DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date FEB - 9 1998

From Acting Director, Division of Programs and Enforcement Policy, Office of Special Nutritionals, HFS-455

2587 '98 MAR 17 P1:4

Subject 75-Day Premarket Notification for New Dietary Ingredients

To Dockets Management Branch, HFS-305

New Dietary Ingredient:	SeaGold™DHA-rich oil
Firm:	Monsanto Company
Date Received by FDA:	December 22, 1997
90-Day Date:	March 22, 1998

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after March 22, 1998.

Sincerely yours,

for James Tanner, Ph.D.
Acting Director,
Division of Programs and
Enforcement Policy
Office of Special Nutritionals
Center for Food Safety and
Applied Nutrition

Attachment

cc:
HFS-22, CCO
HFS-450 (r/f, OSN w/control slip:TRAC#56528 & cpy incoming)
HFS-456 (r/f, Latham, Moore)
r/d:HFS-456:JELatham:jel:01/30/98:DocName:#56528.mem:Disc4

95S-0316

RPT 17

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

FEB -9 1998

Dr. Wayne Stargel, Pharm.D.
Vice President, Regulatory Affairs
Monsanto Company
5200 Old Orchard Road
Skokie, Illinois 60077

Dear Dr. Stargel:

This is to notify you that your submission pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the act) dated December 19, 1997, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., SeaGold™DHA-rich oil) was received by the Food and Drug Administration (FDA) on December 22, 1997. Your submission will be kept confidential for 90 days from the date of receipt, and after March 22, 1998, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have questions concerning this matter.

Sincerely yours,

James Tanner, Ph.D.
Acting Director
Division of Programs and
Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

Monsanto

Monsanto Company
5200 Old Orchard Road
Skokie, IL 60077
Phone: (847) 982-7000

December 19, 1997

Notification of New Dietary Ingredient

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
200 C Street, SW
Washington, DC 20204

To the Food and Drug Administration (FDA):

Pursuant to the Dietary Supplement Health and Education Act of 1994 (DSHEA), 21 U.S.C. § 350b (a) (2), and consistent with the new final regulations published by the FDA in the Federal Register of September 23, 1997 (62 Fed. Reg. 49886-49892), 21 C.F.R. § 190.6, "Requirement for premarket notification," Monsanto hereby submits the following information concerning a new dietary ingredient that Monsanto intends to begin marketing for use in dietary supplements. The new ingredient contains, with the possible exception of one component present in small amounts, only components already present in the food supply. Pursuant to the applicable provisions of the DSHEA, 21 U.S.C. § 350b (a) (2), Monsanto will not introduce the ingredient or deliver it for introduction into interstate commerce until at least 75 days after the date on which FDA receives this notification.

(1) NAME AND ADDRESS OF MANUFACTURER

The name and complete address of the manufacturer of the new dietary ingredient are as follows:

Manufacturer:
Monsanto Company
800 N. Lindbergh Blvd.
St. Louis, MO 63167

Direct correspondence to:
Robert C. Peterson
Monsanto Company
Worldwide Regulatory Affairs
5200 Old Orchard Road
Skokie, IL 60077

(2) NAME OF NEW DIETARY INGREDIENT

The name of the new dietary ingredient is as follows:

SeaGold™ DHA-rich oil. DHA refers to 4,7,10,13,16,19-docosahexaenoic acid.

(3) DESCRIPTION

The description of the new dietary ingredient is as follows:

SeaGold™ DHA-rich oil is a yellow to light orange-colored oil derived from the heterotrophically grown marine microalgae, *Schizochytrium* sp. The oil is refined, bleached, deodorized, and contains natural tocopherols, ascorbyl palmitate and lecithin or other safe and suitable components for stabilization. d,l-α-tocopheryl acetate may also be added to increase the intake of Vitamin E.

Labeling for the new dietary ingredient will specify that it should be used at up to 1 gram of DHA-rich oil per day to increase the intake DHA.

(4) BASIS FOR THE SAFETY OF DHA-RICH OIL

Summary

SeaGold™, a DHA rich oil, is a new dietary ingredient for use in dietary supplements, derived from the heterotrophically grown marine microalgae, *Schizochytrium* sp. DHA is the most abundant polyunsaturated fatty acid (PUFA) component of the oil (approx. 35% w/w). The oil is intended for use as an ingredient in dietary supplements to increase DHA intake. The suggested intake is up to 1 gram of oil containing up to 350 mg DHA per day. Research has indicated it may be desirable to increase n-3 PUFA intake. Current estimated intake of long chain (LC) n-3 PUFA (eicosapentaenoic acid (EPA) plus DHA) is 75-100 mg/d (Raper *et al*, 1992; Douglass *et al*, 1995). Recommended intakes as high as 800 mg of EPA plus DHA per day have been made (British Nutrition Foundation, 1992).

The microalgal oil source, *Schizochytrium* sp., is a thraustochytrid, a member of the kingdom Chromista. *Schizochytrium* sp. occurs widely in the marine environment and is an indirect component of the human food chain through consumption of fish and other marine animals that feed on the *Schizochytrium* sp. microalgae. There have never been any reports of toxic compounds being produced by members of the thraustochytrids.

The safety of DHA-rich oil is based on the inherent safety of the fatty acid and sterol components of the oil. The safety of these components is based on their history of safe use in food, the small quantities expected to be consumed, extensive knowledge of their metabolism, published safety studies, and the absence of reports of toxicity. The safety is further supported by published studies on a microalgal oil nutritional supplement of similar composition, by the historical safe use of fish oil nutritional supplements of similar

composition, and by corroborative safety studies of the dried microalgal source of the DHA-rich oil.

Manufacture of DHA-rich oil

DHA-rich oil is extracted from dried microalgae which are produced from a fermentation process using a microalgae from the genus *Schizochytrium* sp. The algae are grown via a pure culture heterotrophic fed-batch fermentation process. The organism used is an improved strain of the original wild-type culture (*Schizochytrium* sp. ATCC 20888). The improved strain was derived using a classical mutagenesis screening program, which employed well-accepted techniques commonly used in industrial microbiologic strain improvement programs. The dried microalgae intermediate product is wet milled using commercial-grade n-hexane via a two-stage counter-current process. The solvent is partially removed from the miscella (mixture of oil and solvent), after which the oil concentration is adjusted to ~45 wt% by adding fresh commercial-grade n-hexane. The miscella is chilled to approximately -1°C and held for at least five hours. After filtering and removal of solvent the oil is refined, bleached and deodorized using standard food industry procedures. The National Research Council (1989) suggested that diets contain approximately 0.4 mg d-α-tocopherol per g of PUFA. Therefore, 3 mg of d,l-α-tocopherol acetate are added per g of DHA-rich oil during processing, an amount that amply satisfies this recommendation.

Composition of DHA-rich oil

The fatty acid composition of the DHA-rich oil was determined by a validated method and is shown in Table 1. The DHA-rich oil contained 3.1 ± 1.0% (average ± std. dev., n=5) unsaponifiable material. The sterols present in the unsaponifiable fraction were qualitatively analyzed by gas chromatography-mass spectrometry. Their approximate proportions were determined by peak area % (see Table 2).

