.D., Pharmacology; University of Minnesota
PROFESSIONAL CERTIFICATION	Diplomate, American Board of Toxicology
EXPERIENCE	29 year in toxicology and regulatory affairs for industry and government in broad aspects of the chemical industry including food additives, foods, food contact materials, cosmetics, lubricants and fuels, coatings, defoamers, anti-microbial pesticides and pharmaceuticals.

GRAS ASSOCIATES, LLC
Bonita Springs, FL (2006 to Present)

Chief Operating Officer and Co Founder

- Serve as Lead Scientist and Panel Chair for GRAS determinations.
- Coordinate drafting and report review by chemists, toxicologists and scientists of other disciplines as needed.
- Ingredients reviewed include natural antioxidants, novel sources of dietary fiber, fats and oils and extracts from exotic fruit.

KRASKA CONSULTANTS, INC.
Bonita Springs, FL (2004 to Present)

Vice President and Principal

- Toxicology and Regulatory Consultant for a variety of lubricant, chemical, food processing companies and trade associations
- Offer services in Toxicology and Product Safety including FDCA, TSCA and FIFRA regulations and filings, International Hazard Communication Support, Product Stewardship, Expert Witness and Litigation Support
- Founder and Technical Consultant for the Defoamer Industry Trade Association
- Toxicology Consultant for the Independent Lubricant Manufacturers Association

THE LUBRIZOL CORPORATION
Wickliffe, OH (1987 to 2004)

MANAGER OF SPECIAL TOXICOLOGY AND REGULATORY PROJECTS (2001 to 2004)

- Toxicology and regulatory consultant for organic growth initiatives and new acquisitions.
- Coordinating $2.8 million inhalation toxicology program on engines emissions with a novel diesel fuel formulation for registration with EPA under the Clean Air Act.
- Coordinating world wide implementation of compliance with revised European hazard communication regulations
- Consultant to Lubrizol defoamer, coating, process chemical, metalworking and lubricant businesses on regulations and toxicology
- Team member studying and planning implementation of sustainable development at Lubrizol.

MANAGER OF TOXICOLOGY AND RISK ASSESSMENT (1987 – 2001)

- Provided leadership and management for corporate toxicologists and product safety specialists.
- Direct responsibility for toxicology testing and evaluation of all Company specialty chemicals and products.
- Manage $1 million annual toxicology and environmental testing budget for regulatory approvals and product stewardship.
- Lead consultant for business units on novel regulatory approvals, product stewardship and risk evaluation.
- Developed and institutionalized product risk assessment process for all Lubrizol businesses.
- Provide leadership role representing Company on trade association task groups involved in legislative and regulatory advocacy.
- Co-team leader for development and implementation of award -winning expert system for writing MSDSs from a product safety database.

BP AMERICA INC (formerly THE STANDARD OIL CO)
Cleveland, OH (1985-1987)

MANAGER OF PRODUCT SAFETY AND REGULATORY COMPLIANCE

- Assumed responsibility for assuring all Company products complied with federal regulations (TSCA, FIFRA, FDCA, USDA).
- Coordinated and expedited all regulatory submissions for premarket approval, reporting rules and rulemaking comment.
- Conscientiously developed Company Product Safety Policies and Manual.
- Critically evaluated Corporate Hazard Communication Program in a decentralizing company.
- Successfully initiated labeling program to comply with OSHA Hazard Communication Standard.

AMERICAN CYANAMID COMPANY, CHEMICALS GROUP
Wayne, NJ (1983-1985)

MANAGER OF TOXICOLOGY PROGRAMS

- Wide range of responsibility for recommending, contracting, monitoring and evaluating mammalian, genetic and aquatic toxicology studies for chemical products.
- Responsible for $250,000 total contract value for testing, quality assurance and consultants.
- Effectively guided regulatory staff in strategy and data requirements for premarket approvals.
- Successfully orchestrated targeted research programs for mechanistic studies on key chemicals for aquatic and mammalian toxicity
- Actively represented Company in a wide spectrum of trade association activities.

FOOD AND DRUG ADMINISTRATION
Washington, DC (1977-1983)

GRAS Review Branch
Division of Food and Color Additives

SUPERVISORY CONSUMER SAFETY OFFICER
(1981-1983)

- Successfully managed group of 3-4 professionals in regulatory program to implement expert panel reviews of GRAS list food ingredients.
- Projects of responsibility included salt, caffeine, BHA, BHT, cellulose, enzymes, rapeseed oil, vitamins, iron, manganese and zinc salts.
- Co-directed agency expertise on toxicology, chemistry, law and policy to propose regulatory action on food uses of DSS. Negotiated consistency with Bureau of Drugs proposal on OTC and Rx uses.
- Advised Branch Chief in matters of policy, consistency and personnel.
- Interacted with industry regarding regulatory opinions and new product approvals.

Petitions Control Branch
Division of Food and Color Additives

CONSUMER SAFETY OFFICER (1977-1981)

- Coordinated scientific review and regulatory response to review food additive petitions submitted by industry for direct additives and food packaging materials.
- Scientific and historical expert for General Counsel, U. S. Attorney and Department of Justice for legal proceedings on cyclamate.
- Expert on food/drug interface of vitamins and dietary supplements.
- Analyzed quality of critical studies on aspartame and served on GLP review committee
- Served as Bureau representative in Interagency Regulatory Liaison Group on phthalate plasticizers.
- Assistant to Bureau Director on advocacy activities on behalf of U.S. industry for WHO programs

PUBLICATIONS

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water-methanol emulsion emissions Toxicology & Industrial Health Vol 22 In Press

Reed, MD, Blair LF, Burling K, Daly I, Gigliotti AP, Gudi R, Mercieca MD. McDonald JD, Naas DJ, O'callaghan JP, Seilkop, SK, Ronsko NL, Wagner VO, Kraska RC Health effects of subchronic exposure to diesel-water emulsion emissions. Inhal Toxicol 17: 851-70 (2005)

Kraska, RC , Industrial Chemicals. Regulation of new and existing chemicals. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Kraska, RC . and Hooper DH, Industrial Chemicals. Hazard Communication, exposure limits, labeling and other workplace and transportation requirements under OSHA, DOT, and similar authorities around the world. In: Gad S.C. editor. *Regulatory Toxicology.* Taylor and Francis Ltd. London 2001.

