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ORIGINAL SUBMISSION

Martek®

Martek Biosciences Corporation

August 18, 2003

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2003
OFFICE OF
FOOD ADDITIVE SAFETY

BY HAND DELIVERY

Mr. Richard E. Bonnette
Consumer Safety Officer
Office of Premarket Approval (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, D.C. 20204

Re: GRAS Exemption Claim for DHA Algal Oil Derived from *Schizochytrium* sp. as a Source of DHA for Use in Foods

Dear Mr. Bonnette:

Pursuant to proposed 21 CFR 170.36, 62 Fed. Reg. 18938 (April 17, 1997), Martek Biosciences Corporation hereby provides notice of a claim that docosahexaenoic acid (DHA) oil from *Schizochytrium* sp. (DHA Algal Oil) is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act (FFDCA). The summary data and information in this notification establish that DHA Algal Oil is generally recognized as safe (GRAS), based on scientific procedures, for use as a food ingredient to increase dietary intake of DHA up to 1.5 grams of DHA per day.

In accordance with the criteria set forth in the GRAS notification proposed regulation found at 62 Fed. Reg. 18938, 18961 (1997), Martek submits the following information as part of its GRAS exemption claim.

Name and Address of Notifier: Martek Biosciences Corporation, 6480 Dobbin Road, Columbia, Maryland 21045.

Common or Usual Name of the Substances: DHA Algal Oil. This product will be marketed under the tradename, DHASCO®-S.

6480 Dobbin Road
Columbia, Maryland 21045

(410) 740-0081
Fax (410) 740-2985
www.martekbio.com

Applicable Conditions of Use: Use as an ingredient in the food categories for menhaden oil (21 CFR 184.1472(a)(3)) at a level that is approximately 29 percent of the levels listed in that regulation. The DHA Algal Oil is intended for use in those additional food categories covered by the GRAS notification submitted for fish oil concentrate at a level that is 50 percent of the levels covered in GRN 000105.

Basis for GRAS Determination: DHA Algal Oil is GRAS on the basis of scientific procedures.

Availability of Data: The data and information that are the basis for the notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at the law offices of Hogan & Hartson, L.L.P., 555 13th Street N.W., Washington DC 20004, or will be sent to FDA upon request.

GRAS Exemption Claim: The use of DHA Algal Oil as a food ingredient to increase dietary intake of DHA up to 1.5 grams of DHA per day is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FFDCA) because Martek, after consulting with a panel of outside experts, has determined that such use is GRAS.

* * * * *

We enclose an original and two copies of this notification for your review. If you have any questions, please contact me at the above phone number and address.

Sincerely,

Sam Zeller, Ph.D.

Enclosures

GRAS Notification for DHA Algal Oil Derived from *Schizochytrium* sp.

I. NAME AND ADDRESS OF NOTIFIER

Manufacturer: Martek Biosciences Corporation
6480 Dobbin Road
Columbia, MD 21045

Contact: Sam Zeller, Ph.D.
(303) 381-8146 (ph)
(303) 381-8181 (fax)
szeller@martekbio.com (email)

II. INFORMATION ON THE IDENTITY OF THE NOTIFIED SUBSTANCE

A. Name of Notified Substance

DHA Algal Oil

B. Tradename

Proposed Tradename: DHASCO®-S

C. Chemical Abstract Service (CAS) Registry Number

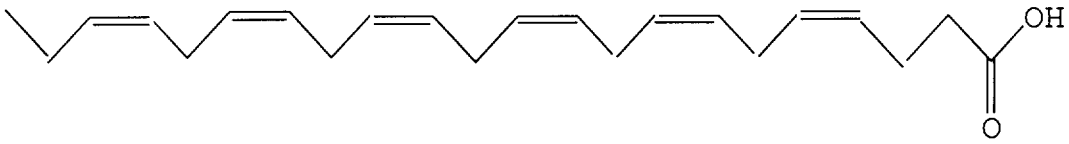
The CAS Number for fatty acids containing 14-22 carbons (C14-C22), and 16-22 carbons (C16-C22) esterified to glycerol is 68424-59-9 (described in the CAS registry as "glycerides", C14-C22 and C16-C22-unsatd.).

D. Empirical Formula

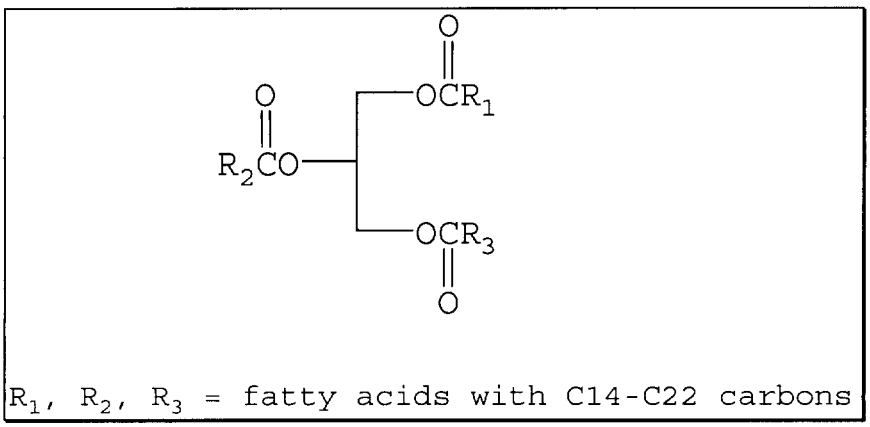
Docosahexaenoic acid (DHA) is a long chain, polyunsaturated fatty acid, with empirical formula $C_{22}H_{32}O_2$. The complete name is 4,7,10,13,16,19-docosahexaenoic acid. The short-hand nomenclature for DHA is often 22:6(n-3). The numbers indicate the number of carbon atoms in the molecule (22), the number of double bonds (6) and the number of carbon atoms from the methyl terminus to the first double bond (3).

E. Structural Formula

The structural formula for docosahexaenoic acid (DHA), is represented by the following:



The structural formula for triglycerides, described by CAS number 68424-59-9, is represented by the following:



F. Specifications and Methods

DHA Algal Oil is a yellow to light orange-colored oil derived from the heterotrophically grown marine alga, *Schizochytrium* sp., intended for use as a food ingredient. The oil is winterized, refined, bleached and deodorized. Antioxidants are added to provide oxidative protection.

Product specifications (Table 1) are set for color, acid value, peroxide value (PV), moisture and volatiles, unsaponifiable content, trans-fatty acids, DHA and DPA(n-6) content, hexane and trace elements (arsenic, copper, iron, mercury and lead). Physical and chemical tests applied to the quality control characterization of the oil are adapted from the Official Methods and Recommended Practices of the American Oil Chemists' Society (AOCS), Fourth ed. (Third Printing) and the Official Methods of Analysis of AOAC International, Seventeenth ed.