Table 1. Fatty acid profile of DHA-rich oil derived from *Schizochytrium* sp. microalgae

FATTY ACID	mg FAME/g oil[1]
Lauric (12:0)	4.0 ± <0.1
Myristic (14:0)	101.1 ± 8.6
Tetradecatrienoic n-3 (TTAn-3)(14:3n-3)	tr-4.5
Palmitic (16:0)	236.8 ± 9.4
Palmitoleic (16:1)	17.6 ± 9.9
Hexadecatrienoic n-6 (HTAn-6)(16:3n-6)	tr-5.0
Stearic (18:0)	4.5 ± 0.5
Vaccenic (VA)(18:1n-7)	tr-13.6
Stearidonic (18:4n-3)	tr-8.5
Dihomo-gamma-linolenic (20:3n-6)	22.1 ± 2.4
Arachidonic (20:4n-6)	9.4 ± 1.7
Eicosatetraenoic n-3 (ETAn-3)(20:4n-3)	tr-4.0
Eicosatetraenoic n-7 (ETAn-7)(20:4n-7)	8.7 ± 0.4
Eicosapentaenoic n-3 (EPA)(20:5n-3)	26.3 ± 6.4
Docosatetraenoic n-9 (DTAn-9)(22:4n-9)	5.4 ± 1.3
Docosapentaenoic n-6 (DPAn-6)(22:5n-6)	135.0 ± 15.0
Docosahexaenoic n-3 (DHA)(22:6n-3)	350.0 ± 24.6
Total FA	936.1 ± 10.1

[1]Fatty acids were converted to fatty acid methyl esters (FAME) prior to quantitation and are reported as fatty acid methyl esters (average values ± standard deviation (n=5)) except when a lot contained a FA below the lowest calibration curve concentration, in which cases, ranges are reported. tr = present (based on similar rf) but below the lowest calibration curve concentration (~4 mg FA/g oil) and therefore not quantified.

Table 2. Sterol profile of DHA-rich oil derived from *Schizochytrium* sp. microalgae

STEROL NAME	*Sterol (% peak area)*[1]
Cholesta-5-en-3-ol (Cholesterol)	25 ± 3
Ergosta-5,22-dien-3-ol (Brassicasterol)	15 ± 3
Ergosta-7,22-dien-3-ol	<5-7
Ergosta-7,24-dien-3-ol	<5-6
Stigmasta-5,22-dien-3-ol (Stigmasterol)	19 ± 2
Stigmasta-5,23-dien-3-ol	8 ± 1

[1] All peaks greater than 5% total peak area are reported as the average ± standard deviation (n=5) except in cases when a lot contained a sterol at <5% of total peak area, in which cases, ranges are reported. The total unsaponifiable fraction is 31 mg/g DHA-rich oil.

Safety of the fatty acid components of DHA-rich oil

The amount of each fatty acid which would be consumed at the proposed use level of up to 350 mg of DHA per day is shown in mg in Table 3.

Table 3. Fatty acid intake from the suggested daily intake of DHA-rich oil

Fatty Acid	Intake (mg) at Proposed Use Level (350 mg DHA)[1]
Lauric (12:0)	4
Myristic (14:0)	101
Tetradecatrienoic n-3 (14:3n-3)	<5
Palmitic (16:0)	237
Palmitoleic (16:1)	18
Hexadecatrienoic (16:3n-6)	<5
Stearic (18:0)	5
Vaccenic (18:1n-7)	<14
Stearidonic (18:4n-3)	<8.5
Dihomo-gamma-linolenic (20:3n-6)	22
Arachidonic (20:4n-6)	9
Eicosatetraenoic n-3 (20:4n-3)	<4
Eicosatetraenoic n-7 (20:4n-7)	9
Eicosapentaenoic (20:5n-3)	26
Docosatetraenoic n-9 (22:4n-9)	5
Docosapentaenoic n-6 (22:5n-6)	135
Docosahexanoic (22:6n-3)	350

[1]Based on the mean of five lots (see Table 1) except for some components of the oil present at trace levels, the highest value across all five lots was used in this table to illustrate the highest likely exposure.

Mammalian cells are able to synthesize saturated and n-9 and n-7 series unsaturated fatty acids *de novo* from acetyl CoA, but lack the Δ12 and Δ15 desaturase enzymes necessary for insertion of a double bond at the n-6 and n-3 positions, respectively, of the fatty acid carbon chain. These n-3 and n-6 FA are *essential* FA, and must be obtained from the diet, usually from vegetable oils, as linolenic acid (18:3n-3) or linoleic acid (18:2n-6). Vegetable oils and fats do not contain C20 or C22 n-6 or n-3 fatty acids in appreciable amounts, although foods of animal origin, particularly egg yolk and meat, liver and other organ meats, contain arachidonic acid and DHA. The longer-chain n-3 fatty acids, eicosapentaenoic acid (EPA) and DHA, are found in highest amounts in high-fat fish and other marine mammals and often constitute the highest concentration of fatty acids within these animals.

Most of the identified fatty acid components in the DHA-rich oil are present in substantial amounts in other foods, and the suggested intake of up to 1g of this oil per day will not significantly increase their intake. The oil is similar in composition to other commercially available oils (Table 4).

Table 4. Typical Fatty Acid Composition % of DHA rich oil and Various Fat and Oils

Fatty Acids	DHA-rich oil[1]	DHASCO oil[2]	Menhaden oil[3]	Salmon oil	Cod-liver oil	Canola oil	Sunflower oil	Corn oil	Olive oil	Palm oil	Butter	Lard
12:0	0.4	4.4	-	-	-	-	-	-	-	0.1	2.3	0.2
14:0	10.1	12.7	9.0	3.3	3.6	-	0.1	0.3	-	1	8.2	1.3
16:0	23.7	9.7	19.0	9.8	10.6	4	5.9	10.9	11	43.5	21.3	23.8
18:0	0.5	1.1	3.8	4.2	2.8	1.8	4.5	1.8	2.2	4.3	9.8	13.5
Sat. Total	(34.6)	(27.9)	33.3	19.9	22.6	7.1	11.7	13.5	-	49.3	50.5	39.2
16:1	1.8	-	13.3	4.82	8.31	-		-	-		-	-
18:1	0.7[4]	27.0	15.5	17	20.7	56.1	19.5	24.2	72.5	36.6	20.4	41.2
Mono. Total	(2.5)	(27.0)	31.2	29	46.7	58.9	20.7	24.2	73.7	37	23.4	45.1
18:2	-	1.2	2.0	1.5	0.9	20.3	65.7	58.0	7.9	9.1	1.8	10.2
18:3	-	-	1.0	1.1	0.9	9.3	0.384	0.7	0.6	0.2	1.2	1.3
18:4	0.6[5]	-	2.4	2.8	-	-	-	-	-	-	-	-
20:3	2.2[6]	-	-	-	-	-	-	-	-	-	-	-
20:4	1.8[7]	-	1.0	0.7	0.9	-	-	-	-	-	-	0.2
20:5	2.6	0	12.5	13	6.9	-	-	-	-	-	-	-
22:4	0.6[8]	-	-	-	-	-	-	-	-	-	-	-
22:5	13.59	-	1.7	3.0	0.9	-	-	-	-	-	-	-
22:6	35.0	40.0	7.9	18.2	11.0	-	-	-	-	-	-	-
n-3, total	(36.2)	(40.0)	-	32.3	18.8	9.3	-	-	0.6	0.2	1.2	1
n-6, total	(16.6)	-	-	2.2	1.9	20.3	65.7	58.0	7.9	9.1	1.8	10.2
Poly. Total	(52.8)	(41.2)	28.5	40.3	22.5	29.6	66.1	58.7	8.4	9.3	3.0	11.2

Data from Bailey's Industrial Oil and Fat Products, Hui YH (ed), John Wiley and Sons, Inc., 1996 unless otherwise indicated

[1]Monsanto derived 1997 analytical data from 5 bench lots, reported as fatty acid methyl esters.