Strother, DE, Mast RW, Kraska RC, Frankos V Acrylonitrile as a carcinogen. Research needs for better risk assessment. Ann NY Acad Sci 534:169-78 (1988)

Petersen DW, Kleinow KM, Kraska RC, Lech JJ Uptake, disposition and elimination of acrylamide in rainbow trout Toxicol Appl Pharmacol 80: 58-65 (1985)

Mast RW, Jeffcoat AR, Sadler BM, Kraska RC and Friedman MA Metabolism, disposition and excretion of [C14] melamine in male Fischer 344 rats. Food Chem Toxicol 21: 807-810 (1983)

SPEAKER Talks given on following topics at national meetings, seminars and workshops
- GRAS Criteria
- REACH and GHS Regulations
- HPV Toxicology Testing
- Risk Assessment and Risk Management
- Lubricant Additive Safety
- Trade Association Environmental Activism
- Product Deselection Lists
- MSDS Expert Systems
- Confidential Business Information under TSCA
- TSCA Section 12(b) Compliance

TRAINING COURSES Training courses given to business, research and legal groups at Lubrizol
- General Regulatory Overview
- TSCA New Chemicals
- FDA Food Additive Requirements
- Product Regulatory Law Course (TSCA, FDCA, OSHA)

Trainer, Toxicology Module, Metalworking Fluids Certificate Course (2005-2006)

TRADE ASSOCIATION ACTIVITIES	Chemical Reporting Task Group (1983-1998) Chemical Manufacturers Association Chairperson (1997-1998) Safety, Health, Environmental and Regulatory Affairs Committee, Independent Lubricant Manufacturers Association (1997 to present) Vice chairperson (2001-2002) Chairperson (2003-2004) Toxicology consultant (2006) Oversight Committee, Metalworking Fluid Product Stewardship Group, Independent Lubricant Manufacturers Association (1997-2004) Health Environmental and Regulatory Task Group, Petroleum Additives Panel (1997-2002) Chairperson, Sensitization Work Group (1999 to 2002) Biocides Panel, AEATF II Protocol Committee and Technical Committee (2003-2006) Team Leader for Metalworking Study (2005-2006) Defoamer Industry Trade Association, Founder and Technical Consultant (2005-2006)
PROFESSIONAL SOCIETY MEMBERSHIPS	Society of Toxicology (SOT) American Standards and Testing Methods (ASTM) Society of Tribology and Lubrication Engineers (STLE) Regulatory Affairs Professionals Society (RAPS) Roundtable of Toxicology Consultants (RTC)

ROBERT S. MCQUATE, Ph. D. **Phone:** **Fax:**
Emails:

WORK HISTORY

2006 - Present	CEO, and Co-Founder, GRAS Associates, LLC, Bend, OR
1988 - Present	President & CEO, R. S. McQuate & Associates, Inc., Bend, OR
2005 - 2006	Chemistry Instructor, Truckee Meadows Community College, Reno, NV
2000 - 2005	Senior Vice President, Scientific & Regulatory Affairs, AminoPath Labs, LLC, Portland, OR
1998 - 2002	Board Member & Business Consultant, National Institute of Standards & Technology, Advanced Technology Program, Gaithersburg, MD
1986 - 1996	Executive Director, Advanced Science & Technology Institute, Eugene & Corvallis, OR
1991 - 1992	Adjunct Professor, Food Science & Technology, Oregon State University, Corvallis, OR
1983 - 1986	Science Director, National Soft Drink Association, Washington, DC
1980 - 1983	Senior Regulatory Scientist and Group Leader of Regulatory and Nutrition, The Dial Company, Inc., Scottsdale, AZ
1977 - 1980	Consumer Safety Officer, Food and Drug Administration, Center for Food Safety & Applied Nutrition, Division of Food and Color Additives, Washington, DC
1974 - 1977	Assistant Professor of Chemistry, Willamette University, Salem, OR

EDUCATION

Postdoctoral Research Fellow with Professor R. G. Wilkins, New Mexico State University, Las Cruces, NM
Ph.D. in Chemistry, The Ohio State University, Columbus, OH
B.S. in Chemistry with Honors, Lebanon Valley College, Annville, PA

PROFESSIONAL EXPERIENCE

CONSULTING SERVICES

CEO, GRAS Associates, LLC; President & CEO, R. S. McQuate & Associates, Inc.

- Provide food ingredient safety evaluations, focusing on independent GRAS evaluations.
- Provide broad-based business consulting services to universities & companies involved in technology commercialization.
- Rapid assimilation of technical and business background for use in formulating commercialization strategies.
- Critically evaluate new technologies and business plans compared to competitive firms and products for economic potential.
- Implement marketing activities to establish strategic alliances and/or licensing agreements.
- Facilitate start-up ventures, including drafting of business plans.
- Utilize negotiation skills to achieve successful execution of deals.

UNIVERSITY EXPERIENCE

Executive Director, Advanced Science & Technology Institute

- Managed industry-university interface program on behalf of University of Oregon, Oregon State University, Oregon Health Sciences University and Portland State University.
- Strategic planning and program implementation; managed staff of up to 8.
- Facilitated linkages between university research community and private sector, working with over 500 faculty members to yield consulting contracts, industrial research sponsorship, technology licensing and business start-ups.
- Aggressively marketed faculty expertise, universities' technologies, and research capabilities through network of contacts, Internet, publications, and conferences.
- Represented universities in broad-based statewide and regional economic development initiatives.

Faculty, Willamette University, Oregon State University, & Truckee Meadows Community College

- Presented introductory and upper level chemistry lecture and laboratory courses.
- Conducted independent research in molecular biology, enzymology, and metal ion catalysis.
- Generated external grant funding to support six research students & acquire equipment.
- Provided food safety guidance to industry.
- Various scientific and chemical education publications.

PRIVATE SECTOR EXPERIENCE

Technical Management, The Dial Company & National Soft Drink Association

- Managed 5-person technical regulatory group with corporate responsibility for compliance with FDA, USDA, EPA, FTC, OSHA, CPSC, and NRC.
- Creatively interpreted regulations to favorably impact company revenues by over $4.2 M annually.
- Special focus on product and ingredient safety; formulated regulatory strategies in anticipation of and in response to agency positions; applied quantitative risk analysis to product safety considerations.
- Provided regulatory support and training to Manufacturing and QA on Good Manufacturing Practices requirements.
- Teamed with Marketing by evaluating advertising, product claims, and labeling for compliance.
- Assessed university research proposals in response to industry solicitations for funding.
- Served as liaison for industry interests on food ingredient safety before FDA officials.
- Served as industry spokesperson with media on technical topics such as NutraSweet addition to soft drinks.

GOVERNMENT EXPERIENCE

Staff, Food & Drug Administration

- FDA representative with regulated food industry officials.
- Managed safety evaluations of food and color additives and GRAS ingredients among FDA scientific divisions and with legal staff.
- Generated food safety notices, proposals, and regulations.
- Evaluated complex net weight food labeling and compliance issues and formulated agency position for Commissioner.
- Participated on special FDA Food Labeling Task Force to develop total food label requirements.
- Formulated recommended agency policy on iron bioavailability nutritional concerns.