G. Manufacturing Process and Flow Charts

DHA Algal Oil is produced *via* an algal fermentation process using an algae from the genus *Schizochytrium.* 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 microbial strain improvement programs.

The algae are grown in a pure culture heterotrophic fed-batch fermentation process, recovered from the fermentation broth, and dried. Antioxidants may be added to the fermentation broth prior to drying the algal biomass.

The resulting dried algae are extracted with hexane to produce a crude oil that is further refined, bleached and deodorized using process operations commonly employed in the vegetable oil industry. An illustration of the manufacturing process can be found in the attached Figures 1, 2, 3, and 4.

The presence of typical food borne microbes is inhibited by a combination of heat treatment applied to the cultured algal cells, the environmental conditions of the oil extraction and processing, and the extremely low water activity of the finished oil product.

1. Fermentation

Frozen cultures are used to inoculate a shake flask, which is then placed on a shaker table. After an incubation period, the mature shake flask is used to inoculate a seed tank, which is allowed to grow prior to being used to inoculate the final fermentor. The growth media used in the final fermentor consists of a carbon source, nitrogen source, bulk nutrients (*i.e.*, sodium, calcium, phosphate, *etc.*), trace minerals, and vitamins. Aeration rates, backpressure, agitation, pH and temperature are controlled during the fermentation process. At the completion of the fermentation, the broth is chilled prior to being transferred to a recovery area.

2. Intermediate Product (Dried Algae) Recovery

The algae cells may be concentrated in the broth and are dried. The dried algae are packaged in suitable containers for transfer to the oil extraction facility. In-process quality control testing is completed to ensure that the dried biomass product meets manufacturing requirements prior to further processing.

3. Oil Extraction

The dried algae are suspended in commercial-grade hexane and wet milled. The de-oiled biomass is separated from the oil-rich hexane phase (miscella) by centrifugation and/or filtration. The miscella is chilled and held for a period of time to allow any saturated fats, or other high melting point components (stearins), to crystallize (winterization). The chilled miscella is centrifuged and/or filtered to remove the solid phase. Hexane is then removed from the miscella, leaving behind the winterized oil.

4. Oil Purification

The winterized oil is heated and pre-treated with acid to hydrate any phosphatides present in the oil. Caustic is added to neutralize any free fatty acids present. The resulting gums (hydrated phosphatides) and soap stock (neutralized fatty acids) are removed using a centrifuge, yielding a refined oil. The refined oil is bleached to remove peroxides, color compounds, and traces of soap stock, phospholipids and metals, and the resulting bleached oil is recovered after filtration.

An additional step may be performed, where the bleached oil is chilled and held to crystallize any remaining stearines or waxes, as necessary to achieve the desired level of clarity. Solids from this step may be removed by centrifugation and/or filtration.

A deodorizer, operated under high temperature and vacuum, is used to remove peroxides and any remaining low molecular weight compounds that may cause off-odors and flavors. Safe and suitable antioxidants are added to the oil to provide oxidative stability. The stabilized oil is packaged under a nitrogen atmosphere to prevent oxidation.

Samples of each lot are submitted to the Quality Control lab for testing. The results of analyses are compared to the manufacturing specifications for DHA Algal Oil to confirm specifications are met prior to product release.

H. Characterization of the Source Organism

DHA Algal Oil is derived from the heterotrophic fermentation of the marine alga, *Schizochytrium* sp. *Schizochytrium* sp. is a thraustochytrid and a member of the Chromista kingdom (Stramenopilia) which includes the golden algae, diatoms, yellow-green algae, haptophyte and cryptophyte algae, and oomycetes. There are no reports of this organism producing toxic chemicals nor is it pathogenic. Chemical and biological analysis of the production strain confirmed the absence of common algal toxins. Field tests confirm the widespread occurrence of thraustochytrids in a typical marine food chain. Consumption by man of thraustochytrids, especially those of the genus *Schizochytrium*, is primarily through consumption of mussels and clams. Indirect consumption, through the marine food chain (fish and shellfish), is more widespread.

Schizochytrium sp. microorganisms are widespread and are commonly found in marine environments throughout the world. The literature indicates that thraustochytrids, especially those of the genus *Schizochytrium*, are regularly consumed as food by a wide range of invertebrates. Based on existing published and unpublished scientific data, there have never been any reports of toxic compounds produced by these microorganisms. Blue-green algae and dinoflagellates produce most of the toxic compounds produced by microalgae, and *Schizochytrium* sp. is in a separate kingdom from both of these types of

microalgae. The two toxic compounds known to be produced in the Chromista (to which *Schizochytrium* sp. belongs) are largely restricted to two genera (domoic acid in *Pseudonitzschia* and prymnesin in *Prymnesium* spp.) which are in a separate class and phylum, respectively, from the thraustochytrids. No evidence of two toxic compounds produced in the Chromista, namely domoic acid and *Prymnesium* toxin, was found in *Schizochytrium* sp. algae using chemical and biological assays.

Martek asked Dr. Colin Ratledge, Professor of Microbial Biochemistry in the Department of Biological Sciences at Hull University in the United Kingdom, a microbiologist, with peer-recognized expertise in oil-producing marine microorganisms, to review the available data on *Schizochytrium* sp. and offer an opinion on whether it is appropriate to use *Schizochytrium* sp. as the source organism for a food ingredient. Dr. Ratledge concludes that *Schizochytrium* is an entirely suitable organism for the production of microbial oils that could be incorporated into foods (Ratledge, 2003).

III. INTENDED CONDITIONS OF USE

DHA Algal Oil is intended to be used as a direct food ingredient to increase dietary intake of the long chain omega-3 fatty acid DHA in food categories and use levels as listed in Table 2. These food ingredient categories are based on the food categories for menhaden oil. In addition, we propose to add DHA to those additional food categories that were reviewed by the agency as part of the GRAS notification for fish oil concentrate (GRN000105). We are not proposing at this time, to include this DHA Algal Oil as an ingredient in infant formula preparations.

The proposed use levels of the DHA Algal Oil are expected to result in a maximum dietary exposure of less than 1.5 grams of DHA per day. Other oil sources of DHA and EPA reviewed under the agency's GRAS program have resulted in maximum use levels of up to 3 grams combined DHA and EPA per day. Martek is limiting the level of DHA in this notice to 1.5 grams per day because this is the maximum level considered GRAS by the panel of independent experts convened by Martek. The expert panel set the 1.5 gram level per day because the panel considered such a level to be an appropriate level for realizing the health benefits of including DHA in the diet. Given the long-standing FDA position that a GRAS ingredient should not be used at a level that exceeds "the amount reasonably required to accomplish the intended physical, nutritional, or other technical effects in food," [1]/ the expert panel did not evaluate whether the DHA Algal Oil would be GRAS at a level in excess of 1.5 grams of DHA per person per day. Data collected by Martek since this GRAS review further supports the position that 1.5 grams of DHA per day is a sufficient level of DHA to accomplish the intended nutritional and other effects of including DHA in

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[1]/ 21 CFR 184.1(b).

the diet. This GRAS notification, therefore, is based on a maximum use level of 1.5 grams of DHA per day.