[2]RBD-DHASCO composition from Martek Home Page, Martek Biosciences Corp., 1996.

[3]National Fish Meal and Oil Association, 1986

[4]Vaccenic acid, 18:1n-7

[5]Stearidonic acid, 18:4n-3

[6]Dihomo-gamma-linolenic acid, 20:3n-6

[7]0.9% arachidonic acid, 20:4n-6, plus 0.9% eicosatetraenoic acid, 20:4n-7

[8]Docastetraenoic acid, 22:4n-9

[9]Docosapentaenoic acid, 22:5n-6

Values in parentheses ( ) were estimated from the table.

Lauric, myristic, palmitic, stearic and palmitoleic acids are present in high amounts in one or more commercial fats and oils. Vaccenic acid is found in animal fats, meats and seafood (Douglass *et al*, 1995). Arachidonic, EPA and DHA are commonly found in seafood (Hui, 1996).

Most of the fatty acids in DHA-rich oil, *i.e.*, lauric, myristic, palmitate, palmitoleic, stearic and arachidonic, are quite common in the Western diet (Bull *et al*, 1983; Jonnalagadda *et al*, 1996), and consumption of up to 1 g of oil per day will not significantly increase their intake. Incremental intakes of four fatty acids, EPA, DHA, VA, and docosapentaenoic acid n-6 (DPAn-6), were determined because these fatty acids are less common in foods. Analysis of the mean daily intake and three-day average intake of EPA, DHA, VA, and DPAn-6 in the US was conducted by Technical Assessment Systems, Washington, DC (Douglass *et al*, 1995). VA intake was substantially higher in all age/sex groups than the amount provided by the proposed use level of the DHA-rich oil product. DPAn-6 intake from the DHA-rich oil product would be higher than the mean intake and approximately equal to the 95th percentile daily intake for eaters (individuals who consume DPAn-6). EPA intake from the DHA-rich oil would be substantially lower than usual intake because EPA is very low in the DHA-rich oil and is common in most fish and seafood. DHA intake from the DHA-rich oil would be higher than from usual diets, but below the 95th percentile intakes for adults. Research has indicated that it may be desirable to increase DHA intake, and recommendations as high as 800 mg of EPA plus DHA per day have been made (British Nutritional Foundation, 1992).

Following process improvements which increased the oil content of the dried microalgae, seven minor fatty acids were identified in the DHA-rich oil in very small amounts. These fatty acids are not commonly reported in foods but are intermediates of fatty acid metabolism. Tetradecatrienoic (14:3n-3) and hexadecatrienoic (16:3n-6) acids are β-oxidation products of α-linolenic acid (18:3n-3) and γ-linolenic acid (18:3n-6) respectively. Stearidonic acid (18:4 n-3) and eicosatetraenoic acid (20:4n-3) are intermediates in the synthesis of EPA and DHA from α-linolenic acid. Dihomo-gamma-linolenic acid (20:3 n-6) is an intermediate in arachidonic acid synthesis from γ-linolenic acid. Eicosatetraenoic acid n-7 (20:4 n-7) is an elongation, desaturation product of cis-vaccenic acid. Docosatetraenoic acid n-9 (22:4 n-9) is an elongation, desaturation product of oleic acid. All of these minor fatty acids are likely to be present at low concentrations in a variety of foods, especially animal derived foods.

The acute safety of some of the fatty acid components of the DHA-rich oil is established by published literature. Shibutani *et al*, (1989) performed acute toxicity studies in both mice and rats using a series of PUFA including stearidonic, arachidonic, EPA and DHA and found toxicity extremely low; LD_{50} values were all more than 10 g/kg.

Literature searches included the following databases: Toxnet databases, Toxline, Toxlit, Medline, Embase, Biosis, NIOSH, Ca Search, Agricola, CAB Abstracts, FSTA and Scisearch. No reports of direct toxicological relevance to humans were found for tetradecatrienoic acid n-3, VA, hexadecatrienoic acid n-6, stearidonic acid, dihomo-gamma-linolenic acid, eicosatetraenoic acid n-3 and n-7, docosatetraenoic acid n-9, DPAn-6 or DHA.

The safety of other commercially available single cell oils has also been investigated. Boswell *et al,* (1996) evaluated a single-cell oil high in DHA produced from *Mortierella alpina*, a microalgae, along with an oil high in arachidonic acid produced from *Crypthecodinium cohnii*, a fungus, and a mixture of the two. The oils were tested by gavage with a very high (20 g/kg) acute dose and 4-week subchronic dose in young Sprague-Dawley rats. The authors determined a no-observable-adverse-effect level of at least 1.25 g/kg/day for the high DHA oil. The oil contained 38.4% DHA which corresponds to 480 mg DHA/kg/day, about 80X the intake of DHA proposed for DHA-rich oil as a dietary supplement ingredient in adults.

Innis and Hansen (1996) supplemented the diets of healthy men with a mixture of a high DHA microalgal oil and a high arachidonic fungal oil (from the same source as above). Subjects received 0, 0.6 g, 1.7 g or 2.9 g DHA per day for 14 days. They found no clinically significant dose-related effects on physical examination or in routine laboratory tests.

Fish are a major dietary source of long chain (LC) PUFA, especially, n-3. Fish are generally considered desirable components of the diet and are considered safe for consumption. For fish oils, no toxicity has been reported in more than 150 studies with intakes up to 18 g/day (Connor, 1994). There was early recognition that bleeding times were longer in subjects consuming high amounts of LC PUFA over time. However, when bleeding time was investigated clinically with low to moderate intakes of fish oil (0.5 to 2.0 g/day of n-3 FA), no significant increases were observed (Connor, 1994). In a recent final rule FDA affirmed that consumption of up to 3 g/day of EPA plus DHA in menhaden oil is generally recognized as safe (GRAS) (FDA, 1997). The total amount of EPA plus DHA in the suggested daily intake of DHA-rich oil will be up to 376 mg, well below 3 grams.

Safety of the sterol components of DHA-rich oil

Eight sterols (including cholesterol) have been identified in DHA-rich oil (Table 5).

Table 5. Sterol components of DHA-rich oil

Common name	Scientific name	Sources	CAS
cholesterol	cholesta-5-en-3-ol	corn[1], fish liver oil[2], egg yolk[2], bran[2]	57-88-5
stigmasterol	stigmasta-5,22-dien-3-ol	vegetable oils[3,4], shellfish[5]	83-48-7
brassicasterol	ergosta-5,22-dien-3-ol	canola[2], vegetable oils[3,4], shellfish[5]	474-67-9
23-dehydrositosterol	stigmasta-5,23-dien-3-ol	corn sheath[1]	38485-29-9
7,24(28)-ergostadienol	ergosta-7,24(28)-dien-3-ol isomer	yeast[6]	17105-77-0
5,6-dihydroergosterol	ergosta-7,22-dien-3-ol	yeast[6]	17608-76-3
	ergosta-8,24(28)-dien-3-ol, 14-methyl	yeast[6]	
24-methylenelophenol	ergosta-7,24(28)-dien-3-ol, 4-methyl	yeast[6]	

Source references:
[1]Guo *et al.*, 1995. [2]Gunstone *et al*, 1994. [3]Warner and Mounts, 1990. [4]Itoh *et al*, 1973.
[5]King *et al*, 1990. [6]Parks, 1978.

