GR

ORIGINAL SUBMISSION

~~REC'D MAY 3 0 2006~~

VIA HAND DELIVERY

May 26, 2006

REC'D MAY 3 0 2006
Cath

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

AAC
CONSULTING GROUP

7361 Calhoun Place,
Suite 500
Rockville, Maryland 20855-2765
301.838.3120
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RE: Submission of GRAS Notification – Omega-3 Triglyceride Concentrate (N-3-TG-2020)

Dear Sir/Madame:

In accordance with proposed 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the agent to the notifier, Twin Rivers Technologies, a GRAS Notification of OMEGA-3 Triglyceride Concentrate (N-3-TG-3020). Also enclosed is a GRAS panel report setting forth the basis for the GRAS determination.

Please let me know if you have any questions.

Sincerely,

Edward A. Steele
President

Enclosures

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OMEGA-3 TRIGLYCERIDE CONCENTRATE (N-3-TG-3020)

I. GRAS Exemption Claim

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A. Claim of Exemption From The Requirement for Premarket Approval Requirements Pursuant to Proposed CFR § 170.36(c)(1)

Omega-3 Triglyceride concentrate (N-3-TG-3020) has been determined to be generally recognized as safe, and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

_____ Date 5/26/06
Edward A. Steele

Agent for:

Twin Rivers Technologies
780 Washington Street
Quincy, MA 02169

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OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

B. Name and Address of Notifier

Twin Rivers Technologies
Attn: David Astrauckas
Vice President, Business Development
780 Washington Street
Quincy, MA 02169
Telephone: 617-745-4271
Facsimile: 617-472-5460
Email: dave.astrauckas@trtlp.com

C. Common or Usual Name of the Notified Substance and Identity of GRAS Substance

The subject that has been determined to be Generally Recognized as Safe (GRAS) and thus is the subject of this GRAS notification is a product derived from fish oil called "N3-TG-3020 Omega-3 Triglyceride Concentrate." This product is manufactured by Twin Rivers Technologies. N3-TG-3020 Omega-3 Triglyceride Concentrate is composed primarily of triglycerides, with lesser amounts of diglycerides. The glycerides making up N3-TG-3020 Omega-3 Triglyceride Concentrate contain a higher proportion of polyunsaturated fatty acids than the fish oil employed as the starting material, with the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) predominating at about 32 percent and 23 percent, respectively, yielding a combined EPA and DHA content of approximately 55 percent by weight. The fish oil employed as the starting material in the production of N3-TG-3020 Omega-3 Triglyceride Concentrate is extracted from multiple edible fish species including salmon, menhaden, anchovy, sardine, jack mackerel, mackerel and tuna.

N3-TG-3020 Omega-3 Triglyceride Concentrate is derived from fish oil by Twin Rivers Technologies employing a proprietary process for the production of a concentrated omega-3 fatty acid triglyceride containing substantially increased levels of EPA and DHA above those found in naturally occurring fish oils. It is described in detail in the section below titled Method of Manufacture. This process can be compared with other industrial fish-oil based practices for processing of marine oils into a refined fish oil concentrate.

D. Conditions of Use and Consumer Exposure

Twin Rivers Technologies intends to market N3-TG-3020 Omega-3 Triglyceride Concentrate for addition to food as a nutrient supplement to increase the dietary intake of the two omega-3 fatty acids, EPA and DHA, and as an alternative to other food-grade oils. N3-TG-3020 Omega-3 Triglyceride Concentrate will be added to the same categories of foods at concentrations providing the same combined EPA and DHA content as are permitted for menhaden under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). Further, N3-TG-3020 Omega-3 Triglyceride Concentrate will be used as the sole source of EPA and DHA in any given food category of this regulation and will not be combined or augmented with any other EPA/DHA-rich oil in making a food product. The

maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36 percent of that of menhaden oil. These proposed uses are presented in Table 1. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions (GRN Nos. 000102, 000105, 000109, 000138 and 000146) including one for a marine oil concentrate with similar increased EPA and DHA levels (GRN No. 000105). Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate.

The estimated mean intake of EPA and DHA combined from all dietary sources in the general population, excluding infants under the age of one year, has been addressed in earlier FDA rulemaking (menhaden oil final rule; 62 FR 30751; June 5, 1997) where FDA estimated that the mean exposure to EPA and DHA from the use of menhaden oil in all food categories would be 2.8 grams/person/day. It was also addressed as well in the subsequent GRAS Notices referenced above. The total cumulative estimated mean intake of EPA and DHA combined from the proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate listed in the foods in Table 1, as well as from all other dietary sources in this general population is also estimated to be 2.8 grams per person per day. This estimate reflects 100 percent market penetration of the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate that are listed in Table 1. Because 100 percent market penetration of this product is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower. Further, this cumulative intake of EPA and DHA combined is still less than the safe limit for EPA and DHA combined of 3 grams per person per day established by the agency. The proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate will result in no incremental increase in the intake of EPA and DHA combined over that described for menhaden oil in the final rule Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005. *[§184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day. (4) To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid].*

E. Basis for the GRAS Determination

The GRAS determination for N3-TG-3020 Omega-3 Triglyceride Concentrate under the proposed maximum use levels listed in Table 1 is based on scientific procedures as described under Title 21 of the Code of Federal Regulations (21CFR)§170.30(b). These scientific procedures have been used to demonstrate that the estimated intake of N3-TG-3020 Omega-3 Concentrate from the intended uses specified in Table 1, in conjunction with intakes of EPA and

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

DHA combined from other dietary sources, is safe, and also GRAS under the Food, Drug, and Cosmetic Act (FDCA). FDA has already established that a combined intake of EPA and DHA of less than 3 grams per person per day is a safe level of intake. In addition, this cumulative intake of EPA and DHA combined was also determined to be GRAS and the safety of this level of intake is generally recognized by experts qualified by both training and experience to evaluate the safety of substances directly or indirectly added to food, and is also based on generally available and accepted information.

Table 1. Proposed Maximum Use Levels in Food as Served of Omega-3 Triglyceride Concentrate (N3-TG-3020) Compared with Current Menhaden Oil Uses Permitted under 21CFR184.1472 and Final Rule Published in FR March 23, 2005(Volume 70, Number 55 PP14530-14532)

Category of food (and 21CFR §170.3(n) paragraph)	Maximum Level of Use of Menhaden Oil under 21CFR184.1472 and FR Vol 70 No 55 March 23 2005 Final Rule	Proposed Maximum Level of Use of N3-TG-3020 Omega-3 Triglyceride Concentrate[a]
Baked goods, baking mixes, §170.3(n)(1)	5.0 percent	1.8 percent
Cereals, §170.3(n)(4)	4.0 percent	1.4 percent
Cheese products, §170.3(n)(5)	5.0 percent	1.8 percent
Chewing gum, §170.3(n)(6)	3.0 percent	1.1 percent
Condiments, §170.3(n)(8)	5.0 percent	1.8 percent
Confections, frostings, §170.3(n)(9)	5.0 percent	1.8 percent
Dairy product analogs, §170.3(n)(10)	5.0 percent	1.8 percent
Egg products, §170.3(n)(11)	5.0 percent	1.8 percent
Fats, oils, §170.3(n)(12), but not in infant formula	12.0 percent	4.3 percent
Fish products, §170.3(n)(13)	5.0 percent	1.8 percent
Frozen desserts, §170.3(n)(20)	5.0 percent	1.8 percent
Gelatins, puddings, §170.3(n)(22)	1.0 percent	0.36 percent
Gravies, sauces, §170.3(n)(24)	5.0 percent	1.8 percent
Hard candy, §170.3(n)(25)	10.0 percent	3.6 percent
Jams, jellies, §170.3(n)(28)	7.0 percent	2.5 percent
Meat products, §170.3(n)(29)	5.0 percent	1.8 percent
Milk products, §170.3(n)(31)	5.0 percent	1.8 percent
Nonalcoholic beverages, §170.3(n)(3)	0.5 percent	0.18 percent

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

Table 1. Proposed Maximum Use Levels in Food as Served of Omega-3 Triglyceride Concentrate (N3-TG-3020) Compared with Current Menhaden Oil Uses Permitted under 21CFR184.1472 and Final Rule Published in FR March 23, 2005(Volume 70, Number 55 PP14530-14532)

Category of food (and 21CFR §170.3(n) paragraph)	Maximum Level of Use of Menhaden Oil under 21CFR184.1472 and FR Vol 70 No 55 March 23 2005 Final Rule	Proposed Maximum Level of Use of N3-TG-3020 Omega-3 Triglyceride Concentrate[a]
Nut products, §170.3(n)(32)	5.0 percent	1.8 percent
Pastas, §170.3(n)(23)	2.0 percent	0.7 percent
Plant protein products, §170.3(n)(33)	5.0 percent	1.8 percent
Poultry products, §170.3(n)(34)	3.0 percent	1.1 percent
Processed fruit juices, §170.3(n)(35)	1.0 percent	0.36 percent
Processed vegetable juices, §170.3(n)(36)	1.0 percent	0.36 percent
Snack foods, §170.3(n)(37)	5.0 percent	1.8 percent
Soft candy, §170.3(n)(38)	4.0 percent	1.4 percent
Soup mixes, §170.3(n)(40)	3.0 percent	1.1 percent
Sugar substitutes, §170.3(n)(42)	10.0 percent	3.6 percent
Sweet sauces, toppings, syrups, §170.3(n)(43)	5.0 percent	1.8 percent
White granulated sugar, §170.3(n)(41)	4.0 percent	1.4 percent

[a]Proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532) are set to provide the same concentrations of EPA+DHA as those provided by menhaden oil; based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate

The safety of consumption of N3-TG-3020 Omega-3 Concentrate for use as an ingredient in food is based on the similarity of this product's composition to other currently marketed fish-oil derived products including an Omega-3 Concentrate, as well as the safety of ingestion of its two major fatty acid constituents, EPA and DHA. Safety of consumption of the whole product was determined by evaluating the source of the product, the production process, the nature and quantity of impurities, product specifications, and the identity and positional distributions of EPA and DHA within the glycerides that comprise the final product. Appropriate specifications have been established to ensure that the final product is food grade, and compositional analysis

of the product supports the presumption that there is no toxicological concern from the ingestion of any product impurities.

The EPA and DHA levels in the Omega-3 Triglyceride Concentrate (N3-TG-3020) are increased above those found in naturally occurring fish oils by a very selective process involving separation of different fatty acid chain lengths from fish oils via either methylation or ethylation into fatty methyl or ethyl esters followed by distilling the short chain, non-omega-3 methyl or ethyl esters away from the longer chain methyl or ethyl esters and then re-esterifying the longer chain methyl or ethyl esters with glycerine to form a triglyceride, whose chain lengths are now concentrated in omega-3 content. Analysis of the triglycerides that comprise the Omega-3 Triglyceride Concentrate (N3-TG-3020) is presented in the GRAS determination document and compared to menhaden oil. The results indicate that these glycerides are structurally unchanged and their positional distributions within the glyceride are comparable to the starting fish oil. Based on this similarity, Omega-3 Triglyceride Concentrate (N3-TG-3020) would be expected to be metabolized by the body in a similar manner as naturally occurring fish oil.

In affirming the GRAS status of menhaden oil under 21CFR§184.1472, the FDA established that a daily intake of EPA and DHA combined not exceeding 3 grams per person per day is safe. The scientific basis to support the establishment of this safe level of intake has been published by the FDA. In 1997, FDA affirmed, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use to ensure that the total daily intake of EPA and DHA would not exceed 3.0 grams per person per day (g/p/d) (62 FR 30751; June 5, 1997; 21 CFR 184.1472). EPA and DHA are the major omega-3 fatty acids in fish oil and together comprise about 20 percent by weight of menhaden oil. FDA established maximum use levels of menhaden oil in certain foods because of concerns over possible adverse effects of fish oil consumption on bleeding time, glycemic control, and LDL cholesterol (62 FR 30751 at 30757; June 5, 1997). In 2002, FDA published a proposed rule to reallocate the uses of menhaden oil in conventional food, while maintaining the total daily intake of EPA and DHA from menhaden oil at a level not exceeding 3.0 g/p/d (67 FR 8744; February 26, 2002). FDA placed specific limitations, including the category of foods, the functional use of the ingredient, and the level of use, to ensure that the consumption of EPA and DHA from conventional food sources would not exceed 3.0 g/p/d. FDA then published a tentative final rule (69 FR 2313; January 15, 2004 **NOTE: the finalized allocation has since been published in a final rule on March 23, 2005, 70FR Number 55 Part 184 at page 14530**), to additionally require that menhaden oil not be used as an ingredient in foods in combination with other added oil that is a significant source of EPA and DHA to ensure that total intake from conventional food sources do not exceed 3.0 g/p/d.