DHA Algal Oil will initially be added to the same food categories as those currently listed in 21 CFR 184.1472(a)(3) (menhaden oil) at maximum use levels that are 29 percent of those specified in that regulation. We derived the 29 percent value because of the following factors:

- Whereas menhaden oil is considered GRAS at a level providing no more than three (3) grams of DHA and EPA per day, the GRAS panel convened by Martek concluded that this DHA Algal Oil is GRAS at a level providing 1.5 grams of DHA per day. The use levels in each food category are decreased by 50 percent so that total daily consumption of DHA from the DHA Algal Oil will be no more than 1.5 grams per day.
- The levels of use are based on the quantity of DHA Algal Oil that can be added to each product. An additional adjustment is needed because the DHA Algal Oil has a different concentration of DHA than that found in menhaden oil. DHA Algal Oil contains approximately 350 mg DHA per gram oil (35 wt%) compared to about 20 percent combined EPA and DHA in menhaden oil. An additional adjustment of 57 percent is needed to accommodate the different concentrations of DHA in the two oils.
- The 29 percent adjustment is calculated by multiplying the 50 percent adjustment that is needed in accordance with the first bullet point above by the 57 percent adjustment that is needed in accordance with the second bullet point above. ((0.50) x (0.57) x 100 = 29 percent).

In addition to food categories currently listed in 21 CFR 184.1472(a)(3), the GRAS notification also covers the addition of DHA Algal Oil to soy protein bars; processed vegetable drinks; hard candy; soft candy; non-dairy and powdered cream substitutes; jams and jellies; milk, dry and powdered mixes; milk-based meal replacements; flavored milk and milk products; and non-dairy milk, imitation and soy milk. These are the same additional food categories found in the GRAS notification for fish oil concentrate (GRN 000105) and for which the agency did not raise any objections to the company's conclusion that its fish oil concentrate would be considered GRAS when used in the food categories identified for menhaden oil and these additional food categories. [2]

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[2] Although we made a 29 percent adjustment to the food category levels listed in 21 CFR 184.1472(a)(3), we only made a 50 percent adjustment to the additional food category levels found GRN 105. The DHA Algal Oil and the concentrated fish oil both contain approximately 35 percent DHA. No adjustment to the use levels, therefore, is needed to compensate for differences in the DHA concentrations of the oils. A 50 percent adjustment to the levels listed in GRN 105 is needed because this GRAS notification covers a maximum use level of DHA per day

The agency ostensibly based this conclusion on data demonstrating that the addition of the fish oil concentrate to these additional food categories would not result in a daily exposure to DHA and EPA of more than 3 grams per day.

FDA recently issued a proposed rule (the menhaden oil proposal; 67 Fed. Reg. 8744, February 26, 2002) that would amend 21 CFR 184.1472(a)(3) by changing the maximum use levels and food categories in which menhaden oil will be used. Consistent with the position taken by the agency in its review of prior GRAS notifications for DHA containing substances, we ask that the agency apply any changes to the food categories in 21 CFR 184.1472(a)(3) to DHA Algal Oil. The maximum use levels in any such revised final rule would need to be adjusted by 29 percent for the reasons discussed above.

Table 2 identifies the maximum use level of DHA Algal Oil in the various food categories discussed above. The estimated mean intake of DHA from the initial intended uses and future intended uses at the maximum use levels of DHA Algal Oil listed in Table 2 by U.S. consumers will be approximately 1.4 g/p/d. This level is 50 percent of that estimated from combined DHA and EPA that would result from the fish oil concentrate that is the subject of GRN 000105.

DHA Algal Oil is intended to be the sole source of DHA in any given food category. It would be possible, however, to blend DHA Algal Oil with other sources of DHA and/or EPA. FDA has determined in its review of other sources of DHA and/or EPA that these oils may be used at a level providing up to 3.0 grams of DHA and/or EPA per day. In the event that a manufacturer blends DHA Algal Oil with another oil that is a source of DHA and/or EPA, such blending would be appropriate provided that (1) the DHA Algal Oil is used at a level consistent with Table 2 (*i.e.,* its use would not result in more than 1.5 grams of DHA exposure per day) and (2) the other oil source of DHA and/or EPA is used at a level that would not result in a cumulative exposure of DHA and EPA of greater than 3.0 grams per person per day.

IV. BASIS FOR THE GRAS DETERMINATION

The GRAS determination for DHA Algal Oil derived from the alga, *Schizochytrium* sp., under the maximum use levels listed in Table 2 is based on scientific procedures as described under 21 CFR 170.30(b).

The company convened an Expert Panel of independent scientists, qualified by scientific training and experience for evaluating the safety of food ingredients, to evaluate the available information on DHA Algal Oil derived from *Schizochytrium* sp., and to determine whether such algal oil is GRAS. The Panel

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of 1.5 grams while the concentrated fish oil notification covered up to 3 grams per day of DHA and EPA.

included Dr. Joseph F. Borzelleca (Professor Emeritus, Medical College of Virginia), Dr. Gary Flamm (Flamm Associates), and Dr. Ian Munro (Cantox Health Sciences International) (Borzelleca et al., 2000).

The Panel evaluated the safety of DHA Algal Oil under conditions of intended use as a food ingredient using appropriate scientific procedures. A comprehensive search of the literature on *Schizochytrium* sp. algae, the individual components of the DHA Algal Oil, and related substances was performed and made available to the panel. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in food products, consumption estimates for all intended uses and comprehensive literature on the safety of individual components of the DHA Algal Oil, similar marine oils, and safety studies conducted on the DHA Algal Oil and the *Schizochytrium* sp. algae. Panel members individually and independently reviewed the appropriate material and collectively made the following determination:

> Based on its critical, independent and collective evaluation of available information and the use of appropriate scientific procedure, the Panel concludes that DHA Algal Oil derived from the alga *Schizochytrium* sp., meeting food grade specifications and manufactured in accordance with current Good Manufacturing Practices (GMP) would be Generally Recognized as Safe (GRAS) when used as a nutritional ingredient in foods in accordance with current GMP equating to a daily intake of 1.5 g DHA.

A. Detailed Safety Information

The safety of DHA Algal Oil derived from *Schizochytrium* sp. algae is based on 1) the history of use of fatty acid and sterol components of the oil as a result of their abundant natural presence in food and the small quantities expected to be consumed; 2) extensive knowledge of the absorption, distribution, metabolism and excretion of the fatty acid and sterol components in mammalian species; 3) published safety information on these and similar compounds; 4) safety data on the oil; and 5) results of toxicology studies on the dried algae. Results of toxicology studies have been published in peer reviewed journals and were conducted by dietary administration or gavage of the oil or source algae in laboratory animals and target species of food producing animals. Safety is further supported by the historical safe use of oil as a dietary supplement and algae as a dietary ingredient in commercial animal species.