PROFESSIONAL AFFILIATIONS

American Chemical Society
Institute of Food Technologists
Licensing Executives Society
Regulatory Affairs Professional Society

BOARD AND COMMITTEE MEMBERSHIPS

External Evaluator, Kansas Technology Enterprise Corporation, Higuchi Biosciences Center (2001)
Judge, Ohio State University Business Plan Competition (2001)
Board of Directors - Universal Pulping, Inc. (1996 - 2004)
Scientific Advisory Committee - Bainbridge Technology Group, Ltd. (1991 - 2000)
Board of Directors - Regional Council of Project SBIR West (1994 - 1996)
Board of Directors - Oregon Environmental Technology Association (1994 - 1995)
Co-Director – Oregon Governor's Task Force on Technology Transfer (1991 - 1992)
Board of Directors - LEAP, Inc. (1988 - 1994)
Board of Directors - Oregon Biosciences Association (1991 - 1993)
Board of Directors - BioForum (1988 - 1991)
Oregon Governor's Biotechnology Industry Advisory Council (1988)

HONORS AWARDS AND FELLOWSHIPS

Governor Barbara Roberts Certificate of Appreciation - Task Force on Technology Transfer (1993)
Governor Neil Goldschmidt Letter of Commendation - Biotechnology Industry Advisory Council (1988)
FDA Award of Merit from FDA Commission Jere Goyan (1980)
Letter of Commendation from FDA Commissioner Donald Kennedy (1979)
Seven Research Grants Awarded as Faculty Member at Willamette University (1974 - 1977)
National Science Foundation - Graduate Research Fellowship, The Ohio State University (1971 - 1973)
Graduated with Honors, Lebanon Valley College (1969)
Petroleum Research Fund - Undergraduate Research Fellowship, Lebanon Valley College (1967 and 1968)
Dean's List Student, Lebanon Valley College (1966 - 1969)
Salutatorian, South Lebanon High School, Lebanon, PA (1965)

Stanley T. Omaye, Ph.D.

Biography

For over 30 years, Dr. Omaye has been at the forefront of research focusing on environmental and nutritional interactions. His goal has been to identify nutrient interactions with environmentally induced disorders and to understand the mechanisms of such interactions and their effects on human health and welfare. Currently, Dr. Omaye's research and educational efforts are targeting: Nutrition and aging - efficacy of antioxidant compounds on atherosclerosis and the oxidation of low-density lipoproteins; Environmental health - health effects of air pollutants, environmental tobacco smoke, and mercury/arsenic; Health promoting bioactive compounds - elected phytochemicals and conjugated linoleic acid; and Improving food safety for vulnerable populations of Nevada. Thus, Dr. Omaye is often called upon to serve regionally, nationally, and internationally as an expert in food, nutrition and toxicology.

FDA Experience

- Past Academic Chairperson and currently Secretary, Nevada Food Safety Task Force – FDA initiated state project which has the task of improving food safety in Nevada, 2002 - present
- Appointed Consultant for the Institute of Medicine, Academy of Sciences, Washington D.C. Work involves the assessment the safety of supplement ingredients, FDA & USDA, 2002.
- USA advisor to the University of Hong Kong regarding, "An Investigation of Nutritional Imbalance and Carcinogenicity", at the request of FDA, 1987-1989.
- PI/CoPI, DOA GLP Compliant studies of new antidotal compounds used in the treatment of various biological and chemical military relevant compounds, 1987-1991.

Education

- Postdoctoral Fellow in Pulmonary Biochemistry and Toxicology; California Primate Research Center, Davis, California, 1976
- Ph.D., Biochemistry/Nutrition, University of California, Davis, California, 1975
- M.S., Pharmacology/Physiology, University of the Pacific, Stockton, California, 1972
- B.A., Chemistry, Sacramento State College, Sacramento, California, 1968

Professional Experience

- Professor, Department of Nutrition, College of Agriculture, Biotechnology and Natural Resources, University of Nevada, Reno. 1991 – present; Department Chair, Department of Nutrition, 1991-1996.
- Letterman Army Institute of Research, Research Toxicologist and Team Leader, Military Trauma Research Division, Presidio of San Francisco, CA. 1989 - 1991.
- Letterman Army Institute of Research, Assistant Chief of Toxicology Division and Research Chemist, Presidio of San Francisco, California. Toxicology and safety evaluation (Under Good Laboratory Practices) of military relevant products. 1987 - 1989.
- U. S. Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, Presidio of San Francisco, California. Project Leader and Research Chemist, Bioanalytical Research Unit (Oct. 1983 - April 1984) and Biochemistry Research Unit (April 1984 - April 1987).
- U. S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Berkeley, California. Project Leader and Research Nutritionist, Nutrients Research Unit. 1980 - 1983.
- Letterman Army Institute of Research, Chief of Applied Nutrition Branch and Research Chemist, Biochemistry Division, Department of Nutrition, Presidio of San Francisco, California. 1976 - 1980.
- California Primate Research Center, Pulmonary Biochemistry Section, Postdoctoral fellow. 1975 - 1976.
- Quality Control Chemist, Campbell Soup Company, Sacramento, California, June 1969 - Sept 1969.

Awards

- Fellow, Academy of Toxicological Sciences, 1987. Recertification granted, 1991, 1997, and 2002.
- Fellow, American College of Nutrition, 1993
- Certified Nutrition Specialist, 1993
- Awarded Sabbatical Leave, University of Nevada, Reno, 2001.
- Recipient of Exemplary Service Plaque for efforts as Division Councilor, Toxicology and Safety Evaluation Division, IFT, 1995-1998.
- Recipient of Leadership Award from the Food Safety Specialty Section, Society of Toxicology, 1995.
- Exemplary Service Plaque from the Toxicology and Safety Evaluation Division of the Institute of Food Technologists, 1994.
- Special Act Award, Letterman Army Institute of Research, Presidio of San Francisco, CA., 1989..

- Letter of Commendation of Commendable Performance of Duties by the Institute Commander, Letterman Army Institute of Research, 1976.
- Recipient of NIH Trainee ship Award in Pulmonary Biochemistry and Inhalation Toxicology (Nat'l. Heart, Lung and Blood Institute), 1975.
- RhoChi (National Honor Pharmacy Society), 1972.
- Recipient of Mary Aaron Scholarship, 1963 - 1965.