The mean exposure to EPA and DHA from menhaden oil in all conventional food categories is estimated to be 2.7 g/p/d (67 FR 8744 at 8746; February 26, 2002). This is a conservative estimate with substantial margin for safety, and the agency believes, consistent with its prior decision on the use of a qualified health claim for DHA and EPA omega-3 fatty acids (October 31, 2000 letter) for dietary supplements, that the addition of menhaden oil to food products has not come close to this conservative mean estimate exposure. FDA further believes that the GRAS uses for which it received a GRAS notification for other sources of EPA and DHA omega-3 fatty acids also provide conservative estimates of exposure and that the addition of

these EPA and DHA sources to food products do not come close to the conservative mean estimates. Not all foods in the marketplace within those permitted food categories would contain menhaden oil or other sources of EPA and DHA omega-3 fatty acids that substitute for other edible fat or oil. Also, because not all foods that a consumer eats every day would contain menhaden or other EPA and DHA oil used as a substitute oil, the actual total daily intakes of EPA and DHA from menhaden or other EPA and DHA oil for an average person should be significantly below 3.0 g/p/d (67 FR 8744 at 8746; February 26, 2002).

On September 8, 2004, the requested use of this qualified health claim for omega-3 fatty acids was extended to conventional foods. The agency believes that there is likely to be some increased consumption of EPA and DHA omega-3 fatty acids based on conventional foods that bear the qualified health claim; however, the amounts of EPA and DHA that can be used and the foods in which such food ingredients can be safely used are limited. The agency has established specific limitations of use under its menhaden oil GRAS rule (62 FR 30751; June 5, 1997), proposed and tentatively finalized reallocation of the use of menhaden oil without changing total exposure levels (67 FR 8744; February 26, 2002, 69 FR 2313; January 15, 2004). **NOTE: the finalized allocation has since been published as a final rule on March 23, 2005, 70FR Number 55 Part 184 at page 14530.** Also, manufacturers that have submitted GRAS notifications for other sources, to which the agency has not objected, have established conditions of use similar to those in the menhaden oil GRAS rule.

Based on the data and information that FDA considered, which includes data and information that FDA relied upon in reaching its conclusions about the safety of EPA and DHA omega-3 fatty acids in its GRAS affirmation of menhaden oil, the data and information in the 1991 proposed (56 FR 60663; November 27, 1991) and 1993 final rules (58 FR 2683; January 6, 1993), and its current scientific literature review for other possible safety concerns, FDA concluded that the use of EPA and DHA omega-3 fatty acids used as a GRAS ingredient, consistent with FDA's GRAS rule for menhaden oil and GRAS notifications to which FDA did not object, and the use as a dietary supplement is safe and lawful under 21 CFR 101.14 provided that daily intakes of EPA and DHA omega-3 fatty acids from conventional food and dietary supplement sources do not exceed 3.0 g/p/d.

The N3-TG-3020 Omega-3 Triglyceride Concentrate that is the subject of this safety assessment is comprised primarily of triglycerides of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). It is known by the commercial name of N3-TG-3020. The intended applications of this omega-3 triglyceride concentrate will be for those uses in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). The maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36% of that of menhaden oil. These proposed uses are presented in Table 1. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above including one for a marine oil concentrate. Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)
be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate. Therefore, N3-TG-3020 Omega-3 Concentrate can be considered safe under its intended conditions of use.

A review of the scientific literature published since the final rule appeared in the Federal Register on March 23, 2005, (70FR Number 55 Part 184 at page 14530) affirming the GRAS status of menhaden oil, confirmed the conclusion reached by the FDA that safety of ingestion of up to 3 grams per person per day of EPA and DHA combined is consistent with current information regarding the safety of consumption of these two omega-3 fatty acids. No new safety issues apart from FDA's original 3 concerns on bleeding time, glycemic control effects in diabetes and elevated LDL levels in populations with hypertriglyceridemia or hypercholesterolemia were identified. Recent research reports focus on the clinical usefulness and efficacy of EPA and DHA.

From the foregoing analysis and rulemaking decisions of FDA on the GRAS affirmation of menhaden oil and of EPA and DHA, as well on the submitted GRAS Notices where the agency had no objection to the conclusions of being GRAS including a specific submission on an omega-3 fish oil concentrate (GRN 000105), Twin Rivers Technologies Omega-3 Triglyceride Concentrate (N3-TG-3020) should also be GRAS for the proposed uses specified in the regulations under the conditions described and at the maximum use levels described in Table 1.

"Based on a critical evaluation of the publicly available data and information summarized in the attached GRAS determination, the Expert Panel members, have individually and collectively concluded that Omega-3 Triglyceride Concentrate, (N3-TG-3020) meeting the specifications cited above, and concentrated as described, is GRAS when used as an ingredient in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation when used at a maximum level of 36 % of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020), when used as described, is GRAS based on scientific procedures."

Therefore, N3-TG-3020 Omega-3 Triglyceride Concentrate meeting the specifications described in AAC's GRAS review titled DETERMINATION OF THE *GRAS* STATUS OF A CONCENTRATED OMEGA-3 TRIGLYCERIDE (N3-TG-3020) FOR USE AS A DIRECT FOOD INGREDIENT IN THE SAME FOOD CATEGORIES AS THOSE CURRENTLY LISTED IN 21CFR§184.1472(a)(3) (MENHADEN OIL) AT MAXIMUM USE LEVELS THAT ARE 36 PERCENT OF THOSE SPECIFIED IN THIS REGULATION and to be used as a food ingredient in the food categories and at the use levels listed in Table 1, and resulting in a

potential intake of less than 3.0 grams per person per day of EPA and DHA combined is safe, and also GRAS, for addition to food."

F. Availability of Information

The detailed data and information that serve as a basis for this GRAS determination will be provided to the FDA upon request, or are available for the Food and Drug Administration's review and copying during reasonable business hours at the offices of:

Edward A. Steele
President
AAC Consulting Group
7361 Calhoun Place, Suite 500
Rockville, MD 20855-2765
Telephone: 301-838-3182
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Email: esteele@aacgroup.com

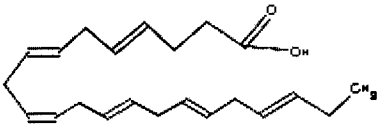
II. Detailed Information about the Identity of the Substance

A. Identity

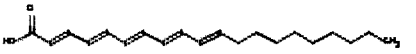
The structures of EPA and DHA are represented by the following chemical formulae:

Figure 1- Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA)

NAME: Docosahexaenoic acid
RN: 25167-62-8
(22:6, n-3, DHA)



NAME: Eicosapentaenoic acid
RN: 25378-27-2
(20:5, n-3, EPA)



The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of about 55 % EPA+DHA in triglyceride form. The chemical formula for Eicosapentaenoic acid is 20:5, n-3, EPA. The empirical formula for EPA is $C_{20}H_{30}O_2$ with a molecular weight of 302.45. The Chemical Abstracts Service (CAS) Number is 25378-27-2. The chemical formula for Docosahexaenoic acid is 22:6, n-3, DHA. The empirical formula for DHA is $C_{22}H_{32}O_2$ with a molecular weight of 328.49. The CAS Number is 25167-62-8.

B. Method of Manufacture

Twin Rivers Technologies manufacturing process for the production of a concentrated omega-3 fatty acid triglyceride containing substantially increased levels of EPA and DHA above those found in naturally occurring fish oils is described below. The process begins with a potential filtration treatment to remove solids from marine oils obtained from several potential sources of edible fish including salmon, menhaden, anchovy, sardine, jack mackerel, mackerel and tuna. This potential filtration step is followed by a potential adsorption step using bleaching earth to reduce odor and possible colors resulting in the production of a filtrate containing the fatty acids and a filter cake which is discarded. The next step involves the application of steam for stripping to reduce free fatty acids. This filtrate (when feed pretreatment via filtration and adsorption is employed) or otherwise unfiltered feedstock is then treated with either methanol or ethanol in the presence of a sodium methoxide catalyst resulting in esterification of the fatty acids and the free glycerine is removed. Next, the free unreacted methanol or ethanol are removed by flash evaporation and the resulting esterified product mix is treated with water wash to remove glycerin, soap, methanol or ethanol, as well as odor and flavor bodies. The water wash containing these components is removed and the esterified mass is then dried to remove any residual water. At this point, fractional distillation is utilized to remove the low boiling C14, C16, C18 methyl or ethyl esters and the remaining esterified mass is subjected to wiped film evaporation or other "Flash Distillation" in order distill the high boiling C18, C20, C22, C24 methyl or ethyl esters. Next, these distilled high-boiling esters are reacted with glycerin in the presence of either potassium carbonate or sodium methoxide to product a highly concentrated long chain triglyceride product (90%) with small amounts of diglycerides (5%) and monoglycerides (5%). This reaction product is then treated with water and potassium citrate as a chelant in two stages to physically refine the product further and remove the soaps and potassium carbonate. The resulting long-chain polyunsaturated fatty acid triglyceride concentrate is dried to

remove moisture, and treated with an adsorbent media to remove any color, odor or peroxides and this residual filter cake is discarded. The resulting concentrate in the form of the filtrate is then further evaporated to remove any residual esters, treated via steam stripping to remove any carry over odor bodies, peroxides, esters and free fatty acids. Finally, approved and acceptable antioxidants are added to retard oxidation and the long-chain polyunsaturated fatty acid omega-3 triglyceride concentrate is placed in storage under nitrogen. This process is similar in many respects to other industrial fish-oil based practices for processing of marine oils into refined fish oils and concentrates described in the GRAS Notices referred to earlier in this document, but also includes specific steps to produce a highly concentrated omega-3 triglyceride product. A schematic of the production process for Omega-3 Triglyceride Concentrate (N3-TG-3020) is shown in the confidential file provided in Appendix I of the GRAS document.

The production process for Omega-3 Concentrate (N3-TG-3020), summarized above, is a process that is similar in many respects to the standard industry practice for the processing of marine oils. Furthermore, this process is well characterized and can consistently yield a food-grade product that is safe for human consumption with the ongoing analytical testing and quality control procedures described below for potential contaminants.

Acceptable analytical methodology is also employed for Omega-3 materials that include measurement of Oxidative by-products (Acid value, Peroxide value, p-Anisidine Value, Totox), PCDDs and PCDFs, PCBs, and heavy metals including lead, cadmium, mercury, and arsenic. Twin Rivers Technologies will be sampling and analyzing this omega-3 product and process to ensure compliance with a voluntary monograph established in 2002 by the Council for Responsible Nutrition as an ongoing measure of quality control. In addition, they will be applying their internal release criteria to this omega-3 product, which also measures low level methyl or ethyl esters to ensure that the wiped film evaporator has suitably removed these constituents.
The members of the expert panel, individually and collectively, have critically evaluated the production process for Omega-3 Concentrate (N3-TG-3020) summarized above and have concluded that this process is similar in many respects to the standard industry practice for the processing of marine oils. Furthermore, the panel also concluded that this process is well characterized and can consistently yield a food-grade product that is safe for human consumption.