1. Source Organism

As discussed above, there are detailed data and information supporting the safety of using this organism as a source for a food ingredient. The expert panel reviewed these data and concluded that they support the use of *Schizochytrium* sp. as the source organism of the DHA Algal Oil.

2. Oil Components

The identified components present in DHA Algal Oil have a demonstrated history of safe consumption. The lipid fraction of *Schizochytrium* sp. algae is comprised mainly of fatty acids and sterols. Fatty acids (Table 3) are found esterified to glycerol (tri- and diacylglycerides) and sterols (steryl esters) and may be present as free fatty acids. Sterols (Table 4) are found as steryl esters and free sterols. Beta-carotene was identified as the primary carotenoid component of the lipid fraction (Zeller et al., 2001).

All fatty acids present in DHA Algal Oil are components of a normal diet or normal metabolites of fatty acids. Recommended use levels will only increase the consumption of two component fatty acids, DHA and docosapentaenoic acid (DPA(n-6)), above that currently consumed from the diet. A comprehensive discussion on the safety of the fatty acid components present in DHA Algal Oil derived from *Schizochytrium* sp. algae along with knowledge of the absorption, distribution, metabolism and excretion of the fatty acids and published safety information on these and similar compounds have previously been provided to the agency as part of a New Dietary Ingredient Premarket Notification filed in December 1997 by Monsanto for SeaGold™ DHA-rich oil—which is the same oil that is the subject of this GRAS notification. [3]/

The non-saponifiable fraction of the DHA Algal Oil consists primarily of squalene, sterols, and carotenoids. These components are all present in the food supply. At the proposed use level for a food ingredient, the estimated consumption of sterols approximates the current consumption of sterols in the general population from other food sources and is likely smaller than some groups within the population such as vegetarians.

Additional information on the safety of the sterol components present in the oil component of *Schizochytrium* sp. algae along with knowledge of the absorption, distribution, metabolism and excretion of sterols and published safety information on these and similar phytosterols has previously been supplied to the agency in the New Dietary Ingredient Premarket Notification submitted by Monsanto in December 1997 for SeaGold™ DHA-rich oil—the same oil that is the subject of this notification. [4]/

3. DHA and DPA(n-6)

Proposed uses of DHA Algal Oil derived from *Schizochytrium* sp. algae as a food ingredient will only increase the consumption of two component fatty acids, DHA and DPA(n-6), above that currently consumed from the diet. FDA has affirmed that the mean consumption of up to 3 g of DHA and EPA (from menhaden oil) per day is GRAS; therefore the proposed consumption of up to 1.5 g DHA per day from DHA Algal Oil is considered safe.

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[3]/ See http://www.fda.gov/ohrms/dockets/dockets/95s0316/rpt0017_01.pdf

[4]/ See http://www.fda.gov/ohrms/dockets/dockets/95s0316/rpt0017_01.pdf.

Based on the fatty acid composition of DHA Algal Oil derived from *Schizochytrium* sp. algae, the DPA(n-6) intake would approximate 0.54 g/p/d assuming all foods listed in Table 2 containing the maximum use level of oil would be consumed daily by a consumer. The actual daily average intake of DPA(n-6) should be significantly less than 0.54 g/p/d based on the unlikely scenario that a consumer would choose all foods in the marketplace within the proposed food categories that contain DHA Algal Oil as a substitute for another edible oil.

DPA(n-6) is a normal component of the human body and in the human diet. Raw and cooked food samples including beef rib eye, chicken breast, chicken thigh, whole egg, pork loin, turkey breast and white tuna have been reported to contain DPA(n-6) (Taber et al., 1998). Numerous examples of commercial seafood containing significant amounts of DPA(n-6) are also cited in the literature (Nichols, et al. 1998). Small amounts of DPA(n-6) are naturally part of the infant diet since this fat is present in human milk. Indeed, the Expert Panel noted that the DPA(n-6) intake level from the proposed use of DHA Algal Oil was approximately equivalent to that consumed by breast fed infants on a mg/kg body weight basis. Breast milk levels most likely reflect a combination of dietary intake and endogenous synthesis (from long chain omega-6 precursors) of this fat.

Plasma, erythrocyte and liver concentrations of DPA(n-6) are highly influenced by dietary intake of this fatty acid. Co-administration of DHA and DPA(n-6) from DHA Algal Oil results in a dose dependent increase in both of these fatty acids (Barclay et al., 2003). The functional impact of incorporation of DPA(n-6) in non-neural tissues appears to be the preservation of long chain omega-6 fatty acids in those tissues, including most importantly arachidonic acid (ARA). Preservation of both DHA and ARA in non-neural tissues is important in many applications including infant and toddler nutrition where ARA is a predominant growth factor.

Dietary DPA(n-6) when co-administered with DHA does not appear to replace DHA in the developing or mature brain. In fact, little if any DPA gets accreted into brain under these circumstances, suggesting that the brain has a high preference for DHA (Tam et al. 2000). Animal studies indicate that in a DHA insufficient animal model, DHA tissue levels are restored and DPA(n-6) declines in response to supplementation with DHA and DPA(n-6) (Barclay et al., 2003).

B. Studies on DHA Algal Oil and Schizochytrium *sp. Algae*

1. Sub-Chronic Feeding Studies

Schizochytrium sp. algae were administered in the diet to rats for at least thirteen weeks. The algae were administered in the diet to groups of twenty male and twenty female Sprague-Dawley derived rats to provide dosages of 0, 400, 1500 and 4000 mg/kg body weight (bw)/day for at least 13 weeks. Untreated controls received basal diet only. An additional group of twenty males and twenty females received rodent diet mixed with fish oil (Arista) to provide a

target dosage of 1628 mg/kg bw/day, an amount of oil comparable to that received by rats administered the highest dose of the algae.

There were no treatment-related effects in clinical observations, body weights or weight gains, food consumption, hematological or urinalysis values, gross necropsy findings or organ weights and there were no deaths. The only treatment-related changes in clinical chemistry parameters were decreases in high-density lipoproteins (HDL) and cholesterol in the algae and fish oil groups when compared to the untreated controls. These changes were expected based on the high polyunsaturated fatty acid content of dried algae and fish oil. There were no microscopic findings suggestive of toxicity. Periportal hepatocellular fat vacuolation (accumulation of fat) was observed only in the livers of female rats in both the algae (all dosages) and fish oil groups. This finding was expected given the higher fat content of both the algae and fish oil diets compared to the basal diet fed to the untreated controls. A slight increase in the incidence, but not severity, of cardiomyopathy was observed only in the 4000 mg/kg bw/day algae males. This finding was not considered adverse because cardiomyopathy occurs spontaneously in rats, and especially male rats of the Sprague-Dawley strain when fed high levels of fat. Since cardiomyopathy does not develop in other species including primates fed high fat diets, its occurrence in rats is considered to have little relevance to human health.