Membership

- Editorial Board Member/Associated Editor, Toxicology, Elsevier, Amsterdam, 1998-Present
- Editor/Co-Founder, Environmental and Nutrient Interactions, Taylor & Francis, 1997
- Editorial Board Member, Society for Experimental Biology and Medicine, New York, NY, 2002-2005
- Editorial Board Member, Nutritional and Environmental Medicine, Taylor & Francis, 2000-Present
- Editorial Board Member, Inhalation Toxicology, Taylor & Francis, 2002-2004.
- Editorial Board Member, Journal of Food Protection, 2003-Present.
- Member, Board of Scientific Advisors of the American Council on Science and Health, 1989-present
- Electronic Journal Editorial Boards: American J. Food Technology; Asian J of Clinical Nutrition; Asian J of Epidemiology; Asian J of Scientific Research; J Pharmacology and Toxicology; Research J of Environmental Sciences; Research J of Environmental Toxicology

Professional Society Memberships, Elected Positions, Advisory and Consulting Activities

- Elected to Councilor, Food Safety Specialty Section, Society of Toxicology, 2007-2010.
- Appointed Food safety Specialty Section Newsletter Editor, Society of Toxicology, 2007-2010.
- Appointed Panel Member, IOM, National Academy of Sciences, 2004-06.
- Invited Panel Member, CSREES Review, Department of Food Science and Toxicology, University of Idaho, 2005.
- Panel member, study group for nutrition and health, USDA CSRS NRI competitive grants, 1996, 2002-2005.
- Panel Member for reviewing grants, USDA, SBIR, 2004
- Invited Speaker Chairperson, Food Safety and Nutraceuticals, Society of Toxicology Annual Meeting, Baltimore, Maryland, 2004.
- Invited Speaker, "Food Toxicology", International Food Protection Conference, Phoenix, AZ, 2004.
- Invited Symposium Chair, 12th World Food Science and Technology Congress. An International Meeting scheduled for 2003 in Chicago, ILL.
- Member or Review Panel, Research Competitiveness Subprogram (RCS) of the Louisiana Board of Regents' R & D Program, 2001 – Present
- Member of Review Panel, Midwest Advanced Food Manufacturing Alliance, 2000 – Present
- Appointed to Assessment Panel for Washoe District Department Health Officer Candidates, Reno/Sparks, Washoe County, Nevada, 1998 and 1999.
- Appointed to the Mead Johnson Award Selection Committee, American Society for Nutritional Sciences, 1998-99.
- Appointed to the Expert Panel on Food Safety and Nutrition, Institute of Food Technologists (three year term), 1998.
- Elected member of the Certification Board for Nutrition Specialists (three year term, 1997.
- Appointed to the Committee for Divisions and Sections, Institute of Food Technologists (three year term), 1996.
- Appointed as Alternative Executive Committee Member, Toxicology and Safety Evaluation Division, Institute of Food Technologists, 1995.
- Nominated to run as Treasurer, Executive Committee, Northern California Chapter of the Society of Toxicology, 1995.
- Appointed to the Program Committee for the Society of Toxicology (three year term), 1994.
- Advisory Committee member for National Association of State Universities and Land-Grant Colleges (NASULGC). Committee authored a position paper on, "Food Safety, Diet, Nutrition and Health", 1994.
- Study Group member/reviewer for SOH/DRG/NIH competitive grants for three years (1993 - 1996).
- Ad Hoc Committee member for NIH Nutrition Study Section, 1993.
- Elected President, Food Safety Specialty Section, Society of Toxicology, 1993 - 1994.
- Elected Chairman, Division of Toxicology and Safety Evaluation, Institute of Food Technologists for 1992 - 1993.
- Nominated to run for the Executive Committee, American College of Toxicology, 1993.
- Member of Team for the evaluation of the Interdepartmental Graduate Toxicology Program, Iowa State University, Ames, Iowa, 1993.
- Technical Advisor, Toxa Chemica, International, Rockville, Maryland. 1993 - 1998.

- Appointed to the Program Committee for the Institute of Food Technologists (three year term), 1991.
- Appointed by the Board of Directors to the Board of scientific Advisors of the American Council on Science and Health, New York, NY, 1989 - present.
- Elected to executive board of the Toxicology and Safety Evaluation Division of IFT in 1989. Elected to two year term.
- Elected Treasurer of the Northern California Chapter of the Society of Toxicology, 1989 (two year term).
- Consultant to the Departments of Community Medicine and Biochemistry, University of Hong Kong for investigations regarding the effect of diet on heavy metal toxicity, 1987 - 1991.
- Provided consultation to the Permanent Senate Subcommittee investigating nutritional quackery, Washington, D. C., 1985.
- Member of program committee for the annual meeting of the Gene and Environmental Toxicologist Society, 1988.
- Member of program committee for the annual meeting of the Northern California Society of Toxicologist, 1988 & 1989.
- Nomination to standing "Membership Committee" of the American College of Toxicology, 1988.
- Nominated to membership committee, Association of Government Toxicologists, Washington DC, 1988.
- Appointed representative from SOT to the Joint Societies Workshop on Food Safety. Served as Chair for writing the position paper on, Naturally Occurring Toxicants. 1988.
- Editor of IFT Toxicology and Safety Evaluation Division Newsletter, 1988 - 1993. Associate Editor between 1986 - 1988.
- Appointed participant sponsor for National Research Council Fellowship, 1987-88. Appointed participant sponsor for 2nd National Research Council Fellowship, 1988-89.
- At the request of FDA acting as USA advisor to the University of Hong Kong regarding, "An Investigation of Nutritional Imbalance and Carcinogenicity", 1987-1989.
- Elected Secretary of CSRS Project W-143 Committee on Nutrient Bioavailability--A Key to Human Nutrition, 1986-1987.
- Appointed to the Interim Executive Committee for the Northern California Chapter of the Society of Toxicology, 1986.
- Appointed to the Committee on making recommendations for the Institute of Food Technologists' on our present state of knowledge of the nutritional problems facing our aged population.
- Appointed to the Committee for National Seminars on "Diet and Health". Held at the University of California, Davis, Sept. 1985.
- Society of Toxicology`s designated liaison representative to the Institute of Food Technologists; 1982 - 1998.
- Society of Toxicology`s designated liaison representative to the American Institute of Nutrition; 1982 - 1998.
- Peer Review Panel/Research Personnel Evaluation, U. S. Department of Agriculture, Agricultural Research Service, Western Region; 1982 - 1987.
- Biological Safety Committee, U.S. Department of Agriculture, Agriculture, Agricultural Research Service, Western Regional Research Center, 1982-1983.
- Internal Grant Review Committee, U.S. Department of Agriculture, Agricultural Research Service (Review application for new and established investigator awards); 1981-1982.
- Served on special committee on the revision of USDA Directive and Manual 232.2: "Laboratory Use of Chemical Substances of Potential Carcinogenic Risk", 1980.