C. Food Grade Specifications for N3-TG-Omega-3 Triglyceride Concentrate

Twin Rivers Technologies has developed specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate to establish and maintain its food-grade status. These specifications are as follows:

Description:	Food-grade N3-TG-3020 Omega-3 Triglyceride Concentrate is
	composed predominantly of triglycerides (approximately 98%), with a
	small amount of diglyceride (about 2%), that are highly enriched in
	polyunsaturated fatty acids, primarily EPA and DHA. It is derived from

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

	fish oil extracted from multiple edible marine fish species (typically sardine, anchovy, menhaden, jack mackerel, mackerel, tuna and salmon) that is prepared in a multi-step proprietary process.
Appearance:	Yellow to light yellow at ambient temperature.
Triglycerides:	Not less than ("NLT") 98%.
Odor and taste:	At worst, a slightly fishy odor and taste.
EPA and DHA:	NLT 500 mg/g and not more than ("NMT") 600 mg/g.
EPA/DHA ratio:	NLT 1.5.
Lovibond Color:	5 MAX
Red, 5 ¼"	
Acid value:	NMT 1%.
Monoglyceride:	0
Diglyceride:	NMT 2%.
Triglyceride:	NLT 98%.
Peroxide value:	NMT 5 meq/kg.
p-Anisidine value:	NMT 20.
Tottox value:	NMT 26 (Anisidine value +2x [peroxide value]
Iodine value:	NLT 284.4
Density:	942 mg/ml.
Tocopherols:	NLT 2.0 mg/g.
Moisture:	NMT 0.1%.
Unsaponifiables:	NMT 2.35 %.
Total PCBs:	NMT 0.09mg/kg.
PCDDs and PCDFs:	NMT 2.0 pg/g.
Heavy Metals	
Lead:	NMT 0.1 mg/kg.
Cadmium:	NMT 0.1 mg/kg.
Arsenic:	NMT 0.1 mg/kg.
Mercury:	NMT 0.1 mg/kg.
Delivery form:	Oil concentrate shipped under inert nitrogen atmosphere.

The members of an expert panel, individually and collectively, have critically evaluated the food-grade specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate summarized above, and have concluded that appropriate product specifications have been established to ensure that the final product is food grade and is safe for human consumption.

D. Testing for Contaminants

In addition to the testing specifications identified above for PCDD (dioxins), PCDFs (furans), total PCBs and heavy metals, Twin Rivers Technologies also examined potential levels of

contaminants in the N3-TG-3020 Omega-3 Triglyceride Concentrate. Organochlorine and organophosphate pesticide levels were below the limit of detection (1, 5, 10, or 25 ng/g (i.e. ppb)). Levels of PCDD and PCDF were 0.40 and 0.76 pg/g respectively. A sample of N3-TG-3020 Omega-3 Concentrate as well as three potential marine oil feedstocks were also analyzed for polycyclic aromatic hydrocarbons. The detection limit for these compounds was 0.5 parts per billion. The analysis included fluorene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, total light PAHs, benzo(b)anthracene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h, i)perylene, total heavy PAHs, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, perylene, coronene and benzo(e)pyrene. Results for all four samples were "none detected" -for the aforementioned compounds. Twin Rivers Technologies will be screening each new lot manufactured from a different (geographically) sourced marine oil starting material for PAHs to ensure there are no outlier issues from the values reported here.

The GRAS panel concluded that the analyses of the N3-TG-3020 Omega-3 Concentrate support the presumption that there is no toxicological concern from any product impurities.

III. Self-Limiting Levels of Use

Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Given the addition of omega-3 fatty acids to such a wide variety of foods and the many choices available to the consumer of these products with and without this addition, the intake of omega-3 fatty acids would be limited in the typical daily diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate. Therefore, N3-TG-3020 Omega-3 Concentrate can be considered safe under its intended conditions of use.

IV. Detailed Summary of the Basis for the Notifier's Determination that N3-TG-3020 Omega-3 Concentrate is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Twin River Technologies to determine the Generally Recognized As Safe (GRAS) status of a concentrated omega-3 triglyceride (N3-TG-3020) for use as a direct food ingredient in the same food categories as those currently listed in 21CFR§184.1472(a)(3) (Menhaden oil) at maximum levels that are 36 percent of those specified in this regulation. Specifically, these categories include the following: baked goods and baking mixes, cereals, cheese products, chewing gum, condiments, confections and frostings, dairy product analogs, egg products, fats and oils (but not in infant formula), fish products, frozen desserts, gelatins and puddings, gravies and sauces, hard candy, jams and jellies, meat products, milk products, nonalcoholic beverages, nut products, pastas, plant protein products, poultry products, processed fruit juices, snack foods, soft candy, soup mixes, sugar substitutes, sweet sauces, toppings and syrups, and white granulated sugar. The Expert Panel also evaluated previously filed GRAS notifications on this

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)
subject with FDA as well as FDA's Tentative, Proposed and Final Rule affirming the Generally Recognized as Safe (GRAS) status of menhaden oil. A comprehensive search of the scientific literature was completed through October 13, 2005, along with an evaluation of FDA's database for safety and toxicity information submitted as part of several GRAS notices and a Citizen's Petition on fish oil, salmon oil, menhaden oil, an omega-3 concentrate (Marinol) and on tuna oil. The Expert Panel independently evaluated this information and other materials deemed necessary or appropriate. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the following decision:

> "Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members, have individually and collectively concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020) meeting the specifications cited above, and when used as an ingredient in the manufacture of products identified in the menhaden oil GRAS Affirmation regulation in which menhaden oil is permitted (21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005 (Volume 70, Number 55 PP14530-14532)) and when used at a maximum level of 36 % of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined, is safe, and GRAS, for addition to food.
>
> It is also the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, the Expert Panel concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020), when used as described, is GRAS based on scientific procedures." Because N3-TG-3020 Omega-3 Triglyceride Concentrate is GRAS for its proposed uses, it is excluded from the definition of a food additive, and thus may be marketed and sold for these uses in the U.S. without the promulgation of a food additive regulation under 21 CFR.

REFERENCES

1. Council for Responsible Nutrition, October 2002. Voluntary Monograph for testing Omega-3 DHA, Omega-3 EPA and Omega-3 DHA & EPA combinations. Available at http://www.crnusa.org/pdfs/O3FINALMONOGRAPHdoc.pdf

2. Federal Register/62FR/ No.108/pp 30751-30757/ Thursday, June 5, 1997-21CFRPart 184 Substances Affirmed as Generally Recognized as Safe: Menhaden Oil. Final Rule.

3. Federal Register January 15, 2004 FR69 10 2313 - Substances Affirmed as Generally Recognized as Safe: Menhaden Oil http://www.cfsan.fda.gov/~lrd/fr040115.html

4. Federal Register - 70FR 14530 March 23, 2005: Substances Affirmed as Generally Recognized as Safe;menhaden oil http://www.cfsan.fda.gov/~lrd/fr050323.html

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

5. GRAS Notice No.GRN 000102 (September 3, 2002): *FDA/CFSAN/OPA*: Agency Response Letter to Jedwards International on small planktivorous pelagic fish body oil. http://www.cfsan.fda.gov/~rdb/opa-g102.html

6. GRAS Notice No.GRN 000105 (October 15, 2002): *FDA/CFSAN/OPA*: Agency Response Letter to Unilever United States, Inc. on Marinol omega-3 concentrate. http://www.cfsan.fda.gov/~rdb/opa-g105.html

7. GRAS Notice No.GRN 000109 (December 4, 2002): *FDA/CFSAN/OPA*: Agency Response Letter to Piper Rudnick on behalf of Clover Corporation on tuna oil. http://www.cfsan.fda.gov/~rdb/opa-g109.html

8. GRAS Notice No.GRN 000138 (April 20, 2004): *FDA/CFSAN/OPA*: Agency Response Letter to JHeimbach on behalf of Ocean Nutrition Canada, Ltd on fish oil from anchovy. http://www.cfsan.fda.gov/~rdb/opa-g138.html

9. GRAS Notice No. GRN 000102 (February 12, 2004): *FDA/CFSAN/OPA*: Agency Response Letter to Martek Biosciences Corporation on algal oil. http://www.cfsan.fda.gov/~rdb/opa-g137.html

10. GRAS Notice No.GRN 000146 (August 17, 2004): *FDA/CFSAN/OPA*: Agency Response Letter to Jedwards International on salmon oil. http://www.cfsan.fda.gov/~rdb/opa-g146.html

11. Letter to Martin Hahn of Hogan & Hartson LLP "Letter Responding to Health Claim Petition Dated November 3, 2003 (Martek Petition): Omega-3 Fatty Acids and Reduced Risk of Coronary Heart Disease" (Docket No. 2003Q-0401), September 8, 2004 Authorizing Qualified Health Claim for Conventional Foods. http://www.cfsan.fda.gov/~dms/ds-ltr37.html

EXPERT PANEL STATEMENT

DETERMINATION OF THE GRAS STATUS OF A CONCENTRATED OMEGA-3 TRIGLYCERIDE (N3-TG-3020) FOR USE AS A FOOD INGREDIENT

The undersigned, an independent panel of recognized experts, qualified by scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Twin River Technologies to determine the Generally Recognized As Safe (GRAS) status of a concentrated omega-3 triglyceride (N3-TG-3020) for use as a direct food ingredient in the same food categories as those currently listed in 21 CFR §184.1472(a)(3) (Menhaden oil) at maximum levels that are 36 percent of those specified in this regulation. Specifically, these categories include the following: baked goods and baking mixes, cereals, cheese products, chewing gum, condiments, confections and frostings, dairy product analogs, egg products, fats and oils (but not in infant formula), fish products, frozen desserts, gelatins and puddings, gravies and sauces, hard candy, jams and jellies, meat products, milk products, nonalcoholic beverages, nut products, pastas, plant protein products, poultry products, processed fruit juices, snack foods, soft candy, soup mixes, sugar substitutes, sweet sauces, toppings and syrups, and white granulated sugar. The Expert Panel also evaluated previously filed GRAS notifications on this subject with FDA as well as FDA's Tentative, Proposed and Final Rules affirming the Generally Recognized as Safe (GRAS) status of menhaden oil. A comprehensive search of the scientific literature was completed through October 13, 2005, along with an evaluation of FDA's database for safety and toxicity information submitted as part of several GRAS notices and a Citizen's Petition on fish oil, salmon oil, menhaden oil, an omega-3 concentrate (Marinol) and on tuna oil. The Expert Panel independently evaluated this information and other materials deemed necessary or appropriate. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

INTRODUCTION

N-3 polyunsaturated fatty acids (PUFA) or omega-3 fatty acids belong to an essential fatty acid family characterized by their first double bond at carbon atom number 3, counted from the methyl or omega end of the carbon chain constituting the backbone of fatty acids. N-3 PUFA are chemically and biologically distinct from n-6 PUFA, where the first double bond is at carbon atom number 6 (Schmidt, 1997).

There are two subgroups of n-3 PUFA or omega-3 fatty acids, as they are often collectively termed. One subgroup, α-linolenic acid, is derived from plant oils such as canola oil, rapeseed oil and linseed oil and is composed of 18 carbon atoms with three double bonds (nomenclature; 18:3). The other group of n-3 PUFA is derived from seafood, and the major marine n-3 PUFAs are eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6). In humans, α-linolenic acid can, to a limited extent, be elongated and desaturated to EPA and DHA. Otherwise, EPA and DHA are only acquired from seafood.

In a recent review by Schmidt *et al.,* (2005), it was reported that the content of marine n-3 PUFA varies between fish species (Hepburn *et al.*, (1986), Soringeur *et al.,* (1997), Kris-Etherton *et al.*, (2002)) being high in fatty fish like mackerel and salmon and low in lean fish such as flounder and cod (Table 1). The content of n-3 PUFA in seafood varies considerably in relation to location, water temperature and the season of capture. Values given in Table 1 should therefore be viewed in this light.

Table 1. The approximate content of n-3 PUFA in seafood

Seafood	n-3 PUFA g/100 g
Mackerel	1.8–5.3
Herring	1.2–3.1
Salmon	1.0–2.0
Trout	0.5–1.6
Tuna	0.5–1.6
Halibut	0.5–1.0
Shrimp	0.2–0.4
Cod and flounder	0.2

The intake of marine n-3 PUFA differs considerably between populations, being very high in traditionally living Eskimos (10–14 g/day), low in most Western populations (<0.2 g/day) and intermediate in areas like Japan and Norway (1–3 g/day).