This study demonstrates that administration of *Schizochytrium* sp. algae and its oil component does not produce any treatment-related adverse effects in Sprague-Dawley rats at dosages up to 4000 mg algae/kg bw/day (or approximately 1600 mg oil/kg bw/day) for 13 weeks. (Hammond et al., 2001a)

2. Developmental Toxicity Evaluation in Rats and Rabbits

The developmental toxicity of *Schizochytrium* sp. algae was assessed in Sprague-Dawley-derived rats (25 per group provided dried algae in the diet at 0.6, 6 and 30% on gestation days [GD] 6-15) and in New Zealand White Rabbits (22 per group, dosed with dried algae at levels of 180, 600 and 1800 mg/kg bw/day by oral gavage on GD 6-19). Fish oil was used as a control at dose levels to provide an equivalent amount of oil to that received by the high dose rabbits. Maternal food consumption, body weights and clinical signs were recorded at regular intervals throughout these studies. Animals were sacrificed on GD 20 (rats) and GD 29 (rabbits) and examined for implant status, fetal weight, sex and morphologic development. No clinical signs of toxicity were observed. Maternal exposure to dried algae during organogenesis did not adversely affect the frequency of post implantation loss, mean fetal body weight per litter, or external, visceral or skeletal malformations in either the rat or the rabbit.

In the rats, neither maternal nor developmental toxicity was observed at any dietary concentration of algae. Thus 22000 mg/kg bw/day of the algae administered in the feed to pregnant rats during organogenesis was the

NOAEL (no observed adverse effect level) for both maternal and developmental toxicity.

In rabbits, no maternal toxicity was expressed at algae dose levels of 180 and 600 mg/kg bw/day. As a possible consequence of the high-fat content of the fish oil and algae, reductions in food consumption and body weight gain and a slight increase in abortions occurred in the fish oil control and 1800 mg/kg bw/day algae groups. Developmental toxicity was not observed at any algae dose level in the rabbit. Based on the results of this study, the NOEL (no observed effect level) for maternal toxicity of algae was 600 mg/kg bw/day, and the NOEL for developmental toxicity was 1800 mg/kg bw/day. (Hammond et al., 2001b)

3. Single Generation Rat Reproduction Study

The reproductive toxicity of *Schizochytrium* sp. algae was examined in Sprague-Dawley-derived rats Crl:CD®(SD)BR (30 per sex per group) provided algae in the diet at concentrations of 0, 0.6, 6.0 and 30%. These dietary levels corresponded to overall average dosages of approximately 400, 3900 and 17800 mg/kg bw/day for F_0 males (pre-mating) and 480, 4600 and 20700 mg/kg bw/day for F_0 females, respectively. Prior to mating, males and females of the F_0 generation were treated for 10 weeks and 2 weeks, respectively. Treatment of males continued throughout mating and until termination (approximately 3 weeks after mating). Treatment of the females was continued throughout gestation and through lactation day 21. The females were killed after raising their young to weaning at 21 days of age. Food consumption was measured weekly throughout the study (except during mating) and body weights were recorded at least weekly during premating, gestation and lactation. Reproductive parameters including estrus cycle duration, mating performance, fertility, gestation length, parturition and gestation index were evaluated. Litter size, and offspring body weights were recorded, offspring viability indices were calculated, and physical development (vaginal opening and preputial separation) was assessed for the F_1 generation. All adult F_0 and F_1 animals were subjected to a detailed necropsy.

The algae treatment had no effects on estrus cycles or reproductive performance including: mating performance, fertility, gestation length, parturition or gestation index. Litter size, sex ratio and offspring viability indices were similarly unaffected and there were no effects of dried algae treatment to the physical development of F_1 animals. (Hammond et al., 2001c)

4. Mutagenicity Studies

A series of studies to assess the genotoxic potential of *Schizochytrium* sp. algae and its oil component were performed. All *in vitro* assays were conducted with and without mammalian metabolic activation. *Schizochytrium* sp. algae and its oil component were not mutagenic in an Ames reverse mutation assay using five different *Salmonella* histidine auxotroph tester strains. Mouse lymphoma suspension assay methodology was found to be

inappropriate for this test material because precipitating test material could not be removed by washing after the intended exposure period and the precipitate interfered with cell counting. The AS52/XPRT assay methodology was not subject to these problems and algae was tested and found not to be mutagenic in the CHO AS52/XPRT gene mutation assay. *Schizochytrium* sp. algae were not clastogenic to human peripheral blood lymphocytes in culture. Additionally, algae did not induce micronucleus formation in mouse bone marrow *in vivo* further supporting its lack of any chromosomal effects. Overall, the results of this series of mutagenicity assays, combined with the rodent and rabbit studies cited above, support the conclusion that *Schizochytrium* sp. algae does not have any genotoxic potential. (Hammond et al., 2002)

5. Historical Safe Use in Target Animal Species

Schizochytrium sp. algae have been utilized in aquaculture applications, including enrichment of DHA in *Artemia* and rotifers used to feed larval fish and shrimp (Barclay and Zeller, 1996; Luizi et al., 1999). Prior to merging with Martek, OmegaTech commercialized a product for aquaculture applications (HUFA2000, a spray-dried form of *Schizochytrium* sp. algae) that has been successfully utilized for over seven years with no adverse effects in shrimp larva culture and finfish (red seabream, Japanese flounder) culture. Use of algae from *Schizochytrium* sp. in these applications promotes larvae survival and growth. Studies have also been performed in juvenile mussels, *Mytilus galloprovincialis*, fed diets of spray-dried algal products, which included *Schizochytrium* sp. algae (Docosa Gold). Mussels fed dried *Schizochytrium* sp. algae as a partial replacement of live microalgae (*Spirulina platensis* or *Hematococcus pluvialis*) grew significantly faster than mussels fed a full live algal ration. Also, mussels fed diets containing dried algae derived from *Schizochytrium* sp. grew significantly faster than mussels fed an equal ration of living Tahitian *Isochrysis galbana*.

Algae from *Schizochytrium* sp. produced by fermentation are Generally Recognized as Safe (GRAS) for use as a feed ingredient incorporated into the feed rations of laying hens and broiler chickens at up to 4.3% of 2.8%, respectively (Abril, et al., 2000). Eggs containing up to 2.5-5.0 times the DHA content of regular market eggs can be produced using *Schizochytrium* sp. algae as an animal nutritional food supplement. These eggs have been commercialized in Europe since 1996 and in the U.S. since 1998 under the Gold Circle Farms® brand. Additional references regarding the use of *Schizochytrium* sp. algae have been described in the literature for use in poultry applications (Herber and Van Elswyk, 1996; Herber-McNeill and Van Elswyk, 1998) poultry eggs and meat applications (Abril and Barclay, 1998; Barclay et al., 1998; Zeller et al. 2001), and for use in enriching swine and dairy products (Marriott et al. 2002a; Marriott et al. 2002b).