Publications (partial list since 2000):

(a) In Refereed Journals (92):

- Chen, L., Yang, W., Jennison, B. L. and **Omaye, S. T.** Air particulate pollution and hospital admissions for chronic obstructive pulmonary disease in Reno, Nevada. *Inhalation Toxicol* 12: 281-298, 2000.
- Zhang, P. and **Omaye, S. T.** $-Carotene and protein oxidation: Effect of ascorbic acid and "-tocopherol. *Toxicology* 146: 37-48, 2000.
- Chen, L., Jennison, B. L., Yang, W., and **Omaye, S. T.** Air pollution and elementary school absenteeism. *Inhalation Toxicol,* 12: 997-1016, 2000.
- Zhang, P. and **Omaye, S. T.** DNA strand breakage and oxygen tension: Effects of $-carotene, "-tocopherol and ascorbic acid. *Food Chem Toxicol* 39: 239-246, 2001.
- Zhang, P. and **Omaye, S. T.** Antioxidant and prooxidant roles for $-carotene, "-tocopherol and ascorbic acid in human lung cells. *Toxicol in vitro* 15: 13-24, 2001.
- Zhang, P. and **Omaye, S. T.** $-Carotene: Interactions with "-tocopherol and ascorbic acid in microsomal lipid peroxidation. *J Nutri Biochem* 12: 38-45, 2001.

- Chen, L., Yang, W., Jennison, B. L., Goodrich, A. and **Omaye, S. T.** Using two different statistical models to compare the associations between PM10 and hospital admissions for COPD. *Toxicol Meths,* 11: 233-246, 2001.
- Chen, L., Yang, W., Jennison, B. L., Goodrich, A. and **Omaye, S. T.** Air pollution and birth weight in Northern Nevada, 1991-1999. *Inhalation Toxicol* 14: 141-158, 2002.
- Nakamura, Y. K. and **Omaye, S. T.** Age-related changes of serum lipoprotein oxidation in rats. *Life Sciences* 74: 1265-1275, 2004.
- Elsayed, N. M. and **Omaye, S. T.** Biochemical changes in mouse lung after subcutaneous injection of the sulfur mustard 2-chloroethyl 4-chlorobutyl sulfide. *Toxicology* 199: 194-206, 2004.
- Flintoff-Dye, N. L. and **Omaye, S. T.** Antioxidant effects of conjugated linoleic acid isomers in isolated human low-density lipoproteins. Nutr Res 25: 1-12, 2005
- Nakamura, Y. K., Read, M. H., Elias, J. W., and **Omaye, S. T.** Oxidation of serum low-density lipoprotein and antioxidant status in young and elderly humans. *Arch Gerotol Geriatrics* 42: 265-276, 2006.
- Cirico, T. L., and **Omaye, S. T.** Additive or synergetic effects of phenolic compounds on human low density lipoprotein oxidation. *Food Chem Toxicol* 44: 510-516, 2006.
- Badr El-Din, N. and Omaye, S. T. Concentration dependent antioxidant activities of conjugated linoleic acid and alpha-tocopherol in corn oil. *J Sci Food Agriculture* (In press), 2007.

(b) Books, Book Chapters and Reviews Articles (34):

- Bidlack, W. R., **Omaye, S. T.**, Meskin, M. S. and Jehner, D. Phytochemicals As Bioactive Agents. Technomic Publishing Co., Inc. Pennsylvania, 2000.
- Dubick, M. A. and **Omaye, S. T.** Modification of atherogenesis and heart disease by grape wine and tea polyphenols. In: Nutraceuticals Handbook, (Wildman, R., Ed.) CRC press, pp. 235-260, 2001.
- Chen, L. and **Omaye, S. T.** Air pollution and health effects in northern Nevada. *Rev Environ Health* 16:51-67, 2001.
- Dubick, M. A. and **Omaye, S. T.** Evidence for grape, wine and tea polyphenols as modulators of atherosclerosis and ishemic heart disease in humans. *Nutraceuticals, Functional & Medical Foods* 3: 67-93, 2001
- Meskin, M. S., Topham, D. K. W., **Omaye, S. T.**, Bidlack, W. R. Phytochemicals in Nutrition and Health, CRC Press, Boca, Raton, FL, 2002.
- **Omaye, S. T.** Metabolic modulation of carbon monoxide toxicity. *Toxicology* 180: 139-150, 2002.
- Hussein, H. S., and **Omaye, S. T.** Introduction to the food safety concerns of Verotoxin-producing *Escherichia coli. Exp Biol Med* 228: 331-332, 2003.
- **Omaye, S. T.** Introduction to food toxicology. In: *Pesticides, Veterinary and Other Chemical Residues in Food.* Watson, D., Ed., Woodhead Publishing Ltd., pp, 1-26, 2004.
- **Omaye, S. T.** *Food and Nutritional Toxicology*, CRC Press, 2004.
- Todd, S. J. T. and **Omaye, S. T.** Maternal environmental tobacco exposure and in utero and adverse birth outcomes. *Inhalation Toxicology*, 2nd edition, Salem, H. and Katz, S. A., Eds., pp, 875-912, CRC Press, Roco Ratan, FL, 2006.
- Dubick, M. A., and **Omaye, S. T.** Grape wine and tea polyphenols in the modulation of atherosclerosis and heart disease. In: Nutraceutical Handbook, 2nd edition (Wildmann, R., Ed.) CRC Press, 2007.

(c) Other Professional Publications (41):

- **Omaye, S. T.**, and Elsayed, N. M. Editorial: A new beginning. *Toxicology*, 48: 81-83, 2000.
- Bendich, A., Elsayed, N. M., and **Omaye, S. T.** Editorial: Lawrence J. Machlin, Ph.D., FACN, 1927 - 2000. *Toxicology* 159: 117-118, 2001
- **Omaye, S. T.** Biotechnology and sustainable agriculture. *Food Techn* 55 (February): 23, 2001.
- **Omaye, S. T.** Preventing BSE in the U.S. *Food Techn* 55 (April): 26, 2001.
- **Omaye, S. T.** Shark-fin soup and methylmercury: To eat or not to eat. *Food Techn* 55 (October): 26, 2001.
- **Omaye, S. T.** Cooperating to meet food safety challenges. *Food Tech* 57: 24, 2003.

(d) Published Abstracts (88):

- Dubick, M. A., Williams, C., **Omaye, S. T.**, Villarreal, R. L., Herndon, D. N., Goodwin, C. W., and Kramer, G. C. High dose ascorbic acid infusion reduces net fluid balance in sheep subjected to a 40% TBSA burn. *J Burn Care & Rehabilitation*, 21: S144, 2000
- Chen, L., Jennison, B. L., Yang, W., and **Omaye, S. T.** Air pollution and elementary school absenteeism. *Toxicologist* 54: 15, 2000
- Chen, L., Jennison, B. L., Yang, W., and **Omaye, S. T.** Particulate air pollution and hospital admissions for chronic obstructive pulmonary disease in Reno, Nevada. *Toxicologist* 54:15, 2000