The eight sterols in DHA-rich oil are considered safe for human consumption based on the following considerations: (1) history of safe use as a result of their abundant natural presence in food (except as discussed below) and the relatively small quantities expected to be consumed; (2) extensive knowledge of the absorption, distribution, metabolism and excretion of phytosterols in mammalian species; (3) safety information as the result of testing these and similar phytosterols; (4) easy identification of rare at-risk individuals (i.e., phytosterolemics); and/or (5) results of corroborative safety studies.

The three principal sterols in the DHA-rich oil, cholesterol, stigmasterol and brassicasterol, are quite common in human foods (Itoh *et al*, 1973; King *et al*, 1990; Weihrauch and Gardner, 1978; Slover *et al*, 1985). Five other sterols are present in the oil in very small amounts, 23-dehydrositosterol, 7,24(28)-ergostadienol, 5,6-dihydroergosterol, ergosta-8,24(28)-dien-3-ol, 14-methyl, and ergosta-7,24(28)-dien-3-ol, 4-methyl. All have been identified in food (Guo *et al*, 1995; Gunstone *et al*, 1994) except 23-dehydrositosterol which has only been identified in a part of the corn plant consumed by animals, not humans (Guo *et al*, 1995).

Phytosterols are nearly ubiquitous in the American diet. Exposure to phytosterols via DHA-rich oil, at the recommended dose would be approximately 30 mg/day (estimated from the unsaponifiable content), less than half of the current intake of the non-vegetarian general population as estimated by Nair *et al*, (1984) and one-eighth of that estimated by Ling and Jones (1995). Since phytosterols are poorly absorbed from the gastrointestinal tract (<5%), the systemic exposure would be significantly less than 30 mg/day.

Because of the structural differences from cholesterol, phytosterols are absorbed to a much lesser extent (<5%) than ingested cholesterol (>40% absorption) and in fact, because of its low degree of absorption, β-sitosterol has been used as a marker in cholesterol balance studies (Björkhem and Skrede 1989; Ling and Jones, 1995). Phytosterols are also preferentially excreted compared to cholesterol (Ling and Jones, 1995; Björkhem and Skrede 1989).

Phytosterols have been regarded as naturally-occurring cholesterol-lowering agents since the 1950s, thus, a substantial body of data exists on their effects following large doses via the oral route. Although the majority of these studies were not classical toxicity studies *per se* and the various effects were noted in terms of effect on cholesterol, no untoward effects have been reported (Ikeda *et al,* 1985; Kitahara *et al,* 1983; Teshima *et al,* 1974; Laraki *et al,* 1991). β-Sitosterol has been reported to have an estradiol equivalent in humans of 51.5% (DES = 86.2%) (Calabrese *et al* 1997). No reports were found of a similar effect for the eight phytosterols substances in DHA-rich oil.

Phytosterolemia or sitosterolemia is a rare lipid storage disease inherited in an autosomal recessive pattern (Ling and Jones, 1995; Bhattacharyya *et al,* 1991). As of 1991, approximately 22 persons with this disease had been identified. Treatment is largely palliative, excluding from the diet vegetable oils, shortening, margarine, nuts, seeds, chocolate, olives, avocados and cereal products with the germ remaining (Bhattacharyya *et al,* 1991).

The conclusion from the information cited above is that consumption of the sterols in DHA-rich oil is safe at the suggested intake level. The at-risk group, the phytosterolemics, are few in number, known to themselves and consumption of phytosterols in small quantities in DHA-rich oil does not pose an acute risk.

Taxonomic Review of *Schizochytrium* sp.

Schizochytrium sp. is a member of the kingdom Chromista (also called stramenopiles) which includes the golden algae, diatoms, yellow-green algae, haptophyte and cryptophyte algae, oomycetes and thraustochytrids. *Schizochytrium* sp. is a thraustochytrid. The earliest research of thraustochytrids placed them in the fungi because of the heterotrophic nature and superficial resemblance to chrytrids (Sparrow, 1936). Current analyses using molecular biology techniques have demonstrated that thraustochytrids are not fungi and they are related to the heterokont algae (Cavalier-Smith, 1994).

An improved strain was developed from the patented wild-type parent strain using a classical mutagenesis/screening program. No recombinant DNA technology was employed. This particular strain was selected for its improved DHA productivity. Under standard nutrient and environmental conditions for growth and DHA production, the improved strain performed equivalently to its parent in terms of overall growth and growth rate, carbon consumption and microscopic morphology. Glucose uptake, growth profile and fermentation times were similar in

triplicate fermentors for both strains. Moreover, microbial identification panels (Biolog Inc.) based on carbon source utilization, though not designed for microalgae, showed a high degree of similarity. These results indicate that no apparent adverse traits were elicited in the improved strain due to mutagenesis.

Thraustochytrids are found throughout the world in estuarine and marine habitats. Their nutritional mode is primarily saprotrophic (obtain food by absorbing dissolved organic matter) and as such are generally found associated with organic detritus (Findlay *et al.*, 1980; Raghukumar & Balasubramanian, 1991). Thraustochytrids have been reported to comprise up to 30% of the microbial community on detritus derived from brown algae (Sathe-Pathak *et al.*, 1993). Thraustochytrids can also comprise a significant portion of the phytoplankton community (e.g., 5.4×10^6 cells per gram dry weight phytoplankton, Raghukamar & Schaumann, 1993).

There are no reports in the literature of human consumption of thraustochytrids or of *Schizochytrium* in particular. The literature indicates that thraustochytrids, especially those of the genus *Schizochytrium* have an extensive worldwide distribution. Field tests by OmegaTech, Boulder, Colorado (B. Barclay, personal communication), confirm the widespread occurrence of thraustochytrids in a typical marine food chain demonstrating that thraustochytrids are consumed by a wide variety of filter feeding organisms, including mussels that are consumed directly by humans. These collective observations suggest that *Schizochytrium* sp. microalgae are indirect components of the human food chain through consumption of shellfish.

It has long been known that a few species of marine and freshwater microalgae produce toxic substances. All of the species known to produce toxins are found in just six of the approximate 76 known orders of microalgae and algae-like microorganisms. The majority of toxins produced in microalgae occur in the species of dinoflagellates (kingdom Protozoa, phylum Dinophyta) and bluegreen algae (kingdom Eubacteria, phylum Cyanobacteria). Dinoflagellate toxins cause paralytic shellfish poisoning and diarrhetic shellfish poisoning. They are produced in the dinoflagellates, accumulated in filter-feeding shellfish which feed on the algae, and then passed on to human or other invertebrate consumers. Toxic cyanobacteria (bluegreen algae) can produce neurotoxic, hepatoxic, and dermatotoxic compounds. Acute lethal toxicity can occur from ingestion of toxic cells or water containing toxins from certain freshwater/brackish water species of *Anabaena, Aphanizomenon, Microcystis, Nodularia* and *Oscillatoria.*

Thraustochytrids are not related to either of the above groups of microalgae (bluegreen or dinoflagellate). Thraustochytrids are members of the class Thraustochytridae and no reports of toxins in any member of this class have ever been published. Within the kingdom Chromista, to which thraustochytrids belong, only two genera of microalgae, *Pseudonitzschia* (phylum: Heterokonta; class: Bacillariophycae) and *Prymnesium* (phylum: Prymnesiophyta) are known to produce toxins. *Pseudonitzschia* produces domoic acid, a potent neurotoxin, which causes amnesic shellfish poisoning in humans. It is a naturally occurring amino acid whose production appears to be limited to a few species of microalgae (diatoms) in the genus *Pseudonitzschia* (and

possibly by one species of *Chrysochromulina*, a flagellated species of golden algae) (Villac *et al.*, 1993). The other toxin, prymnesin, is produced by two species of *Prymnesium* (*P. parvum* and *P. patelliferum*). Prymnesin toxins exhibit a broad spectrum of activity including lethal effects on gill breathing animals, cytotoxic effects on erythrocytes, nucleated mammalian cells, protozoa and bacteria.