6. Laying Hen Study

A target animal safety trial with laying hens was conducted using *Schizochytrium* sp. algae at dose levels of 165, 495, and 825 mg DHA/hen/day.

Each treatment consisted of 64 laying hens divided into eight replicates (cages) per group for a total of 320 animals on study. As required by FDA laying hen target animal safety protocols, all of the hens were preconditioned for one month prior to the start of the dosing period by feeding a basal commercial type layer feed. Body weights, feed conversion, egg production, egg weight, shell thickness, and interior quality were measured at the end of each of the four months during the dosing period. Eggs were also collected and analyzed at the end of months 2 and 4 for their weight, shell thickness, interior egg quality, and fatty acid profile. At the end of the 4-month dosing period, terminal sacrifices were conducted and two randomly selected hens from each dose level and replicate were evaluated for hematological and histopathological changes. Hematological analyses included the following: red blood cell count, hematocrit, differential leukocyte count and hemoglobin. As dietary omega-3 fatty acids are known to decrease platelet reactivity, blood-clotting time was also determined. Gross necropsy was completed on all layers found dead during the trial or killed for scheduled evaluation. Weights were determined for the following organs: liver, kidney, heart, bursa of Fabricus, brain, spleen, thymus, bone marrow, and ovaries. Tissues were collected for histopathology, preserved, and evaluated. Breast tissue samples were evaluated for fatty acid profile by gas chromatography. Consequence of experimental diets was determined via statistical analysis of feed consumption/efficiency, egg production, egg weight, egg quality, body weight, organ weight, and histopathology.

There were no significant differences in any of the organ weights measured and there were no significant differences in the feathering score between any of the treatments. The results of the histopathological examination also indicated that no alterations could be observed in the tissues examined that would differentiate between treatment groups. There were also no significant differences between treatments for any of the hematological analyses

It was concluded, based on results from this study, that *Schizochytrium* sp. algae are safe as a feed ingredient for laying hens at 3040 mg/kg bw/day dried algae delivering approximately 532 mg DHA/kg bw/day. (Abril et al., 2000)

7. Broiler Chicken Study (Unpublished)

A target animal safety trial with broiler chickens was conducted with two thousand two hundred and forty birds, sexed at day of hatch, wing banded, and randomly assigned to one of four dietary treatments. In addition to a control broiler ration, dietary treatments of *Schizochytrium* sp. algae delivered 82, 240, and 408 mg DHA/bird per day. Each dietary treatment contained 560 broilers divided among eight replicates (n=70; 35 males; 35 females). All rations were pelletized for feeding to the birds. Group body weights for each pen were determined on days 0, 21, and 49 of the feeding trial. Feed consumption was evaluated for each pen on days 21, 42, and 49 of the trial and used to determine feed efficiency for feeding periods 0-21 days and 0-49 days. On days 4 and 49, birds (n=2 per replicate) were bled for hematological analyses and sacrificed for

histopathologic evaluation. Hematological analyses included the following: red blood cell count, hematocrit, differential leukocyte count, and hemoglobin. As dietary n-3 FA are known to decrease platelet reactivity, blood-clotting time was also determined. Gross necropsy was completed on all broilers found dead during the trial or killed for scheduled evaluation. Weights were determined for the following organs: liver, kidney, heart, bursa of Fabricus, brain, spleen, thymus, bone marrow, and ovaries. Tissues were collected for histopathology, preserved, and evaluated. Breast samples were evaluated for fatty acid profile by gas chromatography. Consequence of experimental diets was determined by statistical analysis of feed consumption/efficiency, body weight, organ weight, and histopathology.

The results of this study indicate that there was no effect of treatment level on any of the evaluated broiler growth performance measures. There was no significant difference between treatment level regarding weight gain, feed intake, or feed conversion. There was no significant difference between treatments on organ weight for the liver, kidney, heart, bursa of Fabricus, brain, spleen, thymus, bone marrow, or ovaries. The histopathological examination also indicated that no alterations could be observed in the tissues examined that would differentiate between treatment groups. There was no significant difference between treatments for any of the hematological analyses conducted.

Based on the results from this unpublished study, it is concluded that *Schizochytrium* sp. algae are safe as a feed ingredient for broiler chickens at 2331 mg/bird/day dried algae delivering approximately 408 mg DHA/bird/day.

8. Swine Study

A target animal study was conducted in swine to determine the potential toxicity of *Schizochytrium* sp. algae. In this study, dried algae were administered in the diet to groups of castrated male growing pigs (mixed commercial breeds Land Race & Large White) reared from early weaning to approximately 250-270 pounds. Over the course of the 120-day study, animals were fed *ad libitum* four treatment diets each designed to optimize weight gain over the growing cycle, and a control diet. *Schizochytrium* sp. algae were incorporated into the diet of the first treatment group at a level delivering 2.680 kg algae per pig over the course of 120 days (a constant, whole-life exposure) equating to 598 g DHA per pig (or approximately 511 mg algae/kg bw/day or 114 mg DHA/kg bw/day). *Schizochytrium* sp. algae were incorporated into finisher diets only (administered over the last 42 days of the growing cycle) to treatment groups 2, 3, and 4 delivering 1.169, 3.391, and 5.746 kg algae per pig (261, 756, and 1281 g DHA per pig). These levels represent approximately 1, 3, and 5 times the anticipated commercial dose and were delivered in a feeding strategy designed to mimic commercial use.

Results of this animal study demonstrated no statistically significant treatment-related effects in clinical observations, body weights, food

consumption, mortality, hematological values, gross necropsy findings, organ weights or histopathology. The only treatment-related changes were higher weight gain and feed conversion efficiency, anticipated results based on the increased fat content in the experimental algae diets.

In summary, this study demonstrates that administration of dried *Schizochytrium* sp. algae (at up to five times the anticipated commercial dose) does not produce any treatment-related adverse effects in commercial strains of swine. (Abril et al., 2003)

9. Availability of DHA in *Schizochytrium* sp. Algae

Availability of DHA when administered in the form of *Schizochytrium* sp. algae was evaluated in swine and rats. Nutritional availability of DHA was assessed by the dose responsiveness of rat and swine tissues to varying levels of *Schizochytrium* sp. algae in their diets. In one study, female and male rats were fed whole-cell *Schizochytrium* sp. algae for 13 weeks providing approximately 32, 324 and 1440 mg DHA/kg bw/day. The fatty acid content of rat sera and brain tissue was analyzed at the end of the 13-week period. In a second study, weanling male pigs were fed *Schizochytrium* sp. algae for 120 days at levels providing 114 mg DHA/kg bw/day and their brain tissue fatty acids were then quantified.