- Zhang, P. and **Omaye, S. T.** Oxygen tension and DNA strand breakage: Effects of antioxidants. *American College of Nutrition Annual Meeting*, Las Vegas, NV, 2000.
- Chen, L., **Omaye, S. T.**, Yang, W., and Jennison, B. L. Comparisons between ARIMA and GAM applications in the same data analysis. *Twelfth Conference of the International Society of Environmental Epidemiology*, University at Buffalo, New York., 2000
- Chen, L., **Omaye, S. T.**, Yang, W., and Jennison, B. L. Particulate air pollution and hospital admissions for COPD: Two statistical models. one result. *Toxicologist* 60: 190, 2001.
- Carughi, A., **Omaye, S.** and Furst, A. Carotenoid levels before and after supplementation with a mixture of vegetable extracts. *FASEB*, 2001.
- Carughi, A., and **Omaye, S.** Plasma carotenoid levels before and after supplementation with mixture of vegetable oleoresins. *Ventura Gordon Conference*, Ventura, California, 2001.
- Yamauchi, M., Fernandez, G. C. J., and **Omaye, S. T.** Multiple linear regression analysis of total mercury levels in blood and urine samples. SAS Annual Meeting, San Diego, CA, 2002.
- Cheng, H., **Omaye, S. T.,** Yang, W., and Calderon, R. Mortality rates of Churchill county, rural Nevada, and Nevada state. Fifth International Conference on Arsenic Exposure and Health Effects, San Diego, CA, 2002.
- Maher, T., **Omaye, S. T.**, Nagle, D., Wren, A. F., Geraci, C. L., Zumwalde, R. D., Miller, A. L., and Hoover, M. D. A case study in partnering to develop a nanotechnology occupational safety program in a nanotechnology manufacturing environment. Second International Symposium on Nanotechnology and Occupational Health, Minneapolis, Minnesota, Oct, 2005.
- Elsayed, N. M., and **Omaye, S. T.** Pulmonary biochemical alterations induced by systemic administration of nitrogen mustard. Society of Toxicology Annual Meeting and published in the "Toxicologists", San Diego, California, April, 2006.
- Wilson, M. P., **Omaye, S. T.**, and Surrendera-Babu, A. Food handling practices and eating preferences of child and adult care providers. Society for Nutrition Education, Annual Meting, San Francisco, California, 2006.

Appendix G

Unpublished Safety Studies on Neptune Krill Oil™

Neptune Krill Oil™ Human Safety Assessment

Evaluation of the Effects of Neptune Krill Oil™ on UVB-Radiation Induced Skin Cancer

Pages 000076-000096 removed under the Privacy Act of 1974.

GRAS ASSOCIATES, LLC
Generally Recognized As Safe
20482 Jacklight Lane Bend, OR 97702-3074 541 678-5522
mcquate@gras-associates.com www.gras-associates.com

January 30, 2008

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

02-04-08 ^11:59 OUT

Attention: Dr. Robert L. Martin

Re: GRAS Notification for High Phospholipid Krill Oil

Dear Dr. Martin:

Attached you will find the content of Appendix G that is associated with the above-referenced GRAS Notification. The reference to the confidential nature of the content of the unpublished reports has been removed, and the noted waiver of confidentiality as outlined in the previous transmittal letter of January 18, 2008 has been removed.

I trust that these actions will remove all questions or concerns regarding confidential content supplied as part of our GRAS notification.

We look forward to your feedback as your review progresses.

Thank you.

Sincerely,

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosure: GRAS Notification – High Phospholipid Krill Oil Appendix G (in triplicate)

000097

GRAS ASSOCIATES, LLC
Generally Recognized As Safe
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mcquate@gras-associates.com www.gras-associates.com

January 30, 2008

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-200)
5100 Paint Branch Parkway
College Park, MD 20740-3835

02-04-08 A11:59 OUT

Attention: Dr. Robert L. Martin

Dear Dr. Martin:

On behalf of Neptune Technologies & Bioressources of Laval (Quebec), Canada, we are submitting for FDA review a GRAS notification for High Phospholipid Krill Oil. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely.

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosure: GRAS Notification – High Phospholipid Krill Oil (in triplicate)

000098

Neptune Krill Oil (NKO™)

Human Safety Assessment

Report to:

NEPTUNE TECHNOLOGIES & BIORESSOURCES INC.

Department of
Research & Development

Submitted by:

JSS medical research inc.

September 30, 2002

recherche médicale JSS medical research

Neptune Krill Oil™
Human Toxicity Assessment
September 30, 2002
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2.1 STUDY OBJECTIVES 1
2.2 LONG TERM SAFETY ASSESSMENT / ANIMAL STUDIES: 1
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1.0 Background

Neptune Krill Oil™ is a complex natural health product extracted from a crustacean, habitant of the Antarctic Ocean known as the Antarctic Krill (Euphausia superba). In general, this product consists of a natural blend of polyunsaturated fatty acids, phospholipids, antioxidants, metals and minerals.

Neptune Krill Oil is intended for human consumption as a dietary supplement, beneficial for the maintenance of a normal and healthy function of body systems.

2.0 Safety Assessment:

2.1 Study Objectives

The objective of the present studies was to investigate the possible toxicity of consumption of Neptune Krill Oil (NKO™) in humans by determining a variety of physical, biochemical and hematological variables. These variables reflect kidney, liver, pancreatic, and hematological, respiratory, intestinal, musculoskeletal and general biological function.

2.2 Long Term Safety Assessment / Animal studies:

2.2.1 Study Design

This was a prospective controlled trial where all animals were submitted to a high dose of NKO™.

2.2.2 Animal model:

The animal model used was C57BL6 Nude Congenic Mice – B6NU-T heterozygote.

Mice were kept in a controlled environment with a 12 hour regulated light – dark cycle.

2.2.3 Treatment:

Animal dose: 16.6% daily diet or 3g per 21.94cm^2 mean body surface

Human equivalent: 9 grams of EPA and DHA per day (70 kg person) which corresponds to 23.1 grams of oil or 7 – 11 times the recommended dose.

Duration: 6 months

Method: All animals were evaluated weekly by a certified vetenerian. At the end of 6 months all animals euthanized by gas exposure and all organs were submitted to histopathological analysis

2.2.4 Results:

Clinical observations: No adverse effects reported

Pathology results: autopsies performed on all systems revealed no pathological findings. The following organs were examined:

a. Brain
b. Lungs
c. Heart
d. Stomach
e. Pancreas
f. Liver
g. Kidneys

h. Uterus / prostate

i. Intestine

j. Skin

3.0 Hman Safety Assessment:

3.1 Study Design

This was a prospective controlled trial where all patients were submitted to a high dose of NKO™.

3.2 Patients

The study enrolled 25 healthy volunteers, 13 women and 12 men aged between 25 and 53 years, with a mean age of 42.

3.3 Materials & Methods

All volunteers were advised to take NKO™ 2 gelcaps three times per day for 2 months. Each gelcap contained 1 gram of NKO™.

3.3.1 NKO™ Contents

Component	mg / gelcap	mg / 6 gelcaps
Omega-3	386	2 316
Phospholipids	416	2 496
Astaxanthin	0.16	0.96

All participants were advised to continue with their usual nutrition habits and to refrain from initiating any special diet.