The thraustochytrids are in a separate subphylum and class from both *Pseudonitzschia* and *Prymnesium* so one would not expect to find domoic acid or prymnesin toxin in *Schizochytrium* sp. However for confirmation, dried microalgae have been analyzed for domoic acid using the standard HPLC method (Lawrence *et al*, 1991). With regard to prymnesin, a bioassay has been developed for this compound utilizing *Artemia* nauplii as the test organism (Vanhaecke *et al*, 1981). The results (Kaneko, 1997) indicate normal growth of *Artemia* culture with all test lots, indicating absence of prymnesin toxin. Furthermore, Monsanto has commercialized a product for aquaculture applications (HUFA2000), a spray-dried form of *Schizochytrium* sp. dried microalgae which has been successfully utilized for over two years with no adverse effects in shrimp larvaculture and finfish (red seabream, Japanese flounder) culture throughout the world. The use of *Schizochytrium* sp. dried microalgae in these applications promotes larval survivability and growth, representing additional evidence of the absence of algal toxins in *Schizochytrium* sp.

Allergic responses to microorganisms by humans can sometimes be related to microbial toxins. There have been no reports in the literature of allergic responses to any members of the kingdom Chromista, including the thraustochytrids.

Composition of DHA-Rich Oil Derived from the original and two subsequent modifications of the fermentation process

The fermentation process was improved in stages during development of the commercial process. As a consequence, three generations of the fermentation process evolved (PB26, AS4, HD1). A comparison of the fatty acid and sterol profile for the three production processes demonstrates the equivalency of components in the oil. Corroborative safety studies on the microalgal source of DHA-rich oil were conducted using dried microalgae from the first and second generation processes. The commercial oil product is produced from a third generation fermentation process. This oil was subjected to an acute gavage study in mice and an Ames test.

Batches of oil were produced from each of the three fermentation processes. Results of fatty acid composition analysis demonstrate that each successive process improvement resulted in an increase in the DHA content in oil (Table 6). As DHA content increased, there was a corresponding decrease in the major saturated fatty acids, myristic and palmitic in the oil (Table 1). Importantly, no fatty acids absent in the first generation process were identified in oil produced in the second and third generation fermentation processes. All fatty acid components were conserved from process generation to process generation, as demonstrated in Table 6. The major sterol components qualitatively remained consistent in all lots of oil evaluated from PB26,

AS4, and HD1 processes as shown in Table 7. Sterols, as quantitatively judged by total unsaponifiable content, were comparable for the first (PB26) and second (AS4) generation processes, while a decrease was observed in the third generation (HD1) process (Table 8). No sterols absent in the first generation process were identified in oil produced in the second and third generation fermentation processes.

In conclusion, the fatty acid and sterol profiles of DHA-rich oil derived from the PB26, AS4, and HD1 fermentation processes are qualitatively similar. No fatty acids or sterols not present in the first generation process were introduced into oil produced from the improved fermentation processes.

Table 6. Fatty Acid Profile of DHA-rich oil Derived from *Schizochytrium* sp. Microalgae

FATTY ACID	**Production Process**		
	PB26	***AS4***	***HD1***
	(mg FAME/g oil)[1]		
Lauric	6.3 ± 0.6	4.3 ± 0.6	4.0 ± <0.1
Myristic	157.5 ± 3.3	125.7 ± 1.1	101.1 ± 8.6
Tetradecatrienoic n-3	tr	tr	tr-4.5
Pentadecanoic	tr	tr-4.0	tr
Palmitic	294.7 ± 5.9	300.6 ± 8.9	236.8 ± 9.4
Palmitoleic	63.0 ± 14.1	68.6 ± 7.1	17.6 ± 9.9
Hexadecatrienoic n-6	tr	tr	tr-5.0
Stearic	8.0 ± <0.1	8.9 ± 0.1	4.5 ± 0.5
Vaccenic	20.8 ± 6.4	38.1 ± 6.8	tr-13.6
Stearidonic n-3	tr	tr	tr-8.5
Dihomo-gamma-linolenic	11.3 ± 1.6	5.9 ± 1.1	22.1 ± 2.4
Arachidonic	tr	tr	9.4 ± 1.7
Eicosatetraenoic n-3	tr	tr	tr-4.0
Eicosatetraenoic n-7	6.0 ± <0.1	4.9 ± 0.1	8.7 ± 0.4
Eicosapentaenoic	4.2 ± 0.4	5.3 ± 0.6	26.3 ± 6.4
Docosatetraenoic n-9	tr	tr	5.4 ± 1.3
Docosapentaenoic n-6	95.3 ± 5.5	93.1 ± 1.8	135.0 ± 15.0
Docosahexaenoic	251.5 ± 2.6	271.7 ± 6.0	350.0 ± 24.6
Total FA	918.5 ± 24.6	928.5 ± 6.1	936.1 ± 10.1

[1]Measured as fatty acid methyl esters (FAME) reported as fatty acid methyl ester average values ± standard deviation (n=3 for PB26 oil lots, n=3 for AS4 oil lots, n=5 for HD1 oil lots) except when a lot contained a FAME below the lowest calibration curve concentration, in which cases, ranges are reported.

tr = present (based on similar rf) but below the lowest calibration curve concentration (4 mg FAME/g oil) and therefore not quantified.

Table 7. Sterol Profile of DHA-rich oil Derived from *Schizochytrium* sp. Microalgae

STEROL NAME	Production Process		
	PB26	***AS4***	***HD1***
	Sterol (% peak area)[1]		
Cholesta-5-en-3-ol (Cholesterol)	26 ± 5	28 ± 2	25 ± 3
Ergosta-5,22-dien-3-ol (Brassicasterol)	8 ± 3	13 ± 2	15 ± 3
Ergosta-7,22-dien-3-ol	tr	7 ± 1	<5-7
Ergosta-7,24-dien-3-ol	5 ± 0	5 ± 1	<5-6
Ergosta-8,24(28)-dien-3-ol, 14-methyl	<5-12	tr	tr
Stigmasta-5,22-dien-3-ol (Stigmasterol)	23 ± 6	13 ± 1	19 ± 2
Stigmasta-5,23-dien-3-ol	13 ± 2	10 ± 1	8 ± 1
Ergosta-7,24(28)-dien-3-ol, 4-methyl	≤5	tr	tr

[1]All peaks greater than 5% total peak area are reported as the average ± standard deviation (n=3 for PB26 oil lots, n=3 for AS4 oil lots, n=5 for HD1 oil lots) except in cases when a lot contained a sterol at <5% of total peak area, in which cases, ranges are reported. tr = present (based on similar rf) but <5% of total peak area.

Cholesta-5-en-3-ol (cholesterol), ergosta-5,22-dien-3-ol (brassicasterol), and stigmasta-5,22-dien-3-ol (stigmasterol) were identified as the major sterols in DHA-rich oil.