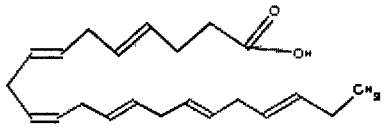
Uauy and Valenzuela (2000) reviewed the biochemical basis for essentiality of n-3 fatty acids and metabolism. They noted that the biochemical pathways for n-3 and n-6 fatty acid desaturation are only present in the chloroplasts of cells. Thus, only algae and some fungi are capable of forming these essential fatty acids (EFAs). Terrestrial and marine plants are the primary source of EFAs in the food chain; fish and other marine animals are able to elongate and desaturate the parent EFAs, forming the long-chain polyunsaturated fatty acids (LCPUFAs).(Simopoulos, 1991). Animal tissues, especially the liver, but also lung, heart, kidney, and brain, are capable of further elongating and desaturating the parent EFAs, generating a family of compounds for the respective families (n-3 and n-6 fatty acids). The competitive desaturation of the n-3, n-6, and n-9 series by ∂-6 desaturase is of major significance because this step is controlled by the interaction of hormones and diet, including the amount of linoleic acid (18:2, n-6, LA) and α-linolenic acid (18:3, n-3, LNA) in the diet. The controlling step in the metabolic pathway for both n-3 and n-6 EFA series is the ∂-6 desaturation of the respective precursor (LNA and LA, respectively). The uniqueness of the biological effects of feeding human milk or marine foods on EFA metabolism is based on the direct supply of

LCPUFA's bypassing the controlling step of the ∂-6 desaturase. Further, the conversion of parent EFAs to LCPUFAs is under active regulation. Therefore, the effects of providing EPA or DHA in the diet cannot be reproduced by providing the equivalent amount of LNA. Excess dietary LA associated with some vegetable oils, particularly safflower, sunflower, and corn oils consumed in most Western countries, may decrease the formation of DHA from LNA because the ∂-6 desaturase is inhibited by excess substrate (Galli and Marangoni, 1997). This review noted that other dietary factors such as overall energy and protein supply, and zinc and copper deficiency, affect EFA metabolism; for example, a zinc deficit is associated with decreased activity of ∂-6 desaturase, and thus too low arachidonic acid (AA) and DHA. Hormones such as insulin increase conversion of EFA to long-chain derivatives.

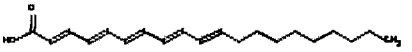
The inhibitory effect of EPA on ∂-5 desaturase activity has also been considered responsible for the lower AA observed when marine oil is consumed and this has been confirmed in molecular studies. Excess LA, as seen in enterally or parenterally fed patients receiving corn oil or safflower oil as the predominant fatty acid supply, will inhibit the elongation/desaturation of the parent EFAs, and thus will lower the LCPUFA supply necessary for membrane synthesis. Marine PUFAs provide minimal preformed AA and substantial amounts of preformed n-3 LCPUFA such as EPA and DHA.

The structures of these compounds are represented by the following chemical formulae:

NAME: Docosahexaenoic acid
RN: 25167-62-8
(22:6, n-3, DHA)



NAME: Eicosapentaenoic acid
RN: 25378-27-2
(20:5, n-3, EPA)



The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of 52.6% EPA+DHA in triglyceride form. As noted earlier, the only source of these omega-3 fatty acids is marine seafood. FDA affirmed the GRAS status of menhaden oil under 21 CFR §184.1472 and established that a daily intake of EPA and DHA combined not exceed 3 grams per person per day is safe. The scientific basis to support the establishment of this safe level of intake was published in the Federal Register at page 30751 (Volume 62, Number 108) on June 5, 1997, as part of the final rule on menhaden oil. As reported above, menhaden oil is one of the key edible fish sources that have a high concentration of EPA and DHA. Menhaden oil is a refined marine oil that is derived from menhaden fish (*Brevoortia* species) which are any of a general species of valuable Atlantic coastal fishes. It consists primarily of fatty acid triglycerides, with small amounts of monoglycerides and diglycerides. The triglycerides are esters of glycerol and fatty acids with chains of 14 to 22 carbon atoms. Menhaden oil differs from edible vegetable oils and animal fats in its high proportion of polyunsaturated fatty acids with 4, 5 and 6 double bonds (about 25 percent). The mean percentages for these polyunsaturated fatty acids in menhaden oil are C18:4 (2.3 percent), C20:4 (2.0 percent), C20:5 (13.1 percent), C22:5 (2.5 percent) and C22:6 (6.7 percent). Note that C20:5 and C22:6 are EPA and DHA, respectively, and are the major source of omega-3 fatty acids from fish oil. Menhaden oil also contains about 33 percent saturated fatty acids and about 31 percent monounsaturated fatty acids. In their review, FDA identified three potential safety issues of menhaden oil consumption above 3 grams per person per day: increased bleeding time by reducing platelet aggregability, a potential concern about glycemic control in non-insulin-dependent diabetics, and potential increases in LDL cholesterol.

An extensive review by the Agency of the available scientific information subsequently addressed these concerns and FDA stated the following:

Bleeding time: *"The totality of the scientific evidence demonstrates that when consumption of fish oils is limited to 3 g/p/d or less of EPA and DHA, there is no significant risk for increased bleeding time beyond the normal range."* A report from an industry-sponsored roundtable discussion on the topic of fish oils and bleeding time (CRN, 2000) also supports the conclusion that EPA and DHA are safe at intake levels at or below 3 g/p/d. On the other hand, ingestion of fish oils providing more than 3 g/d of

EPA and DHA have generally been found to produce increases in bleeding time that are statistically significant. At this time, there are insufficient data to evaluate the clinical significance of this finding. Because of the lack of data and because of the potential risk of excessive bleeding in some individuals with intakes at higher levels, FDA concludes that the safety of menhaden oil is generally recognized as safe only at levels that limit intake of EPA and DHA to 3 g/p/d.

Glycemic control in non-insulin dependent diabetics: *"Based on the available information, FDA concludes that consumption of EPA and DHA in fish oils at 3 g/p/d by diabetics has no clinically significant effect on glycemic control, although higher amounts of EPA and DHA (4.5 g/p/d and above) remain of concern. Therefore, FDA concludes that 3 g/p/d of EPA and DHA is a safe level with respect to glycemic control."*

LDL Cholesterol: In the 1997 final rule, FDA noted that many studies on hypertriglyceridemic or hypercholesterolemic subjects, and some studies on normal subjects, reported an increase in LDL cholesterol or apo B (apolipoprotein B, a principal component of LDL) following fish oil supplementation (58 FR 2682 at 2705). Because increases in LDL cholesterol predict increased risk of coronary heart disease, FDA recently reevaluated those studies, as well as newer studies published since the health claim final rule, to address the question of whether 3 g/p/d of EPA and DHA derived from menhaden oil is generally recognized as a safe level with respect to its effect on LDL cholesterol. The agency considered the reported effects of fish oil on LDL cholesterol levels in healthy persons with normal cholesterol levels, as well as in persons with diabetes mellitus, hypertension, abnormal blood lipid levels, and cardiovascular disease.

As a result of its reevaluation, FDA found that although reported study results are variable, there appears to be a trend toward increased LDL cholesterol values with increased fish oil consumption in all population subgroups, with the magnitude of the increase appearing greater and more consistent in populations with abnormal blood lipid levels, hypertension, diabetes, and cardiovascular disease. They also noted that in the earlier 1993 health claims final rule, most reports of increased LDL were in studies where large amounts of fish oils were given (i.e., 5 g or more per day of EPA plus DHA), and any safety concern relating to changes in LDL cholesterol might be suitably addressed by restricting the intake of DHA and EPA (58 FR 2682 at 2705).

"Based on these levels, FDA has determined that the mean intake of menhaden oil, if menhaden oil were to be used at the maximum allowable level in all permitted food categories, would be less than 3 g of DHA and EPA per day. Further, menhaden oil would substitute for other dietary fats, some of which have similar effects on LDL cholesterol. Based on its evaluation, the agency concludes that the petitioned levels of menhaden oil are safe with respect to the effect on LDL cholesterol."

On February 26, 2002, the National Fish Meal and Oil Association (NFMOA) amended its original 1986 filing for menhaden oil (GRASP 6G0316) to include suggested maximum use levels of menhaden oil for each food category in which menhaden oil can be used under 21 CFR §10.20 and §10.30 requesting that the agency amend §184.1472

Menhaden oil (21 CFR §184.1472) by reallocating the uses of menhaden oil in food, while maintaining the total daily intake of EPA and DHA from menhaden oil at a level not exceeding 3.0 g/p/d. FDA noted "The maximum limit of 3.0 g/p/d on the total daily intake of EPA and DHA has been considered a reasonable safeguard against the possible adverse effects stated above and to date no new information available has caused the agency to alter this limit." The reallocation is performed by: (1) reducing the maximum levels of use of menhaden oil in some of the currently listed food categories; (2) adding additional food categories along with assigning maximum levels of use in these new categories; and (3) eliminating the listing of subcategories, for example, cookies and crackers, breads and rolls, fruit pies and custard pies, and cakes, and including them under broader food categories, i.e., baked goods and baking mixes.

The NFMOA provided exposure analyses that contained estimates of EPA and DHA intake from menhaden oil for the revised uses of the currently listed food categories and the proposed uses of the new food categories. The NFMOA stated that the estimated daily exposure to EPA and DHA from those uses of menhaden oil is 2.7 g/p/d. NFMOA concluded that menhaden oil is GRAS for the revised uses of the currently listed food categories and the proposed uses of the new food categories, because the total daily intake of EPA and DHA from those uses of menhaden oil would not exceed 3.0 g/p/d, consistent with the June 1997 final rule.

In support of FDA's conclusions, on April 3, 2000 (Docket Number 91N-0103) a detailed review of the published literature was submitted on behalf of a joint task group of the Consumer Healthcare Products Association (CHPA), Council for Responsible Nutrition (CRN) and the National Fisheries Institute (NFI) on health claims for omega-3 fatty acids (EPA and DHA) in response to the FDA's request for scientific information and data in order to evaluate the evidence for the relationship between omega-3 fatty acids (EPA and DHA) and heart disease. As part of this review of all available clinical trials, the task group evaluated the three potential safety issues identified by the Agency and concluded that studies published since FDA's review in 1993 have been examined and no new information has become available to invalidate FDA's previous conclusions regarding safety of omega-3 fatty acids (EPA and DHA) up to 3g/day as stated in the GRAS affirmation of the menhaden oil petition.

During this interim period, the omega-3 fatty acids have also been the subject of several GRAS Notices submitted to FDA for use as products that would be substantially equivalent to menhaden oil and that would be substituted in its place in the approved food categories at levels equivalent to the EPA and DHA from menhaden oil. In each case, FDA responded that they had no questions on the proposed use and did not object to the GRAS notice. These submissions and FDA's responses are presented below.

1. GRAS Notice No. 000102: Response dated September 3, 2002 to a submission by Jedwards International on a small planktivorous pelagic fish body oil (SPPFBO) informing FDA of their view that this product is GRAS, through scientific procedures for use as a direct food ingredient in the same food categories as those

currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) at maximum use levels that are two thirds of those specified in that regulation.

As with the use of menhaden oil, the maximum levels of use of SPPFBO are designed to assure that the combined daily intake of two fatty acids that are components of SPPFBO (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In an amendment dated July 15, 2002, Jedwards noted that SPPFBO will be used as the sole added source of EPA and DHA in any given food category and is not to be combined or augmented with any other EPA/DHA-rich oil in making a food product.

Jedwards described generally available information about the identity and composition of SPPFBO. SPPFBO is derived from small planktivorous pelagic fish, primarily sea-harvested sardine (*Sardina pilchardus*) and anchovy (*Cetengraulis mysticetus*). Jedwards compared sardine and anchovy with menhaden and concluded that they are all plankton-eating fish that vary mainly in size. Because sardines and anchovies are commonly consumed fish, Jedwards considers that SPPFBO is already part of the diet.

Jedwards described SPPFBO as a complex mixture of glycerides, fatty acids, unsaponifiables and phospholipids. It is predominantly a mixture of triglycerides with small amounts of mono- and diglycerides. Like other fish oils, SPPFBO differs from edible vegetable oils and animal fats in its relatively high proportions of polyunsaturated fatty acids, such as EPA (which has five double bonds) and DHA (which has six double bonds). Jedwards also provided comparative fatty acid composition data of SPPFBO to that of menhaden oil and contrasted the fatty acid compositions of these two fish oils with that of a vegetable oil (i.e., soybean oil).