3.4 Outcome Measures

3.4.1 General Parameters tested

1. Upon initiation:
   a. Complete blood count and biochemical blood test
   b. Vital signs
   c. Medical history
2. Every month:
   a. Monitoring of vital signs
   b. Complete blood count and biochemical blood test
   c. Adverse events / Regurgitation

3.4.2 Biochemical parameters tested (analyzed at an independent laboratory):

a. Complete blood count
b. PTT
c. Creatinine
d. Glucose
e. Alkaline Phosphatase
f. Albumin
g. Amylase
h. Bilirubin total

i. Bilirubin direct

j. Cholesterol

k. Triglycerides

l. HDL + LDL

m. Cholesterol / HDL ratio

n. Urea

o. TSH

Patients were advised to stop if they notice any of the following symptoms occurring without a logical reason:

a. Low blood pressure (< 90/65)

b. High blood pressure (3 or more points above your usual blood pressure)

c. Difficulty breathing

d. Bleeding

e. Loss of consciousness (faint, dizzy)

f. Unusual migraines

g. Unusual body pain

h. Fatigue, weakness

i. Weight gain

j. Significant alterations in your blood test

3.5 Results:

- ✓ No serious side effects were observed.
- ✓ Of the25 healthy volunteers enrolled in the study, one female patient with known salt intolerance withdrew due to a moderate increase in water accumulation.
- ✓ Of the remaining 24 patients two women complained of rapidly increasing greasiness of their facial skin and requested to be withdrawn from the study.
- ✓ The 22 remaining volunteers completed the 2-month period without any noticeable physical or laboratory adverse events.
- ✓ Among the 25 healthy volunteers participating in the trial there were no complaints of regurgitation or unpleasant aftertaste.

3.5.1 Observed benefits:

- ✓ Increased ability to concentrate
- ✓ Decreased seasonal allergy symptoms
- ✓ Increased skin hydration
- ✓ Improved hair texture
- ✓ Decreased joint discomfort
- ✓ Minimized PMS emotional and physical symptomatology

The table below describes the changes in the laboratory tests between the baseline and follow up assessments:

Parameter:	Mean Change Baseline – Follow UP	Std. Deviation	95% Confidence Interval of the Difference		P Value
			Lower	Upper	
Hemoglobin	1.9565	6.65704	-.9222	4.8352	.173
Hematocrit	.0179	.01775	.0172	.0176	.6994
Platelets	-14.2174	53.77390	-37.4710	9.0362	.218
PTT	1.3391	3.96023	-.3734	3.0517	.119
Glucose	.0883	.65303	-.1941	.3707	.524
Urea	.0652	.84294	-.2993	.4297	.714
Creatinine	7.4348	7.25061	5.0021	9.8675	.650
Cholesterol	*.4675*	*.65512*	*.1909*	*.7441*	*.002*
Triglycerides	*.1937*	*.36865*	*.0381*	*.3494*	*.017*
HDL	*-.1387*	*.21955*	*-.2315*	*-.0460*	*.005*
LDL	*.3662*	*.70315*	*.0693*	*.6632*	*.018*
CHOL/HDL	*.5604*	*.72054*	*.2562*	*.8647*	*.001*
Albumin	*1.4091*	*2.64861*	*.2348*	*2.5834*	*.021*

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Bilirubin Total	.2174	1.95301	-.6272	1.0619	.599
Bilirubin Direct	-.1182	.42047	-.3046	.0682	.202
ALPP	1.1818	8.30741	-2.5015	4.8651	.512
GGT1	.9565	9.97943	-3.3589	5.2719	.650
Amylase	***3.4348***	***5.07960***	***1.2382***	***5.6314***	***.004***
NA	.5000	2.01778	-.3946	1.3946	.258
K	-.1545	.58369	-.4133	.1042	.228
TSH	-.0354	.40912	-.2216	.1508	.696

These results show that the values for following parameters: Cholesterol, LDL, Albumin and Amylase decreased significantly during the study. For HDL there was a statistically significant increase between baseline and the final follow up. There were no adverse events observed in the study sample. These results show that NKO™ is well tolerated without any expected adverse reactions when consumed on a high dose by humans.

3.6 Conclusion:

The results of the present toxicity assessment confirm that Neptune Krill Oil (NKO™) can be considered as safe for human consumption even at double the highest recommended dose.

Note: People with a known allergy to fish or seafood were not tested in this study. The precaution remains that in the case of the above allergies, previous professional allergy testing is advised prior to consumption of Neptune Krill Oil (NKO™).

Evaluation of the Effects of Neptune Krill Oil ™ on UVB-Radiation Induced Skin Cancer

recherche médicale

Final Report to:

JSS

NEPTUNE TECHNOLOGIES & BIORESSOURCES INC.

medical research

Submitted by:
JSS medical research inc.

November 18th, 2002

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Evaluation of the Effects of Neptune Krill Oil™
on UV-Radiation induced Skin Cancer Prevention

Introduction

Euphasia Pacifica, commonly known as Krill, make up the order of Euphausiacea, subclass of Malacostraca, class of Crustacea and phylum of Anthropoda. They are small shrimp or prawn-like crustaceans, habitants of oceans off the West coast of Vancouver Island, Russia, Ukraine and Japan. Approximate area of distribution at the Circum-polar in Antarctic waters is 35 million square kilometers. Approximately 90 species have been recognized, most of which range in length from 8 to 70 mm (mean length 16 mm.). Considering a population size of 500 million tones, Krill are the most important Zooplankton group in the world oceans after Copepods. Major predators are Baleen whales (blue, fin, mink), crabeater seals, fur seals, Adelie and macaroni penguins, petrels, fulmars and shearwaters, squid and fish. In Japan humans consume them as an ethnic delicacy.

NKO™ is extracted from the body of Krill with a innovative process of lipid extraction which produces a dehydrated residue. The most significant components of NKO™ are the Omega-3 fatty acids, containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), all-trans retinol or vitamin A, vitamin E, Astaxanthin and Canthaxanthin. Scientific evidence suggests that Omega-3 and antioxidants may have both preventive and

therapeutic properties for systemic disease, mainly cardiovascular and neoplastic.

Neoplastic disease is the second most frequent cause of morbidity and mortality in North America. In the year 2000, mortality is expected to rise to 552,200 Americans, which amounts to over 1,500 people per day. The incidence of malignancies is expected to rise to 1,220,100 this year. Since 1990, near 13 million new cases have been diagnosed excluding non-invasive cancers and squamous cell skin cancers. The incidence of squamous cell skin cancer alone within the year 2000 is expected to rise to 1.3 million (Source: American Cancer Society).

Since 1993 it has been observed that cancer mortality is decreasing while the incidence is increasing. Therefore, the number of cancer survivors is rising along with a respective rise in morbidity. Consequently, the health resources consumed by neoplastic disease are progressively elevating. According to the Pharmaceutical Industry Profile for the year 2000, reported by the Pharmaceutical Research and Manufacturers of America, 14,869 million dollars are spent on prescription drugs for neoplastic disease alone, covering 19.7% of the annual market.