The sera data from the rat study indicated excellent availability of the fatty acids in the algae. Swine data also support ready availability of DHA from algae. In summary, these studies demonstrate that DHA content of tissues increases in rat and swine when *Schizochytrium* sp. algae are administered into the diet. (Barclay et al., 2003).

C. Data Inconsistent with GRAS Position

Martek is unaware of any data that would be inconsistent with a finding that the DHA Algal Oil is GRAS when used as a source of DHA in the foods and at the levels covered by this GRAS notification.

D. Basis for GRAS Position

A review of the data summarized in this GRAS notification establish that there is general recognition, among experts qualified by scientific training and experience to evaluate the safety of substances added to food, that there is reasonable certainty that the DHA Algal Oil is not harmful under its intended conditions of use. Indeed, Martek convened an Expert Panel to review the available data and information and this panel reached the following conclusion.

> Based on its critical, independent and collective evaluation of available information and the use of appropriate scientific procedure, the Panel concludes that DHA Algal Oil derived from the alga *Schizochytrium* sp., meeting food grade specifications and

manufactured in accordance with current Good Manufacturing Practices (GMP) would be Generally Recognized as Safe (GRAS) when used as a nutritional ingredient in foods in accordance with current GMP equating to a daily intake of 1.5 g DHA.

The data in this notification and the exposure data presented to the agency for other GRAS sources of DHA and EPA, establish that the uses of DHA Algal Oil in the food categories and at the levels specified in this notification will not exceed 1.5 grams of DHA per day.

V. CONCLUSION

Martek believes that the data and information establish that DHA Algal Oil derived from *Schizochytrium* sp. is GRAS on the basis of scientific procedures when used as a source of DHA in the various food products, and at the levels, specified in Table 2.

VI. REFERENCES

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Table 1. Product specifications for DHA Algal Oil from **Schizochytrium** *sp.*

PHYSICAL AND CHEMICAL TESTS	SPECIFICATION
Color	Yellow to light orange
Acid Value	Not more than 0.5 mg KOH/g
Peroxide Value (PV)	Not more than 5.0 meq/kg oil
Moisture and Volatiles	Not more than 0.1%
Unsaponifiables	Not more than 4.5%
Trans-fatty acids	Not more than 2%
DHA content (%FAME)	Between 32 and 45%
DPA(n-6) content (%FAME)	Between 10 and 20%
Hexane	Not more than 10 mg/kg
ELEMENTAL ANALYSIS	
Arsenic	Not more than 0.5 mg/kg
Copper	Not more than 0.1 mg/kg
Iron	Not more than 0.5 mg/kg
Mercury	Not more than 0.2 mg/kg
Lead	Not more than 0.2 mg/kg

KOH : potassium hydroxide
meq: milliequivalents
FAME: fatty acid methyl ester

TABLE 2. Maximum Intended Use Levels of DHA Algal Oil from* Schizochytrium *sp. [1]

Category of Food	Initial Intended Use Level (%)	Future Intended Use Level (%)
Cookies, crackers (1) [2]	1.45	-
Breads, rolls (white and dark) (1) [2]	0.29	-
Fruit pies, custard pies (1) [2]	2.03	-
Cakes (1) [2]	2.9	-
Baked goods and baking mixes (1)	-	1.45
Cereals (4)	1.16	1.16
Fats and oils (12) (not including infant formula)	5.8	3.48
Yogurt (31) [3]	1.16	-
Milk products (31)	-	1.45
Cheese products (5)	1.45	1.45
Frozen dairy products (20)	1.45	1.45
Meat products (29)	2.9	1.45
Egg products (11)	1.45	1.45
Fish products (13)	5.8	1.45
Condiments (8)	1.45	1.45
Soup mixes (40)	0.87	0.87
Snack foods (37)	1.45	1.45
Nut products (32)	1.45	1.45
Gravies and sauces (24)	1.45	1.45
Soy protein bars (33) [4]	1.45	-
Plant protein products (33)	-	1.45
Processed vegetable drinks (36)	0.29	0.29
Hard candy (25)	2.9	2.9
Soft candy (38)	1.16	1.16
Non-dairy and powdered cream substitutes (10) [5]	1.45	-
Jams and jellies (28)	2.03	2.03
Milk, dry and powdered mixes (31) [5]	0.85	-
Milk-based meal replacements (31) [5]	0.29	-
Flavored milk and milk products (31) [5]	0.15	-
Non-dairy milk, imitation and soy milk (10) [5]	0.3	-
Dairy product analogs (10)	-	1.45
Nonalcoholic beverages (3)	-	0.15
Pastas (23)	-	0.58
Poultry products (34)	-	0.87
Processed fruit juices (35)	-	0.29
White granulated sugar (41)	-	1.16
Sugar substitutes (42)	-	2.9
Chewing gum (6)	-	0.87

Category of Food	Initial Intended Use Level (%)	Future Intended Use Level (%)
Gelatins and puddings (22)	-	0.29
Confections and frostings (9)	-	1.45
Sweet sauces, toppings, and syrups (43)	-	1.45

[1] The food categories correspond to those listed in 21 CFR 170.3(n). The number in parenthesis following each food category is the paragraph listing of that food category in §170.3(n) (21 CFR 170.3(n))
[2] Subsumed by "baked goods and baking mixes"
[3] Subsumed by "milk products"
[4] Subsumed by "plant protein products"
[5] Subsumed by "dairy product analogs"

Table 3. Fatty Acid Profile of DHA Algal Oil from* Schizochytrium *sp.

Fatty Acid	Concentration (mg/g)
Laurate (12:0)	4.0 ± <0.1
Myristate (14:0)	101.1 ± 8.6
Tetradecatrienoate (14:3n-3)	tr-4.5
Palmitate (16:0)	236.8 ± 9.4
Palmitoleate (16:1)	17.6 ± 9.9
Hexadecatrienoate (16:3n-6)	tr-5.0
Stearate (18:0)	4.5 ± 0.5
Vaccenate (18:1n-7)	tr-13.6
Octadecatetraenoate (18:4n-3)	tr-8.5
Dihomogamma-linolenate (20:3n-3) & Eicosatetraenoate (20:4n-7)	22.1 ± 2.4
Arachidonate (20:4n-6)	9.4 ± 1.7
Eicosatetraenoate (20:4n-3)	8.7 ± 0.4
Eicosapentaenoate (20:5n-3)	26.3 ± 6.4
Docosatetraenoate (22:4n-9)	5.4 ± 1.3
Docosapentaenoate (22:5n-6)	135.0 ± 15.0
Docosahexaenoate (22:6n-3)	350.0 ± 24.6

Fatty acid concentration expressed as average value (n=5 lots) ± standard deviation

Table 4. Sterol Profile of DHA Algal Oil from* Schizochytrium *sp.