Jedwards described the method of manufacture of SPPFBO. The fish are cooked and pressed. The oil is separated from the expressed liquor and further processed using standard methods in accordance with current good manufacturing practice. The oil is refined by winterization, neutralization, bleaching, and deodorization. The methods and equipment used to process SPPFBO are standard in the processing of other edible oils and fats. Jedwards describes the manufacture of SPPFBO as similar to that of menhaden and other fish oils. Jedwards provided product specifications with limits on saponification value, iodine number, unsaponifiable matter, free fatty acids, peroxide value, lead, and mercury.

Jedwards acknowledged that FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil Final Rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR

§184.1472(a)(3)). Likewise, Jedwards concluded that there appear to be no risks associated with the consumption of SPPFBO, provided that consumption is limited to a combined total intake for EPA and DHA of 3 g/p/d, with SPPFBO as the sole source of added oils containing EPA and DHA. Because SPPFBO contains higher levels of EPA and DHA than menhaden oil, Jedwards intends to use SPPFBO at maximum levels that are two thirds of the maximum levels of use described in the regulation for menhaden oil.

Jedwards concluded that SPPFBO is GRAS because its manufacture, composition and intended use are similar to the manufacture, composition, and intended use of menhaden oil.

Based on the information provided by Jedwards, as well as other information available to FDA, the agency had no questions at that time regarding Jedwards' conclusion that SPPFBO is GRAS under the intended conditions of use, provided that the levels of use do not exceed two thirds of the levels of use described in 21 CFR §184.1472 and that a combined intake of EPA and DHA from all added sources does not exceed 3 g/p/d.

2. GRAS Notice No. GRN 000105: Response dated October 15, 2002 from FDA to a submission from Unilever United States on a fish oil concentrate (Marinol Omega-3 Concentrate) informing FDA of their view that this product is GRAS, through scientific procedures for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) at maximum use levels that are 57 percent of those specified in that regulation.

Further, Unilever also intended for their fish oil concentrate to be used in several additional food categories (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) at specified maximum use levels based on FDA's recently issued proposed rule (the menhaden oil proposal; 67FR 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 may be used. Unilever noted that any changes to 21 CFR §184.1472(a)(3) would also apply to fish oil concentrate, with maximum use levels that are 57 percent of those specified in that regulation.

As with the use of menhaden oil, the maximum levels of use of fish oil concentrate are designed to assure that the combined daily intake of two fatty acids that are components of fish oil concentrate (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In an amendment dated August 28, 2002, *Unilever noted that fish oil concentrate would be used as the sole added source of EPA and DHA in any given food category and would not be combined or augmented with any other EPA/DHA-rich oil in making a food product.*

As part of its notice, Unilever included a summary of conclusions of a panel of individuals (Unilever's GRAS panel) who evaluated the data and information that are the basis for Unilever's GRAS determination. Unilever's GRAS panel evaluated dietary

exposure, the method of manufacture, specifications, contaminant levels, and a literature review of toxicological studies. Unilever's GRAS panel concluded that fish oil concentrate, meeting food grade specifications, is GRAS for its intended use.

Unilever also described generally available information about the identity and composition of fish oil concentrate. Fish oil concentrate is a mixture that is predominantly triglycerides, with small amounts of mono- and diglycerides. Fish oils, including fish oil concentrate, differ from edible vegetable oils and animal fats in their relatively high proportions of polyunsaturated fatty acids, especially DHA and EPA. Unilever provides a representative fatty acid profile for fish oil concentrate, which shows that fish oil concentrate contains EPA at a level of approximately 20 percent (by weight) of total fatty acids and DHA at a level of approximately 18 percent of total fatty acids. By comparison, menhaden oil contains EPA at a level of approximately 12 percent and DHA at a level of approximately 8 percent.

Unilever also described the manufacture of fish oil concentrate. The product is manufactured using starting material extracted from edible marine fish species that normally include anchovy, sardine, jack mackerel and mackerel. The fish oil extract is bleached and filtered, followed by enzymatic hydrolysis using a lipase. The lipase selectively hydrolyzes saturated and monounsaturated fatty acids from the triglycerides that are components of the fish oil to give free fatty acids (predominantly saturated and monounsaturated) and glycerides enriched in polyunsaturated fatty acids (i.e., EPA and DHA). The glycerides and the free fatty acids are separated by short-path molecular distillation. The glycerides are further processed by adjusting the pH with a base, bleaching, adding antioxidants, filtering, and deodorizing to generate the fish oil concentrate final product. Unilever's GRAS panel evaluated the production process and concluded that the process is similar to the standard industry practice for processing marine oils. Unilever also provided product specifications for fish oil concentrate.

Unilever acknowledged that FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR §184.1472(a)(3)). In Unilever's view, a review of the literature published since FDA issued the menhaden oil final rule confirms FDA's conclusion that intake of EPA and DHA is safe as long as the combined daily intake does not exceed 3 g/p/d.

Unilever also provided a per capita estimate of daily intake of EPA and DHA from the use of fish oil concentrate in each of the food categories for the initial intended use levels listed above. Unilever calculated these estimates using data submitted to FDA in Citizen Petition GRP 0Z0425. The data in GRP 0Z0425 were obtained from USDA's Continuing

Surveys of Food Intakes by Individuals, 1989-1992 3-day survey. By summing the intake of EPA and DHA from each of the food categories, Unilever estimated exposure to EPA and DHA from use of fish oil concentrate in the combined food categories at the initial intended use levels to be 2.8 g/person/day**. Unilever estimated daily intake of EPA and DHA from the use of fish oil concentrate from the future intended use levels (see additional products above) to be 2.7 g/person/day. *Because fish oil concentrate is intended to be used as an alternative to menhaden oil, Unilever concluded that there will be no increase in exposure to EPA and DHA from its use. FDA concurred with their own independent assessment (See ** Note below).*

Based on the information provided by Unilever, as well as other information available to FDA, the agency had no questions at the time regarding Unilever's conclusion that fish oil concentrate is GRAS under the intended conditions of use, provided that combined intake of EPA and DHA from all added sources does not exceed 3 g/p/d.

** **NOTE**: FDA normally considers exposure to an ingredient for the eaters only population (individuals in the survey who consumed at least one of the foods in which the ingredient is intended to be used during the survey period), rather than the entire per capita population (all individuals who participated in the survey). However, because of the number of food categories in which fish oil concentrate is intended to be used, the eaters only population is nearly identical to the per capita population; 98 percent of the individuals surveyed are eaters of at least one of the foods. FDA independently estimated intake of EPA and DHA from the proposed use of fish oil concentrate and concluded in this response that the additional food categories in Unilever's initial intended use not currently listed in 21 CFR §184.1472(a)(3) (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, non-dairy milk, imitation and soy milk) do not contribute significantly to exposure and that intake of EPA and DHA will remain below 3 g/person/day.

3. GRAS Notice No. 000109: Response dated December 4, 2002 to a submission by Anthony Young of Piper Rudnick, LLP on behalf of the Clover Corporation on tuna oil informing FDA of their view that this product is GRAS, through scientific procedures, for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) at maximum use levels that are 62 percent of those specified in that regulation.

As with the use of menhaden oil, the maximum levels of use of tuna oil are designed to assure that the combined daily intake of two fatty acids that are components of tuna oil (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In an amendment dated July 30, 2002, Clover noted that tuna oil would be used as the sole added source of EPA and DHA in any given food category and would not be combined or augmented with any other EPA/DHA-rich oil in making a food product.

In GRN 000097, Clover included the report of a panel of individuals (Clover's GRAS panel) who evaluated the data and information that are the basis for Clover's GRAS determination. Clover's GRAS panel reviewed data and information about tuna oil relating to the identity, manufacture, stability, conditions of use, estimated daily intake, and published toxicological studies conducted with fish oils. Clover's GRAS panel concluded that tuna oil is GRAS when meeting appropriate specifications and used to provide up to 4 g/p/d of EPA plus DHA. In GRN 000109, Clover specified lower maximum intended use levels to provide a maximum of 3 g/p/d of combined EPA and DHA and stated that it does not intend to market tuna oil for any other food ingredient uses. Clover concluded that tuna oil is GRAS for use in foods at levels proposed in this GRAS Notice.

Clover described generally available information about the identity and composition of tuna oil. Clover described fish oils as complex mixtures, which, like edible vegetable oils, consist of glycerides, fatty acids, phospholipids, and an unsaponifiable fraction. Clover indicated that tuna oil, like other fish oils, is similar to other edible vegetable oils and animal fats, differing principally in the higher proportion of long chain polyunsaturated fatty acids DHA (C22:6 n-3) and EPA (C20:5 n-3). Tuna oil mainly contains triglycerides, with small amounts of mono- and diglycerides. Tuna oil differs from other fish oils in the ratio of EPA to DHA, with a ratio of approximately 1:4 in tuna oil and 1:0.6 in menhaden oil.

Clover described the method of manufacture for tuna oil. Clover manufactures tuna oil from "raw tuna oil", a by-product of the edible tuna canning industry. Tuna species harvested for raw tuna oil production include: skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*), albacore (*Thunnus alulunga*), and bigeye (*Thunnus obesus*) from the South and Central Pacific Ocean. These species are all considered to be widely consumed by humans. Raw tuna oil is manufactured from tuna heads, off-cuts from filleting, skin, frame, red muscle meat, and belly flaps. These byproducts are ground and cooked, and the oil is separated from solids and water by centrifugation. Metal ions that could initiate oxidation of the oil are removed with the aqueous phase. The raw tuna oil is refined to make the final tuna oil products using some or all of the processing techniques of winterization, degumming, neutralization, bleaching and deodorization, processes that are standard in the edible oil industry. Clover describes the manufacture of several of its tuna oil-containing products.

Clover indicated that most non-triglyceride substances are removed during the various processing steps used to manufacture tuna oil. Clover also provided results from analyses of several lots of tuna oil that measured the concentrations of various environmental contaminants and pesticides in tuna oil. Clover concluded that the data show that the processing steps used to manufacture tuna oil remove or reduce all contaminants to levels that are either below the level of detection or are within "all known regulatory levels for these compounds." Clover also provided specifications for tuna oil.

Clover specified the intended maximum use levels of tuna oil and stated that the use levels were set using 22 percent as the average amount of EPA and DHA in menhaden oil

and 32.5 percent as the EPA and DHA content of tuna oil. Clover estimated combined intake of EPA and DHA from the proposed use of tuna oil would be 2.76 g/p/d, based on the mean content of EPA and DHA in tuna oil of 32.5 percent and the maximum proposed use levels.

Clover also acknowledged that FDA raised concerns about consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and increase blood glucose levels in non-insulin dependent diabetics (62 FR 30751). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. Clover cites a recent review article that concludes that "studies published since FDA's review in 1993 have been examined and no new information has become available to invalidate FDA's previous conclusions regarding safety of omega-3 fatty acids (EPA and DHA) up to 3g/p/d as stated in the GRAS affirmation of the menhaden oil petition."

Based on the information provided by Clover, as well as other information available to FDA, the agency had no questions at the time regarding Clover's conclusion that tuna oil is GRAS under the intended conditions of use, provided that the levels of use do not exceed 62 percent of the levels of use described in 21 CFR 184.1472 and that a combined intake of EPA and DHA from all added sources does not exceed 3 g/p/d.