Ultraviolet radiation plays an unequivocal role in the etiology of skin cancers such as basal and squamous cell carcinoma and melanoma. Exposure of skin to ultraviolet radiation can cause both short and long term adverse effects. The short-term effects are sunburn and erythema due to photosensitivity of skin. The long-term effects include aging and carcinogenesis. Studies suggest

that UVR-induced inflammation is caused by an increased production of prostaglandins and cytokines. UVR generates free radicals, which cause membrane damage, including skin damage. Retinols have been proven to have prophylactic effects against UV radiation induced skin cancer (Tsambaos D., Sampalis F: UV-radiation induced skin cancer: Inhibition by oral arotinoids. Gior. Ital. Chir. Dermatol. Oncol. 2:409 - 412, 1987).

Oxidation is the chemical process by which an ion from an atom or molecule steals of one or more of another's electrons altering the chemical structure irreversibly. The chemicals that exhibit this tendency for stealing electrons are referred to as oxidizing agents. We are constantly exposed to oxidative stress in our everyday environment by air pollution, tobacco smoke, exposure to chemicals, and exposure to ultraviolet (UV) light or other forms of ionizing radiation (Møller *et al.* 1996; Papas 1999). These oxidants change the chemical structure of DNA and proteins causing various pathological conditions including aging (Harman 1981; Ames and Shigenaga 1992), atherogenesis (Steinberg *et al.* 1989; Esterbauer *et al.* 1992), and carcinogenesis (Moody and Hassan 1982; Marnett 1987; Breimer 1990). Antioxidants protect the human body from oxidative damage by scavenging of radicals to prevent or terminate chain reactions and quenching of singlet oxygen and dissipating the energy as heat. astaxanthin has been proven to be twice as effective as beta-carotene (and about 80 times more effective than vitamin E) in quenching singlet oxygen in chemical solution (Di Mascio *et al* . 1991); and about 50% more effective than beta-carotene and zeaxanthin, in preventing fatty acid peroxidation in chemical solution (Terao 1989). In a membrane model, astaxanthin was found to be more

effective at scavenging peroxyl radicals than was beta-carotene (Palozza and Krinsky 1992).

A systemic photoprotective agent, which would neutralize free radicals, could prevent UVR-induced skin damage and short as well as long term adverse events. Neptune Krill Oil™ (NKO™) is a marine oil composed of a natural mixture of essential nutrients. It is characterized by its high content of phospholipids with substantially high quantities of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) both occupying the two fatty acid chains of the same molecule and potent antioxidants comprised of vitamins A and E, esterified astaxanthin, and a marine source novel flavonoid.

Omega-3 polyunsaturated fatty acids (EPA & DHA) and Omega-9 (Oleic acid) along with the high content of antioxidants in NKO™ justify further investigations of possible anticarcinogenic properties of a NKO™ preparation. The objective of this trial was to evaluate the photoprotective potential of NKO™ against UVB-induced skin cancer.

Materials and Methods

This was a randomized controlled pre-clinical trial. The animal model used was C57BL6 Nude Congenic Mice – B6NU-T heterozygotes because of the species-specific genetic susceptibility to skin cancer.

Mice were kept in a controlled environment with a 12 hour regulated light – dark cycle. In order to obtain an acceptable significant reduction = 25%

(δ = 0.33), statistical significance of 95% ($P \leq 0.05$) and a certainty of 80%, 96 mice were included in the study.

Male and female mice were divided equally. In order to establish efficacy of NKO™ for the prevention of skin cancer, the test was conducted as a randomized double blind controlled trial. The mice were randomized in two groups of 48 mice. One group was treated with NKO™ and the other with active control or Soya oil. Each group was subdivided in three subgroups of 16 mice each depending on the mode of treatment; oral, topical or topical/oral respectively. The diet of the orally treated groups was supplemented with 10% of their daily intake with NKO™ or Soya oil. The daily oral dose given to the mice was equivalent to that of 2 g of NKO™ per day for a 70 kg man or a 60 kg woman. All mice were exposed to 30 minutes of UVB radiation per day. The distance between the mice and the lamps was set at 30cm. The dosage of radiation exposure was calibrated daily. According to animal specifications, the maximum time of exposure required for the development of cutaneous malignancy is 20 weeks at which time all remaining animals were euthanized by ether. All specimens were histologically examined for the presence of malignant pathology.

Results

Table 1: Outcome by Group and Method of Administration

	Outcome						Total	
Group	Cancer		Pre-Malignant		Normal			
	N	%	N	%	N	%	N	%
Krill Oral	**3**	**18.8%**	**3**	**18.8%**	**10**	**62.5%**	**16**	**100.0%**
Krill Topical and Oral	3	18.8%	5	31.3%	8	50.0%	16	100.0%
Krill Topical	2	12.5%	5	31.3%	9	56.3%	16	100.0%
Placebo Oral	6	37.5%	3	18.8%	7	43.8%	16	100.0%
Placebo Oral and Topical	6	37.5%	2	12.5%	8	50.0%	16	100.0%
Placebo Topical	6	37.5%	5	31.3%	5	31.3%	16	100.0%
Total:	26	27.1%	23	24.0%	47	49.0%	96	100.0%

Statistical Significance Testing for Incidence of Cancer

1. Krill Oral vs. Krill Topical vs. Krill Oral/Topical: ***P = 0.42***

Clinical Significance Testing for Incidence of Cancer

1. Krill Oral vs. Placebo Oral: ***49.7% reduction of incidence***
2. Krill Topical vs. Placebo Topical: ***49.7% reduction of incidence***
3. Krill Oral/Topical vs. Placebo Oral/Topical: ***66.6% reduction of incidence***

Table 2: Outcome by Overall Treatment Group

Group	Outcome						Total	
	Cancer		Pre-Malignant		Normal			
	N	%	N	%	N	%	N	%
Krill	8	16.7%	13	27.1%	27	56.3%	48	100.0%
Placebo	18	37.5%	10	20.8%	20	41.7%	48	100.0%
Total:	26	27.1%	23	24.0%	47	49.0%	96	100.0%

Overall Statistical Significance Testing for Incidence of Cancer

Krill vs. Placebo : ***P = 0.04***

These results show that overall Neptune Krill Oil™ significantly prevents the incidence of skin cancer. The analysis comparing different modes of administration showed that all three methods were effective within clinical significance. A different study would need to be designed for the specific evaluation of the three different modes of administration, in order to determine the preferred application method of choice.

Conclusion

The results of the present study clearly indicate that Neptune Krill Oil™ can significantly prevent skin cancer induced by chronic exposure to ultraviolet radiation.

SUBMISSION END