Table 8. Unsaponifiable Content of DHA-rich oil Derived from *Schizochytrium* sp. Microalgae

Process	Percent Unsaponifiable[1]	
	Average	Std. Dev.
PB26	5.5	1.2
AS4	5.0	0.5
HD1	3.1	1.0

[1]%Unsaponifiable reported on weight basis as the average ± standard deviation (n=3 for PB26 oil lots, n=3 for AS4 oil lots, n=5 for HD1 oil lots).

Thus, the oils present in the dried microalgae used as test articles in the safety studies were comparable to the oil to be sold commercially.

Summary of Corroborative Safety Studies with Dried Microalgae

Several safety studies have been conducted with *Schizochytrium* dried microalgae. These studies were generally done according to FDA's *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food* (the Redbook) (1982) guidelines and generally done in compliance with FDA Good Laboratory Practice (GLP) regulations.

The safety studies provide corroborative safety information on the source microalgae and the oil it contains since the fatty acid and sterol components have a history of safe use in food. These corroborative studies have been conducted on *Schizochytrium* dried microalgae produced in three separate production processes, i.e., fermentation processes. During these process generations, improvements were implemented to increase the oil and DHA content and decrease the ash content of the dried microalgae and to add supplemental vitamin E (d,l-α-tocopheryl acetate) to the oil. Vitamin E provides a supplemental nutritional source of antioxidant given the high LC PUFA content of oil. Compositional analyses indicate that the same fatty acids and sterols are present in oil produced during the three generations of the production process (Tables 7 & 8). Process improvements did not result in the introduction of new fatty acids or sterols into the oil.

Corroborative safety studies:

1. A battery of mutagenicity studies was carried out with intact microalgal cells (Ames, In Vitro Human Lymphocytes, Mouse lymphoma assays) from the first generation production process.[1]

2. A battery of mutagenicity studies was carried out with lysed microalgal cells from the second generation production process (Ames, AS52/XPRT Gene Locus , Mouse Micronucleus assays).

3. An Ames test was conducted with oil extracted from *Schizochytrium* dried microalgal cells from the third generation production process.

4. An acute gavage study in mice was conducted with oil extracted from microalgae (third generation production process) administered at a dosage of 2000 mg/kg.

5. A one-generation rat reproduction study was carried out with *Schizochytrium* dried microalgae (first generation production process) administered at average dosages up to 17,800 mg/kg/day (males) and 20,500 mg/kg/day (females) when animals were fed up to 30% dried microalgae in the diet.[1]

6. A dietary teratology study with *Schizochytrium* dried microalgae (first generation production process) was conducted in the rat. Rats were fed up to 30% *Schizochytrium* dried microalgae in the diet (up to 22,000 mg/kg/day).[1]

7. A rabbit teratology study was conducted in which up to 1800 mg/kg/day *Schizochytrium* dried microalgae (second generation production process) was administered by gavage.[2] This study included a control group fed a similar amount of fat as fish oil.

8. A 13 week rat feeding study was carried out with dried microalgae from the second generation production process at dosages up to 4000 mg/kg/day.[3] This study included a control group fed a similar amount of fat as fish oil. The 4000 mg/kg dosage provided an approximately 100 fold safety margin for use of DHA-rich oil as a dietary supplement in man. Since the oil content of *Schizochytrium* dried microalgae across the first two production generations averaged around 40% w/w, rats fed 4000 mg/kg/day dried microalgae consumed approximately 1600 mg/kg/day oil.

[1]The records for test article characterization and test diet analyses were not maintained in accordance with GLP regulations for the safety studies carried out with *Schizochytrium* dried microalgae from the first generation production process.

[2]In an earlier dietary teratology study, pregnant rabbits were fed high levels of dried microalgae in the diet (up to 12% w/w). These high dietary levels caused palatability and digestibility problems in rabbits resulting in marked and sustained reductions in food consumption and loss of body weight. This study could not be completed as the fetal skeletons were inadvertently macerated during staining. This study was repeated using standard oral gavage dosing.

[3]In an earlier 13 week rat feeding study, dried microalgae were added to test diets at very high concentrations (up to 30% w/w, an average intake of 18,000 mg/kg/day overall) which caused kidney changes, (e.g., mineralization) due to the high ash content of dried microalgae. Fat accumulation in the liver and cardiomyopathy due to the high fat content of dried microalgae also were observed. This study was repeated at lower dietary levels.

Results of corroborative safety studies establish that the dried microalgae and the oil derived from it were not mutagenic in bacterial and mammalian test systems and were not teratogenic in a rat dietary teratology study and in a rabbit gavage teratology study. Oil extracted from *Schizochytrium* dried microalgae was not toxic when administered by gavage as a single high dose to mice. There was no evidence that *Schizochytrium* dried microalgae interfered with reproductive performance or progeny development in a rat one-generation dietary reproduction study. *Schizochytrium* dried microalgae were also fed to rats for 13 weeks, and there was no evidence of toxicity with only anticipated findings in clinical chemistry parameters and microscopic changes commonly observed in rats following consumption of diets high in fatty acids. Similar findings were observed in a fish oil control group in this study. These corroborative studies support the safe use of the *Schizochytrium* sp. dried microalgae as a source of DHA-rich oil to be used as an ingredient in dietary supplements.

Conclusion

The above information was reviewed by a panel of experts who concluded (statement attached) that the use of SeaGold™ DHA-rich microalgal oil in dietary supplements at up to 1 gram of oil per day will reasonably be expected to be safe. Safe use is based on knowledge of the components of SeaGold™ DHA-rich oil, their metabolism, their prior history of safe use in foods and consumption in the diet, the small quantities expected to be consumed as dietary supplements, published safety studies of components of the oil and of similar oils, and the absence of reports of toxicity of the components of the DHA-rich oil. The safety is further corroborated by safety studies done with the dried microalgal source of the DHA-rich oil.

Respectfully submitted,

Wayne Stargel

Wayne Stargel, Pharm.D.
Vice President, Regulatory Affairs
Nutrition and Consumer Products

REFERENCE LIST

Bhattacharyya AK, Connor WE, Lin DS, McMurray MM, Shulman RS. (1991). Sluggish sitosterol turnover and hepatic failure to excrete sitosterol into bile cause expansion of body pool of sitosterol in patients with sitosterolemia and xanthomatosis. *Arterioscler Thromb.* 11(5):1287-1294.

Bjorkhem I, Skrede S. (1989). Familial diseases with storage of sterols other than cholesterol: cerebrotendinous xanthomatosis and phytosterolemia. Scriver CR, ed. *The Metabolic Basis of Inherited Diseases.* 8th ed. New York: McGraw-Hill. p.1283-1302.

Boswell K, Koskelo E-K, Carl L, et al. (1996). Preclinical evaluation of single-cell oils that are highly enriched with arachidonic acid and docosahexaenoic acid. *Food Chem Toxicol.* 34:585-593.

British Nutrition Foundation. (1992). *Unsaturated Fatty Acids : Nutritional and Physiological Significance.* London: Chapman & Hall.

Bull NL, Day MJL, Burt R, Buss DH. (1983). Individual fatty acids in the British household food supply. *Hum Nutr Appl Nutr.* 37A:373-377.

Calabrese EJ, Baldwin LA, Kostecki PT, Potter TL. (1997). A toxicologically based weight-of-evidence methodology for the relative ranking of chemicals of endocrine disruption potential. *Regul Toxicol Pharmacol.* 26(1 pt 1):36-40.