4. GRAS Notice No. 000138: Response dated April 20, 2004 to a submission by Dr. James Heimbach of JHeimbach LLC. on behalf of Ocean Nutrition Canada, Ltd.(ONC) on a fish oil predominantly derived from anchovy informing FDA of their view that this product is GRAS, through scientific procedures, for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) and those projected in the tentative final rule that would amend 21 CFR §184.1472 (69 FR at page 2313; January 15, 2004).* ONC describes two formulations of its fish oil: an oil and a microencapsulated oil product. At the time, ONC intended for the oil to be used in the same food categories as those currently listed in 21 CFR §184.1472(a)(3) (menhaden oil) at levels that are 67 percent of the levels specified in that regulation. In the microencapsulated form of its product, fish oil constitutes approximately 55-60 percent (by weight) of the dry powder. ONC, therefore, intended for the microencapsulated oil product to be used in the same food categories as those currently listed in the menhaden oil regulation at levels that are 120 percent of the levels specified in that regulation.**

*** **NOTE**: Subsequent to receiving ONC's notice, FDA issued a tentative final rule that would amend 21 CFR §184.1472 (69 FR 2313; January 15, 2004).FDA issued this tentative final rule to amend its regulations by reallocating the uses of menhaden oil in food that were currently established at the time in §184.1472. *FDA also tentatively concluded that these uses of menhaden oil are generally recognized as safe (GRAS), but only when menhaden oil is not used in combination with other added oils that are*

significant sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Because FDA's proposed rule of February 26, 20002 did not include a condition of use for other added oils, FDA issued this tentative final rule to give interested persons the opportunity to comment on this limitation.

The Final Rule affirming menhaden oil as generally recognized as safe (GRAS) was published in the Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005 with no changes to the tentative rule. As noted here: §184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day:

Category of food	Maximum level of use in food (as served)
Baked goods, baking mixes, § 170.3(n)(1) of this chapter.	5.0 percent
Cereals, § 170.3(n)(4) of this chapter.	4.0 percent
Cheese products, § 170.3(n)(5) of this chapter.	5.0 percent
Chewing gum, § 170.3(n)(6) of this chapter.	3.0 percent
Condiments, § 170.3(n)(8) of this chapter.	5.0 percent
Confections, frostings, § 170.3(n)(9) of this chapter.	5.0 percent
Dairy product analogs, § 170.3(n)(10) of this chapter.	5.0 percent
Egg products, § 170.3(n)(11) of this chapter.	5.0 percent
Fats, oils, § 170.3(n)(12) of this chapter, but not in infant formula.	12.0 percent
Fish products, § 170.3(n)(13) of this chapter.	5.0 percent
Frozen dairy desserts, § 170.3(n)(20) of this chapter.	5.0 percent
Gelatins, puddings, § 170.3(n)(22) of this chapter.	1.0 percent
Gravies, sauces, § 170.3(n)(24) of this chapter.	5.0 percent
Hard candy, § 170.3(n)(25) of this chapter.	10.0 percent
Jams, jellies, § 170.3(n)(28) of this chapter.	7.0 percent
Meat products, § 170.3(n)(29) of this chapter.	5.0 percent
Milk products, § 170.3(n)(31) of this chapter.	5.0 percent
Nonalcoholic beverages, § 170.3(n)(3) of this chapter.	0.5 percent
Nut products, § 170.3(n)(32) of this chapter.	5.0 percent
Pastas, § 170.3(n)(23) of this chapter.	2.0 percent
Plant protein products, § 170.3(n)(33) of this chapter.	5.0 percent
Poultry products, § 170.3(n)(34) of this chapter.	3.0 percent
Processed fruit juices, § 170.3(n)(35) of this chapter.	1.0 percent
Processed vegetable juices, § 170.3(n)(36) of this chapter.	1.0 percent
Snack foods, § 170.3(n)(37) of this chapter.	5.0 percent
Soft candy, § 170.3(n)(38) of this chapter.	4.0 percent

Soup mixes, § 170.3(n)(40) of this chapter.	3.0 percent
Sugar substitutes, § 170.3(n)(42) of this chapter.	10.0 percent
Sweet sauces, toppings, syrups, § 170.3(n)(43) of this chapter.	5.0 percent
White granulated sugar, §170.3(n)(41) of this chapter.	4.0 percent

To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid.

As with the use of menhaden oil, the maximum levels of use of ONC's fish oil are designed to assure that the combined daily intake of two fatty acids that are components of its fish oil and microencapsulated fish oil product (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In its notice dated September 26, 2003, ONC notes that its fish oil would be used as the sole added source of EPA and DHA in any given food category and that its fish oil would not be combined or augmented with any other EPA/DHA-rich oil in making a food product.

In GRN 000138, ONC included a summary of conclusions of a panel of individuals (ONC's GRAS panel) who evaluated the data and information that are the basis for ONC's GRAS determination. ONC's GRAS panel evaluated dietary exposure, source of the substance, method of manufacture, specifications, contaminant levels, and information from recent published toxicological and human studies. ONC's GRAS panel concluded that fish oil and microencapsulated fish oil product, meeting food grade specifications, are GRAS under the intended conditions of use.

ONC described the intended use of fish oil in foods and provided a table that lists the food categories and intended use level in each food category. ONC's fish oil is intended to be used as a direct food ingredient in food categories listed in 21 CFR §184.1472(a)(3) at maximum use levels that are 67 percent of the levels specified in that regulation. ONC intends for the microencapsulated fish oil product to be used in the same food categories as those currently listed in the menhaden oil regulation at levels that are 120 percent of the levels specified in that regulation. ONC estimates that the mean intake of EPA and DHA combined from the proposed uses of ONC's fish oil would not exceed 3 g/p/d.

ONC also described generally available information about the identity and composition of fish oil (predominantly anchovy). ONC's fish oil is a mixture of fatty acids, primarily in the form of triglycerides. ONC presented the fatty acid profiles for fish oil, herring oil, sardine oil, and salmon oil to show that its fish oil is similar to other edible fish oils. ONC's fish oil contains EPA at a level of approximately 18.5 percent (by weight) of total fatty acids and DHA at a level of approximately 11.8 percent (by weight) of total fatty acids, for a combined level of EPA and DHA of approximately 30 percent (by weight) of total fatty acids. By comparison, menhaden oil contains a combined level of EPA and DHA of approximately 21 to 22 percent of total fatty acids.

ONC also described the manufacture of its fish oil (predominantly anchovy). ONC's fish oil is extracted from multiple edible marine fish species caught off the coast of Peru, including anchovy (95-99 percent), sardine (1-5 percent), jack mackerel, Pacific mackerel, and other occasional species. Processing of the crude fish oil extract includes: 1) winterization; 2) deodorization; 3) treating with activated carbon (discussed in more detail below); 4) bleaching; and 5) blending with mixed tocopherols and other appropriate antioxidants. Optional steps include alkali refining (also called neutralization), and treatment with citric acid. Microencapsulated fish oil product is manufactured by encapsulating it in a crosslinked gelatin/polyphosphate shell. ONC provides specifications for the crude oil, the food-grade oil, and the food-grade, mircroencapsulated oil product.

In a series of meetings between FDA and ONC on February 13, 2004, and March 31, 2004, FDA and ONC discussed the potential for contamination of ONC's product with polycyclic aromatic hydrocarbons (PAHs). At the latter meeting, and reiterated in an amendment from ONC dated April 2, 2004, ONC provided data to demonstrate that an activated carbon treatment, described in the original notice as 'optional,' successfully reduces the levels of PAH in oils, including its fish oil. ONC stated that it is revising its standard procedure by removing the word 'optional' and requiring that activated carbon be used as a standard part of the production of the fish oil product that is the subject of GRN 000138.

ONC acknowledged that FDA has previously raised concerns about the consumption of high levels of two fatty acids (i.e., DHA and EPA), which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30571; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 grams per person per day (g/p/d). To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR §184.1472(a)(3)). In ONC's view, a review of the scientific literature published since FDA issued the menhaden oil final rule confirms FDA's conclusion that consumption of EPA and DHA is safe as long as the combined daily intake does not exceed 3 g/p/d.

Based on the information provided by ONC, as well as other information available to FDA, the agency had no questions at the time regarding ONC's conclusion that fish oil (predominantly anchovy) is GRAS under the intended conditions of use.

5. GRAS Notice No. 000146: Response dated August 17, 2004 to a submission by Jedwards International on salmon oil informing FDA of their view that this product is GRAS, through scientific procedures for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) and at the same maximum use levels when not combined or

augmented with any other food ingredient containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Jedwards International noted that FDA recently issued a proposed rule (the menhaden oil proposal; 67 FR 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 may be used. Jedwards asked in their notice that the agency apply any changes in 21 CFR 184.1472(a)(3) to the intended use of salmon oil. FDA issued a tentative final rule amending 21 CFR §184.1472 (69 FR 2313; January 15, 2004).

As with the use of menhaden oil, the maximum use levels of Jedwards' salmon oil are designed to assure that the combined daily intake of two fatty acids that are components of salmon oil (i.e., EPA and DHA) would not exceed 3 grams/person/day (g/p/d). FDA has affirmed the GRAS status of menhaden oil (21 CFR §184.1472 (a)(3)) provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. Similarly, Jedwards concluded that salmon oil is safe for use as a direct ingredient provided that the combined daily intake of EPA and DHA from consumption of salmon oil-containing foods does not exceed 3 g/p/d and salmon oil is added as the sole source of EPA and DHA in any given food category.

As part of its notice, Jedwards evaluated the data and information that are the basis for its GRAS determination. Jedwards discusses the source and chemical composition of salmon oil, the GRAS affirmed status of menhaden oil (21 CFR §184.1472), and the scientific literature on fish oils. According to Jedwards, salmon oil is manufactured in the same manner as menhaden oil and is virtually chemically identical in composition; hence salmon oil is a safe substitute for menhaden oil in various food categories listed in 21 CFR §184.1472 (a)(3) and subsequently updated in FDA's tentative final rule for menhaden oil (69 FR 2313; January 15, 2004).

Jedwards described generally available information about the identity and composition of salmon oil. Salmon oil is a complex mixture of glycerides, fatty acids, unsaponifiables, and phospholipids from the body of salmon, primarily *Salmo salar.* Salmon oil consists mainly of a mixture of triglycerides of various long chain (14-22 carbons) fatty acids, with small amounts of mono- and diglycerides. Jedwards noted that salmon oil, like menhaden oil, contains approximately 20 percent by weight of omega-3 fatty acids. According to Jedwards, the relative proportions of EPA and DHA, expressed on a weight percent basis, are typically 12 percent EPA and 8 percent DHA for menhaden oil and 8 percent EPA and 12 percent DHA for salmon oil. Jedwards also provided analytical results to show the EPA and DHA content of the salmon oil as well as the typical distributions of saturated (22%), monounsaturated (39%), and polyunsaturated (35%) fatty acids in the salmon oil ingredient. According to Jedwards, individual lots of salmon oil typically differ in the proportions of EPA and DHA, but lots are blended to achieve a standardized amount of 8 percent EPA and 12 percent DHA.

Jedwards stated that salmon oil is processed according to standardized procedures for fish oils. Fish are cooked and pressed, with the oil separated from the expressed liquor. The

oil is further processed in accordance with current good manufacturing practice using standard refining methods employed in the processing of edible oils that include winterization, neutralization, bleaching, and deodorizing. Jedwards briefly described two purification processes used in the method of manufacture to remove persistent organochlorine pollutants and other contaminants potentially present in fish tissue. Salmon oil is filtered through clay to remove heavy metal contaminants and through activated charcoal to remove organic contaminants. Jedwards provided specifications for maximum levels of environmental contaminants in salmon oil.

Jedwards acknowledged that FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR 184.1472(a)(3)). Jedwards stated that, in accordance with the menhaden oil limitations, the proposed use of salmon oil would correspond with a dietary intake of EPA plus DHA that does not exceed 3 g/p/d. Further, salmon oil is not to be combined or augmented with any other food ingredient containing EPA and DHA.

Based on the information provided by Jedwards, as well as other information available to FDA, the agency had no questions at this time regarding Jedwards' conclusion that salmon oil is GRAS under the intended conditions of use.

IDENTITY, COMPOSITION AND SPECIFICATIONS

FATTY ACID COMPOSITION OF N3-TG-3020 OMEGA-3 TRIGLYCERIDE CONCENTRATE

Twin Rivers Technologies has generated fatty acid profiles for several batches of N3-TG-3020 Omega-3 Triglyceride Concentrate in order to show that its final product is comparable to other food-grade oils, and also to demonstrate that the production process does not structurally alter any of the component fatty acids, or generate any unique byproducts. These profiles were generated based on results from analyses employing AOCS Method Ce-1b-89 (Marine Oil Fatty Acid Composition by GLC). A representative fatty acid profile for N3-TG-Omega-3 Triglyceride Concentrate is displayed in Table 2 below and compared to menhaden oil.