Sterol Name	Concentration (%Peak Area)
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
Stigmastadien-3-ol	8 ± 1
Total Sterols ≈31 mg /g oil	

Sterol concentration expressed as average value (n=5 lots) ± standard deviation

000030

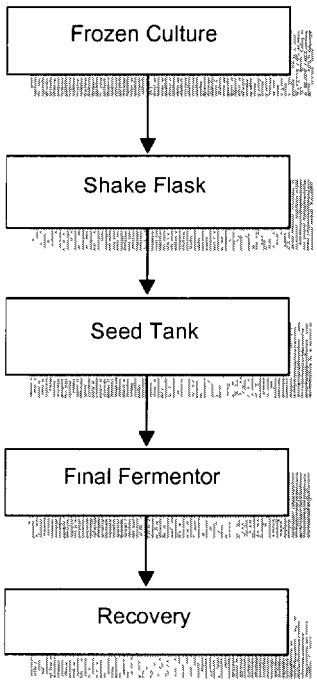

Figure 1. Overview of the fermentation process

000031

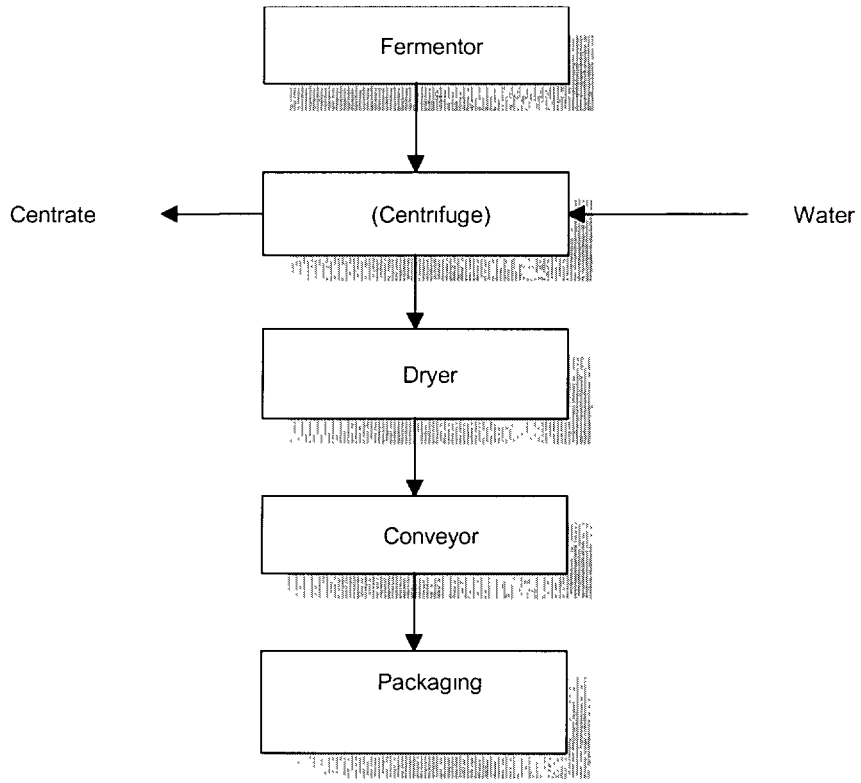

Figure 2. Overview of the dried algae product recovery process

000032

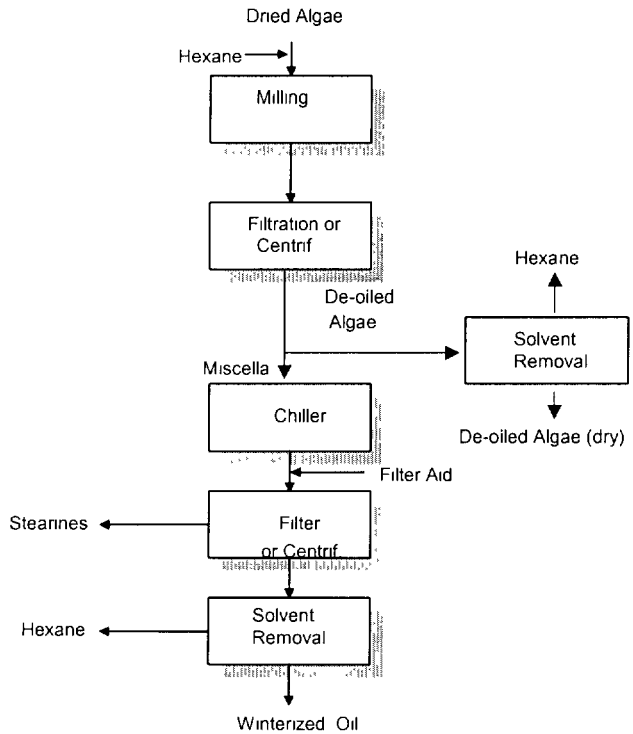

Figure 3. Overview of the DHA Algal Oil extraction process

000033

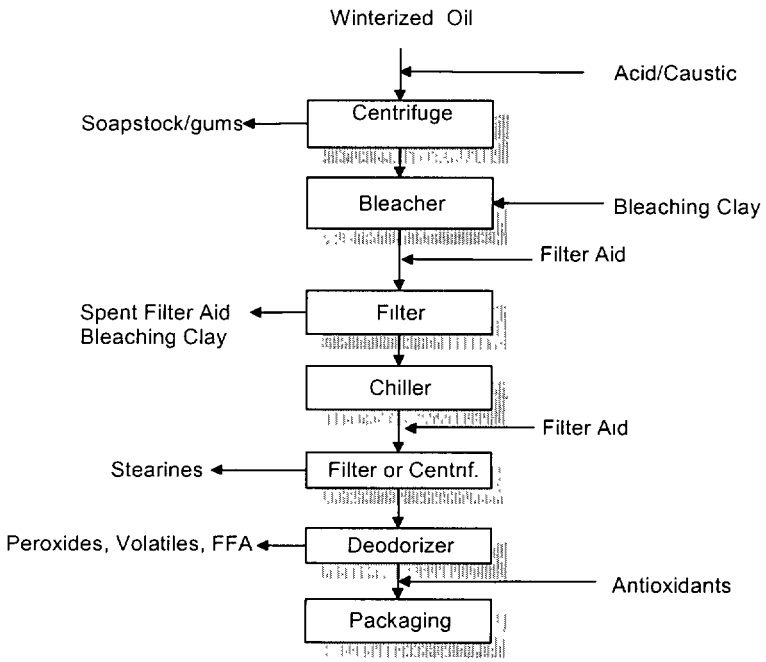

Figure 4. Overview of the DHA Algal Oil purification process

DIET MOISSIMMOS