Cavalier-Smith T, Allsopp MTEP, Chao EE. (1994). Thraustochytrids are chromists, not fungi: 18s rRNA signatures of Heterokonta. *Philos Trans R Soc London B: Biol Sci.* 346(1318):387-397.

Connor WE. (1994). Omega-3 fatty acids and heart disease. Kritchevsky D, Carroll KK, eds. *Nutrition and Disease Update : Heart Disease.* Champaign, IL:AOCS Press. p.1-42.

Douglass JS, Server BE, Reich A.G. , Chew S. (1995). Mean daily intake and three-day average intake of 5,8,11,14,17-eicosapentaenoic acid (EPA), 4,7,10,13,16,19-docosahexaenoic acid (DHA), 11-octadeconoic acid (VA), and 4,7,10,13,16-docosapentaenoic acid (DPA) by the U.S. population and population subgroups. *TAS, Inc.* Report(Prepared for Kelco).

FDA. (1997). Substances affirmed as generally recognized as safe: Menhaden oil. *Fed Register, June 5, 1997.* 62(FR):30751-30757.

Findlay RH, Fell JW, Coleman NK. (1980). Biochemical indicators of the role of fungi and thraustochytrids in mangrove detrital systems. Moss ST, ed. *Biology of Marine Fungi.* London:Cambridge University Press. p.91-103.

Gunstone FD, Harwood JL, Padley FB, eds. (1994). *The Lipid Handbook.* London:Chapman & Hall. p.31-32,259.

Guo DA, Venkatramesh M, Nes WD. (1995). Developmental regulation of sterol biosynthesis in Zea mays. *Lipids.* 30(3):203-219.

Hui YH, ed. (1996). *Bailey's Industrial Oil and Fat Products.* 5th Edition. ed. v.1. New York:John Wiley & Sons. p.444-495.

Ikeda I, Nakashima-Yoshida K, Sugano M. (1985). Effects of cycloartenol on absorption and serum levels of cholesterol in rats. *J Nutr Sci Vitaminol.* 31:375-384.

Innis SM, Hansen JW. (1996). Plasma fatty acid responses, metabolic effects, and safety of microalgal and fungal oils rich in arachidonic and docosahexaenoic acids in healthy adults. *Am J Clin Nutr.* 64(2):159-167.

Itoh T, Tamura T, Matsumoto T. (1973). Sterol composition of 19 vegetable oils. *J Am Oil Chem Soc.* 50:122-125.

Jonnalagadda SS, Mustad VA, Yu S, Etherton TD, Kris-Etherton PM. (1996). Effects of individual fatty acids on chronic diseases. *Nutr Today.* 31(3):90-106.

Kaneko T. (1997). Toxicity Study on DHAINT using *Artemia* nauplii to detect presence/absence of prymnesin toxin. *Monsanto Internal Report.* (Notebook: No.4-395).

King I, Childs MT, Dorsett C, Ostrander JG, Monsen ER. (1990). Shellfish: proximate composition, minerals, fatty acids, and sterols. *J Am Diet Assoc.* 90(5):677-685.

Kitahara M, Obata A, Kaneda T. (1983). Hypocholesterolemic effect of triterpene alcohols with plant sterols on plasma cholesterol in rats. *Dev Food Sci II (Fat Sci).* Pt. A:259-270.

Koletzko B, Mrotzek M, Bremer HJ. (1988). Fatty acid composition of mature human milk in Germany. *Am J Clin Nutr.* 47(6):954-959.

Laraki L, Pelletier X, Debry G. (1991). Effects of dietary cholesterol and phytosterol overload on Wistar rat plasma lipids. *Ann Nutr Metab.* 35(4):221-225.

Lawrence JF, Charbonneau CF, Menard C. (1991). Liquid chromatographic determination of domoic acid in mussels, using AOAC paralytic shellfish poison extraction procedure: collaborative study. *J Assoc Off Anal Chem.* 74(1):68-72.

Ling WH, Jones PHJ. (1995). Dietary phytosterols: A review of metabolism, benefits and side effects. *Life Sci.* 57(3):195-206.

Martek Biosciences Corporation. (1996). *RBD-DHASCO ® (40% Docosahexaenoic Acid [DHA]).* http://www.martekbio.com/rbd_dhasco.html. [7/21/97].

Nair PP, Turjman N, Kessie G, et al. (1984). Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Dietary cholesterol, ß-sitosterol, and stigmasterol. *Am J Clin Nutr.* 40(4 Suppl):927-30.

National Fish Meal and Oil Association. (1986). *Petition to the Food and Drug Administration Requesting Affirmation of Menhaden Oil and Partially Hydrogenated Menhaden Oil As Generally Recognized As Safe for Use in Foods.*

National Research Council. (1989). Vitamin E. *Recommended Dietary Allowances.* 10th ed. Washington, D.C.:National Academy Press. p.99-107.

Raghukumar S, Balasubramanian R. (1991). Occurrence of thraustochytrid fungi in corals and coral mucus. *Indian J Mar Sci.* 20(3):176-181.

Raghukumar S, Schaumann K. (1993). An epifluorescence microscopy method for direct detection and enumeration of the fungi-like marine protists the thraustochytrids. *Limnol Oceanogr.* 38(1):182-187.

Raper NR, Cronin FJ, Exler J. (1992). Omega-3 fatty acid content of the US food supply . *J Am Coll Nutr.* 11(3):304-8.

Sathe-Pathak V, Raghukumar S, Raghukumar C, Sharma S. (1993). Thraustochytrid and fungal component of marine detritus. I. Field studies on decomposition of the brown alga *Sargassum cinereum* J. Ag. *Indian J Mar Sci.* 22(3):159-167.

Shibutani Y, Ishikawa T, Ohtsuka Y. (1989). Toxicity studies of 5, 8, 11, 14, 17-eicosapentaenoic acid ethyl ester. *Pharm Res.* 20(4):801-807.

Slover HT, Thompson RH, Davis CS, Merola GV. (1985). Lipids in margarines and margarine-like foods. *J Am Oil Chem Soc.* 62(4):775-786.

Sparrow FK. (1936). Biological observations on the marine fungi of Woods Hole waters. *Biol Bull Mar Biol Lab.: Woods Hole.* 70:236-263.

Teshima S, Kanazawa A, Yoshioka M, Kitahara K. (1974). Hypocholesterolemic effect of 24-methylenecholesterol and 7-cholestenol in the rat. *J Steroid Biochem.* 5:69-72.

Villac MC, Roelke DL, Villareal TA, Fryxell GA. (1993). Comparison of two domoic acid-producing diatoms: a review. *Hydrobiologia.* 269-270:213-224.

Warner K, Mounts TL. (1990). Analysis of tocopherols and phytosterols in vegetable oils by HPLC with evaporative light-scattering detection. *J Am Oil Chem Soc.* 67(11):827-831.

Weihrauch JL, Gardner JM. (1978). Sterol content of foods of plant origin. *J Am Diet Assoc.* 73:30-47.

Expert Panel Meeting

November 25, 1997

DHA-rich microalgal oil

The Expert Review Panel ("the Panel") members' qualifications in the fields relevant to this determination are noted in their curricula vitae attached hereto. The Panel members are Michael Pariza, Panel Chairman; John Erdman; Gary Flamm; William Harris; and Stephen McNamara.