Table 2. Representative Fatty Acid Profiles for N3-TG-3020 Omega-3 Triglyceride Concentrate Compared to Menhaden Oil

	Menhaden oil	N3-TG-3020
Fatty Acid	**Amount (in relative area percent)**	
C12:0	0.107	
C14:0	8.704	0.304
C14:1		
C15:0		
C16:0	18.779	2.663
C16:1	12.011	1.454
C16:4N3		
C17:0	2.280	0.241
C17:1	0.760	0.274
C18:0	3.233	3.077
C18:1N11	6.977	4.463
C18:1N9	2.883	2.770
C18:2	1.249	1.543
C18:3N6	0.502	0.397
C19:0	0.371	0.238
C19:1	0.644	0.257
C18:3N3	1.148	0.824
C18:4N3	2.039	1.576
C20:0	0.269	1.017
C20:1	1.174	1.573
C20:2	0.161	0.286
C20:3N6	0.312	0.511
C20:4N6	1.423	4.838
C20:3N3	0.140	0.251
C20:4N3	1.292	1.929
C20:5N3 (EPA)	**12.742**	**30.289**
C22:1N11	0.295	0.808
C22:1N9	0.236	2.311
C21:5N3	0.725	1.440
C22:4N6	0.393	0.778
C24	0.429	0.721
C22:5N3	2.822	4.709
C22:6N3 (DHA)	**8.898**	**21.020**
Unidentified	7.003	7.434

The fatty acid profile of N3-TG-3020 Omega-3 Triglyceride Concentrate, summarized above, is generally consistent with naturally occurring marine oils. The compositional analysis of the product supports the presumption that N3-TG-3020 Omega-3 Triglyceride

Concentrate is metabolized by the body similarly to naturally occurring fish oil and poses no novel safety concerns.

SPECIFICATIONS

Specifications for concentrated omega-3 triglyceride (N3-TG-3020) are shown in Table 3.

Table 3. Food Grade Specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate

Twin Rivers Technologies has developed specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate to establish and maintain its food-grade status. These specifications are as follows:

Description:	Food-grade N3-TG-3020 Omega-3 Triglyceride Concentrate is composed predominantly of triglycerides (approximately 98%), with a small amount of diglyceride (about 2%), that are highly enriched in polyunsaturated fatty acids, primarily EPA and DHA. It is derived from fish oil extracted from multiple edible marine fish species (typically sardine, anchovy, menhaden, jack mackerel, mackerel, tuna and salmon) that is prepared in a multi-step proprietary process.
Appearance:	Yellow to light yellow at ambient temperature.
Triglycerides:	Not less than ("NLT") 98%.
Odor and taste:	At worst, a slightly fishy odor and taste.
EPA and DHA:	NLT 500 mg/g and not more than ("NMT") 600 mg/g.
EPA/DHA ratio:	NLT 1.5.
Lovibond Color:	5 MAX
Red, 5 ¼"	
Acid value:	NMT 1%.
Monoglyceride:	0
Diglyceride:	NMT 2%.
Triglyceride:	NLT 98%.
Peroxide value:	NMT 5 meq/kg.
p-Anisidine	
value:	NMT 20.
Tottox value:	NMT 26 (Anisidine value +2x [peroxide value]
Iodine value:	NLT 284.4
Density:	942 mg/ml.
Tocopherols:	NLT 2.0 mg/g.
Moisture:	NMT 0.1%.
Unsaponifiables:	NMT 2.35 %.
Total PCBs:	NMT 0.09mg/kg.
PCDDs and	

PCDFs:	NMT 2.0 pg/g.
Heavy Metals	
Lead:	NMT 0.1 mg/kg.
Cadmium:	NMT 0.1 mg/kg.
Arsenic:	NMT 0.1 mg/kg.
Mercury:	NMT 0.1 mg/kg.
Delivery form:	Oil concentrate shipped under inert nitrogen atmosphere.

The food-grade specifications for Omega-3 Triglyceride Concentrate (N3-TG-3020) summarized above are considered appropriate product specifications to ensure that the final product is safe for human consumption. Further, analyses of the product support the presumption that there is no toxicological concern from any product impurities.

MANUFACTURING PROCESS AND DESCRIPTION

Twin Rivers Technologies manufacturing process for the production of a concentrated omega-3 fatty acid triglyceride containing substantially increased levels of EPA and DHA above those found in naturally occurring fish oils is described below. The process begins with a potential filtration treatment to remove solids from marine oils obtained from several potential sources of edible fish including salmon, menhaden, anchovy, sardine, jack mackerel, mackerel and tuna. This potential filtration step is followed by a potential adsorption step using bleaching earth to reduce odor and possible colors resulting in the production of a filtrate containing the fatty acids and a filter cake which is discarded. The next step involves the application of steam for stripping to reduce free fatty acids. This filtrate (when feed pretreatment via filtration and adsorption is employed) or otherwise unfiltered feedstock is then treated with either methanol or ethanol in the presence of a sodium methoxide catalyst resulting in esterification of the fatty acids and the free glycerine is removed. Next, the free unreacted methanol or ethanol are removed by flash evaporation and the resulting esterified product mix is treated with water wash to remove glycerin, soap, methanol or ethanol, as well as odor and flavor bodies. The water wash containing these components is removed and the esterified mass is then dried to remove any residual water. At this point, fractional distillation is utilized to remove the low boiling C14, C16, C18 methyl or ethyl esters and the remaining esterified mass is subjected to wiped film evaporation or other "Flash Distillation" in order distill the high boiling C18, C20, C22, C24 methyl or ethyl esters. Next, these distilled high-boiling esters are reacted with glycerin in the presence of either potassium carbonate or sodium methoxide to product a highly concentrated long chain triglyceride product (90%) with small amounts of diglycerides (5%) and monoglycerides (5%). This reaction product is then treated with water and potassium citrate as a chelant in two stages to physically refine the product further and remove the soaps and potassium carbonate. The resulting long-chain polyunsaturated fatty acid triglyceride concentrate is dried to remove moisture, and treated with an adsorbent media to remove any color, odor or peroxides and this residual filter cake is discarded. The resulting concentrate in the form of the filtrate is then further evaporated to remove any residual esters, treated via steam stripping to remove any carry over odor bodies, peroxides, esters and free fatty acids. Finally, approved and acceptable antioxidants are added to retard oxidation and the long-chain polyunsaturated fatty acid omega-3 triglyceride concentrate is placed in

storage under nitrogen. This process is similar in many respects to other industrial fish-oil based practices for processing of marine oils into refined fish oils and concentrates described in the GRAS Notices referred to earlier in this document, but also includes specific steps to produce a highly concentrated omega-3 triglyceride product. A schematic of the production process for Omega-3 Triglyceride Concentrate (N3-TG-3020) is shown in the confidential file provided in Appendix I.

The production process for Omega-3 Concentrate (N3-TG-3020), summarized above, is a process that is similar in many respects to the standard industry practice for the processing of marine oils. Furthermore, this process is well characterized and can consistently yield a food-grade product that is safe for human consumption with the ongoing analytical testing and quality control procedures described below for potential contaminants.

Acceptable analytical methodology is employed for Omega-3 materials that include measurement of oxidative by-products (acid value, peroxide value, p-anisidine value, Totox), PCDDs and PCDFs, PCBs, and heavy metals including lead, cadmium, mercury, and arsenic. Twin Rivers Technologies will be sampling and analyzing this omega-3 product and process to ensure compliance with the specifications shown in Table 2 which also include those found in a voluntary monograph established in 2002 by the Council for Responsible Nutrition as an ongoing measure of quality control. In addition, they will be applying their internal release criteria to this omega-3 product, which also measures low level methyl or ethyl esters to ensure that the wiped film evaporator has suitably removed these constituents.

Potential Contaminants

Pesticides

A sample of a single lot of N3-TG-3020 Omega-3 Triglyceride Concentrate was screened for twenty-five organochlorine pesticides and their isomers, seventeen additional chlorinated hydrocarbons and eighteen organophosphate pesticides. None of these pesticides were found above the detection limit in any of the samples analyzed. The limits of detection achieved across these large categories of chemicals, depending upon the specific pesticide being considered were generally 1, 5, 10 or 25 ppb, while the limits of detection (LOD) for technical chlordane was 50-100 ppb and for PCBs, 250-500 ppb. These results are presented in Appendices II and III. Twin Rivers Technologies will be screening each new lot manufactured from a different (geographically) sourced marine oil starting material for these same pesticides to ensure there are no outlier issues from the values reported here.

Dioxins and Furans

A single lot of N3-TG-3020 Omega-3 Triglyceride Concentrate was analyzed for PCDDs (dioxins) and PCDFs (furans) using a modified version of USEPA Method 1613B. This lot yielded very low levels of PCDDs and PCDFs of 0.40 and 0.76 pg/g (WHO-TEQ), assuming that any non-detected congeners were present at their respective limits of detection. These results are presented in Appendix I. Twin Rivers Technologies will be

screening each new lot manufactured from a different (geographically) sourced marine oil starting material for dioxins and furans to ensure there are no outlier issues from the values reported here.

PAHs

A single lot of N3-TG-3020 Omega-3 Triglyceride Concentrate plus three potential feedstock marine oils were analyzed for polycyclic aromatic hydrocarbons (PAHs). The detection limit for these compounds was 0.5 parts per billion, including fluorene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, total light PAHs, benzo(b)anthracene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h, i)perylene, total heavy PAHs, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, perylene, coronene and benzo(e)pyrene. Results for all four samples were "none detected" -for the aforementioned compounds. Twin Rivers Technologies will be screening each new lot manufactured from a different (geographically) sourced marine oil starting material for PAHs to ensure there are no outlier issues from the values reported here.

The analytical results for the contaminants summarized above support the presumption that there are no toxicological concerns from any product impurities.

USE AND EXPOSURE

Kris-Etherton *et al.,* (2000) analyzed polyunsaturated fatty acid intake in adults and compiled Table 4 which is presented below. It is clear from this data that omega-3 fatty acid consumption in the United States is between 0.1-0.2 g/day. To date, no official dietary recommendations have been made for n–3 fatty acids in the United States. Recommendations for total PUFA intake, however, have been made: 1–2% of energy from linoleic acid is required to prevent a fatty acid deficiency (Holman, 1970) and total PUFA intake should remain at 7% of energy (NRC Diet and Health, 1989) and not exceed 10% of energy (National Cholesterol Education Program, 1993)

TABLE 4. Polyunsaturated fatty acid intake of adults in the United States by sex and age[1]

Sex and age (y)	Total 1987–1988	Total 1989–1991	18:2n–6 1987–1988	18:2n–6 1989–1991	18:3n–3 1987–1988	18:3n–3 1989–1991	18:4n–3 + 20:4n–6 1987–1988	18:4n–3 + 20:4n–6 1989–1991	20:5n–3 + 22:6n–3 1987–1988	20:5n–3 + 22:6n–3 1989–1991
					g/d					
Males										
12–19	16 ± 0.3	18 ± 1.1	14 ± 0.3	16 ± 0.9	2 ± 0.03	2 ± 0.07	0.1 ± 01	0.1 ± 01	0.1 ± 0.01	0.1 ± 0.07
20–39	17 ± 0.2		15 ± 0.2		2 ± 0.02		0.1 ± 01		0.1 ± 0.01	
20–49		18 ± 0.4		16 ± 0.4		2 ± 0.03		0.1 ± 01		0.2 ± 0.01
>40	16 ± 0.2		14 ± 0.2		2 ± 0.02		0.1 ± 01		0.1 ± 0.01	
50–69		16 ± 0.5		15 ± 0.4		1 ± 0.03		0.1 ± 01		0.2 ± 0.03
Females										
12–19	12 ± 0.3	13 ± 0.4	11 ± 0.2	12 ± 0.4	1 ± 0.03	1 ± 0.03	0.1 ± 0	0.1 ± 01	0.1 ± 0	0.1 ± 0.02
20–39	12 ± 0.2		11 ± 0.1		1 ± 0.01		0.1 ± 0		0.1 ± 0	
20–49		13 ± 0.2		11 ± 0.2		1 ± 0.02		0.1 ± 01		0.1 ± 0.01
>40	11 ± 0.1	12 ± 0.3	10 ± 0.1		1 ± 0.01		0.1 ± 0		0.1 ± 0	
50–69				11 ± 0.3		1 ± 0.02		0.1 ± 01		0.2 ± 0.02
Males and females										
>70		12 ± 0.3		11 ± 0.3		1 ± 0.02		0.1 ± 01		0.2 ± 0.00

[1] ± SE of 3-d intakes. Data for 1987–1988 adapted from Jonnalagadda *et al.*, (1995). Data for 1989–1991 adapted from Allison *et al.*, (1999).