The Panel has been asked by the Monsanto Company ("the Company") to examine the product, 4,7,10,13,16,19-docosahexaenoic acid (DHA) -rich microalgal oil from *Schizochytrium* sp. marine microalgae (known by the trade name "SeaGold™") ("the Product"), and to render an opinion on whether (a) the Product is safe and is generally recognized by experts in food safety, nutrition, and food technology to be safe for use as a dietary ingredient in dietary supplements at a level of up to 1 gram of oil per day and, (b) consumption of the Product may help maintain a healthy cardiovascular system.

During the course of the review the Panel has examined relevant documents provided by the Company concerning the composition of the Product, the presence of the components of the Product in foods, human experience concerning consumption of the components of the Product, the intended use of the Product, the taxonomy of the source microalgae, safety data relating to the source microalgae, safety information on similar products, results of corroborating safety studies on the source of the oil, results of studies on the effects of DHA on the cardiovascular system, and other relevant information including the knowledge and experience of the individual Panel members.

The undersigned member of the Panel states as follows:

1. The Product is composed primarily of fatty acids, including DHA, as triglycerides and of small amounts of sterols, as shown in the attached Appendix.

2. The Product consists of oil alone or oil in combination with other safe and suitable food ingredients.

3. The Product is manufactured under food cGMP procedures using a conventional oilseed-based extraction and purification of the oil from dried microalgae, which are produced in a pure culture, controlled-environment fermentation system.

4. All of the fatty acids in the Product are common in the food supply or are known metabolites of fatty acid metabolism. All of the sterols in the Product are common in the food supply except 23-dehydrositosterol, which is present in trace amounts and is a known constituent of the sheath of the corn plant.

5. The safety of the Product is corroborated by safety studies on the dried microalgae that are the source of the oil and by evidence that the microalgae do not produce toxins and that this species is not related to other microalgae that produce toxins.

6. The Product can provide a nutritional benefit by providing a rich source of DHA, a long-chain omega-3 fatty acid which may have beneficial effects on cardiovascular health.

7. As a result of this review, the Panel concludes that the Product, meeting food grade specifications, is both safe and generally recognized by experts to be safe by scientific procedures for use as a dietary ingredient in dietary supplements at a level of up to 1 gram of oil per day. Furthermore, the Panel concludes that consumption of the Product may help maintain a healthy cardiovascular system.

Michael W. Pariza — 07 Dec 97

Michael Pariza, PhD, University of Wisconsin, Microbiology/Food Safety — Date

Expert Panel Meeting

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5. The safety of the Product is corroborated by safety studies on the dried microalgae that are the source of the oil and by evidence that the microalgae do not produce toxins and that this species is not related to other microalgae that produce toxins.

6. The Product can provide a nutritional benefit by providing a rich source of DHA, a long-chain omega-3 fatty acid which may have beneficial effects on cardiovascular health.

7. As a result of this review, the Panel concludes that the Product, meeting food grade specifications, is both safe and generally recognized by experts to be safe by scientific procedures for use as a dietary ingredient in dietary supplements at a level of up to 1 gram of oil per day. Furthermore, the Panel concludes that consumption of the Product may help maintain a healthy cardiovascular system.

John W Erdman Jr — 12/4/97

John Erdman, PhD, University of Illinois, Food Science/Nutrition — Date

Expert Panel Meeting

November 25, 1997

DHA-rich microalgal oil

The Expert Review Panel ("the Panel") members' qualifications in the fields relevant to this determination are noted in their curricula vitae attached hereto. The Panel members are Michael Pariza, Panel Chairman; John Erdman; Gary Flamm; William Harris; and Stephen McNamara.

The Panel has been asked by the Monsanto Company ("the Company") to examine the product, 4,7,10,13,16,19-docosahexaenoic acid (DHA) -rich microalgal oil from *Schizochytrium* sp. marine microalgae (known by the trade name "SeaGold™") ("the Product"), and to render an opinion on whether (a) the Product is safe and is generally recognized by experts in food safety, nutrition, and food technology to be safe for use as a dietary ingredient in dietary supplements at a level of up to 1 gram of oil per day and, (b) consumption of the Product may help maintain a healthy cardiovascular system.

During the course of the review the Panel has examined relevant documents provided by the Company concerning the composition of the Product, the presence of the components of the Product in foods, human experience concerning consumption of the components of the Product, the intended use of the Product, the taxonomy of the source microalgae, safety data relating to the source microalgae, safety information on similar products, results of corroborating safety studies on the source of the oil, results of studies on the effects of DHA on the cardiovascular system, and other relevant information including the knowledge and experience of the individual Panel members.

The undersigned member of the Panel states as follows:

1. The Product is composed primarily of fatty acids, including DHA, as triglycerides and of small amounts of sterols, as shown in the attached Appendix.

2. The Product consists of oil alone or oil in combination with other safe and suitable food ingredients.

3. The Product is manufactured under food cGMP procedures using a conventional oilseed-based extraction and purification of the oil from dried microalgae, which are produced in a pure culture, controlled-environment fermentation system.

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Gary Flamm, PhD, Flamm Associates, Food Safety/Toxicology — Date 12/10/97

Expert Panel Meeting

November 25, 1997

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William Harris, PhD, Mid-America Heart Institute, Nutrition/Lipidology — Date 12/10/97

Expert Panel Meeting

November 25, 1997

DHA-rich microalgal oil

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Stephen H. McNamara — December 5, 1997

Stephen McNamara, Hyman, Phelps & McNamara, Food Law Advisor — Date

Appendix

Table 1

Fatty acid profile of DHA-rich oil derived from Schizochytrium sp. marine microalgae

FATTY ACID	mg FAME/g oil[1]
Laurate	4.0 ± 0.1
Myristate	101.1 ± 8.6
Tetradecatrienoate n-3	tr-4.5
Palmitate	236.8 ± 9.4
Palmitoleate	17.6 ± 9.9
Hexadecatrienoate n-6	tr-5.0
Stearate	4.5 ± 0.5
Vaccenate	tr-13.6
Octadecatetraenoate n-3	tr-8.5
Homogammalinolenate	22.1 ± 2.4
ARA n-6	9.4 ± 1.7
Eicosatetraenoate n-3	tr-4.0
Eicosatetraenoate n-7	8.7 ± 0.4
EPA n-3	26.3 ± 6.4
Docosatetraenoate n-9	5.4 ± 1.3
DPA n-6	135.0 ± 15.0
DHA n-3	350.0 ± 24.6
Total FA =	936.1 ± 10.1

[1] Fatty Acid Methyl Esters (FAME) reported as average values ± standard deviation (n=5) except when a lot contained a FAME below the lowest calibration curve concentration, in which cases, ranges are reported.

tr = present but below the lowest calibration curve concentration (4 mg FAME/g oil) and therefore not quantified.

Table 2
Sterol profile of DHA-rich oil derived from Schizochytrium sp. marine microalgae

STEROL NAME	% peak area[1]
Cholesta-5-en-3-ol (Cholesterol)	25 ± 3
Ergosta-5,22-dien-3-ol (Brassicasterol)	15 ± 3
Ergosta-7,22-dien-3-ol	<5-7
Ergosta-7,24-dien-3-ol	<5-6
Stigmasta-5,22-dien-3-ol (Stigmasterol)	19 ± 2
Stigmasta-5,23-dien-3-ol	8 ± 1

1 All peaks greater than 5% total peak area are reported as the average (n=5) except in cases when a lot contained a sterol at <5% of total peak area, in which cases, ranges are reported.