The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of triglycerides of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is known by the commercial name of N3-TG-3020. The intended applications of this omega-3 triglyceride concentrate will be for the same uses in foods for which menhaden oil is permitted under 21 CFR §184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). The maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36% of that of menhaden oil. These proposed uses are presented in Table 5. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above including one for a marine oil concentrate. Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate.

The estimated mean intake of EPA and DHA combined from all dietary sources in the general population, excluding infants under the age of one year, has been addressed in earlier FDA rulemaking (menhaden oil final rule; 62 FR 30751; June 5, 1997) where FDA estimated that the mean exposure to EPA and DHA from the use of menhaden oil in all food categories would be 2.8 grams/person/day. It was also addressed as well in the subsequent GRAS Notices referenced above. The total cumulative estimated mean intake of EPA and DHA combined from the proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate listed in the foods in Table 5, as well as from all other dietary sources in this general population is also estimated to be 2.8 grams per person per day. This estimate reflects 100 percent market penetration of the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate that are listed in Table 5. Because 100 percent market penetration of this product is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower. Further, this cumulative intake of EPA and DHA combined is still less than the safe limit for EPA and DHA combined of 3 grams per person per day established by the agency. The proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate will result in no incremental increase in the intake of EPA and DHA combined over that described for menhaden oil in the final rule Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005. [§184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day.(4) To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid].

Table 5. Proposed Maximum Use Levels in Food as Served of Omega-3 Triglyceride Concentrate (N3-TG-3020) Compared with Current Menhaden Oil Uses Permitted under 21§CFR184.1472 and Final Rule Published in FR March 23, 2005(Volume 70, Number 55 PP14530-14532		
Category of food (and 21CFR §170.3(n) paragraph)	**Maximum Level of Use of Menhaden Oil under 21CFR184.1472 and FR Vol 70 No 55 March 23 2005 Final Rule**	**Proposed Maximum Level of Use of N3-TG-3020 Omega-3 Triglyceride Concentrate[a]**
Baked goods, baking mixes, §170.3(n)(1)	5.0 percent	1.8 percent
Cereals, §170.3(n)(4)	4.0 percent	1.4 percent
Cheese products, §170.3(n)(5)	5.0 percent	1.8 percent
Chewing gum, §170.3(n)(6)	3.0 percent	1.1 percent
Condiments, §170.3(n)(8)	5.0 percent	1.8 percent
Confections, frostings, §170.3(n)(9)	5.0 percent	1.8 percent
Dairy product analogs, §170.3(n)(10)	5.0 percent	1.8 percent
Egg products, §170.3(n)(11)	5.0 percent	1.8 percent
Fats, oils, §170.3(n)(12), but not in infant formula	12.0 percent	4.3 percent
Fish products, §170.3(n)(13)	5.0 percent	1.8 percent
Frozen desserts, §170.3(n)(20)	5.0 percent	1.8 percent
Gelatins, puddings, §170.3(n)(22)	1.0 percent	0.36 percent
Gravies, sauces, §170.3(n)(24)	5.0 percent	1.8 percent
Hard candy, §170.3(n)(25)	10.0 percent	3.6 percent
Jams, jellies, §170.3(n)(28)	7.0 percent	2.5 percent
Meat products, §170.3(n)(29)	5.0 percent	1.8 percent
Milk products, §170.3(n)(31)	5.0 percent	1.8 percent
Nonalcoholic beverages, §170.3(n)(3)	0.5 percent	0.18 percent
Nut products, §170.3(n)(32)	5.0 percent	1.8 percent
Pastas, §170.3(n)(23)	2.0 percent	0.7 percent
Plant protein products, §170.3(n)(33)	5.0 percent	1.8 percent
Poultry products, §170.3(n)(34)	3.0 percent	1.1 percent
Processed fruit juices, §170.3(n)(35)	1.0 percent	0.36 percent
Processed vegetable juices, §170.3(n)(36)	1.0 percent	0.36 percent
Snack foods, §170.3(n)(37)	5.0 percent	1.8 percent
Soft candy, §170.3(n)(38)	4.0 percent	1.4 percent
Soup mixes, §170.3(n)(40)	3.0 percent	1.1 percent
Sugar substitutes, §170.3(n)(42)	10.0 percent	3.6 percent
Sweet sauces, toppings, syrups, §170.3(n)(43)	5.0 percent	1.8 percent
White granulated sugar, §170.3(n)(41)	4.0 percent	1.4 percent

[a]Proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532) are set to provide the same concentrations of EPA+DHA as those provided by menhaden oil; based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate

SAFETY STUDIES AND DISCUSSION

Menhaden Oil

There have been many published studies evaluating the safety of omega-3 fatty acids and these have all been evaluated by FDA in arriving at the determination that menhaden oil and its component fatty acids including EPA and DHA are GRAS as food ingredients subject to the limitations in 21 CFR §184.1472 and the final rule affirming as Generally Recognized as Safe (GRAS) menhaden oil published on March 23, 2005, 70 FR Number 55 Part 184 at page 14530. FDA also permitted the use of a Qualified Health Claim on dietary supplements containing EPA and DHA on October 31, 2000 as well as for conventional foods on September 8, 2004. In the October 31, 2000 letter, FDA concluded that the use of EPA and DHA omega-3 fatty acids as dietary supplements is safe and lawful under 21 CFR § 101.14, provided that daily intakes of EPA and DHA omega-3 fatty acids do not exceed 3 g/p/d from conventional food and dietary supplement sources. Further, FDA concluded that in order to help ensure that a consumer does not exceed an intake of 3 g/p/d of EPA and DHA omega-3 fatty acids from consumption of a dietary supplement with the qualified claim, an EPA and DHA omega-3 fatty acid dietary supplement bearing a qualified claim should not recommend or suggest in its labeling, or under ordinary conditions of use, a daily intake exceeding 2 grams EPA and DHA.

It is instructive to review FDA's rationale, discussion and conclusions on this to determine the safety of consumption of EPA and DHA in either supplement or conventional food forms. These are excerpted below and demonstrate that FDA is comfortable with the safety of the omega-3 fatty acids EPA and DHA as long as consumption is below 3 grams/person/day. This analysis is fully applicable to Twin Rivers Technologies Omega-3 Triglyceride Concentrate (N3-TG-3020) in terms of its inherent use and safety.

Safety Review Excerpted from FDA September 8, 2004 Letter Outlining How They Determined Safety of Omega-3 Fatty Acids to Permit a Qualified Health Claim for Conventional Foods

Under 21 CFR §101.14(b)(3)(ii), if the substance is to be consumed at other than decreased dietary levels, the substance must be a food or a food ingredient or a component of a food ingredient whose use, at levels necessary to justify a claim, must be demonstrated by the proponent of the claim to FDA's satisfaction to be safe and lawful under applicable food safety provisions of the Federal Food, Drug, and Cosmetic Act.

The Wellness petition (Docket No. 2003Q-0401) stated that omega-3 fatty acids, as EPA and DHA, have been a naturally occurring ingredient in foods consumed safely in the United States prior to January 1, 1958, and that there is no evidence that when consumed either in foods or as dietary supplements there is a cumulative effect in the diet that is unsafe. The Martek petition (Docket No. 2003Q-0401) stated that omega-3 fatty acids occur in conventional foods with a long history of safe use, such as fish, and are generally recognized as safe (GRAS) when used as direct food ingredients intended to increase

omega-3 fatty acids. Some comments to the petition expressed an interest in using the omega-3 fatty acid qualified health claim for foods that contain EPA and DHA as a food ingredient from sources including fish oil and algal oil.

> In order to meet the safe and lawful requirement for health claims (21 CFR §101.14(b)(3)(ii)), the use of EPA and DHA omega-3 fatty acid, when used in conventional food or as a dietary supplement at levels necessary to justify the claim, must be demonstrated, to FDA's satisfaction, to be safe and lawful. FDA evaluates whether the substance is "safe and lawful" under the applicable food safety provisions of the Act. For conventional foods, this evaluation involves considering whether the ingredient that is the source of the substance is GRAS, approved as a food additive, or authorized by a prior sanction issued by FDA (see 21 CFR §101.70(f)). Dietary ingredients in dietary supplements, however, are not subject to the food additive provisions of the act (see section 201(s)(6) of the Act (21 U.S.C. §321(s)(6)). Rather, they are subject to the adulteration provisions in section 402 of the Act (21 U.S.C. 342) and, if applicable, the new dietary ingredient provisions in section 413 of the Act (21 U.S.C. 350b), which pertain to dietary ingredients that were not marketed in the United States before October 15, 1994. The term "dietary ingredient" is defined in section 201(ff)(1) of the act and includes vitamins; minerals; herbs and other botanicals; dietary substances for use by man to supplement the diet by increasing the total daily intake; and concentrates, metabolites, constituents, extracts, and combinations of the preceding types of ingredients.

In 1997, FDA affirmed, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use to ensure that the total daily intake of EPA and DHA would not exceed 3.0 grams per person per day (g/p/d) (62 FR 30751; June 5, 1997; 21 CFR §184.1472). EPA and DHA are the major omega-3 fatty acids in fish oil and together comprise about 20 percent by weight of menhaden oil. FDA established maximum use levels of menhaden oil in certain foods because of concerns over possible adverse effects of fish oil consumption on bleeding time, glycemic control, and LDL cholesterol (62 FR 30751 at 30757; June 5, 1997). In 2002, FDA published a proposed rule to reallocate the uses of menhaden oil in conventional food, while maintaining the total daily intake of EPA and DHA from menhaden oil at a level not exceeding 3.0 g/p/d (67 FR 8744; February 26, 2002). FDA placed specific limitations, including the category of foods, the functional use of the ingredient, and the level of use, to ensure that the consumption of EPA and DHA from conventional food sources would not exceed 3.0 g/p/d. FDA then published a final rule on March 23, 2005, 70 FR Number 55 Part 184 at page 14530), to additionally require that menhaden oil not be used as an ingredient in foods in combination with other added oil that is a significant source of EPA and DHA to ensure that total intake from conventional food sources do not exceed 3.0 g/p/d.

In addition, FDA has not objected to certain GRAS notifications for additional sources of EPA and DHA as food ingredients (fish oils other than menhaden oil) (GRAS Notice Nos: GRN 000097, GRN 000102, GRN 000105, GRN 000109, GRN 000137, GRN 000138). These GRAS notices proposed maximum use levels consistent with those

specified in the tentative final rule affirming, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use.

FDA has also responded without objection to a GRAS notification on algal oil DHA from Martek Biosciences Corporation. Martek estimated that the use of algal oil in a number of food categories at the maximum proposed use levels would result in a mean exposure of no more than 1.5 grams of DHA per day (GRAS Notice No. GRN 000137).

> The mean exposure to EPA and DHA from menhaden oil in all conventional food categories is estimated to be 2.7 g/p/d (67 FR 8744 at 8746; February 26, 2002). This is a conservative estimate with substantial margin for safety, and the agency believes, consistent with its prior decision on the use of a qualified health claim for DHA and EPA omega-3 fatty acids (October 31, 2000 letter), that the addition of menhaden oil to food products has not come close to this conservative mean estimate exposure. FDA further believes that the GRAS uses for which it received a GRAS notification for other sources of EPA and DHA omega-3 fatty acids also provide conservative estimates of exposure and that the addition of these EPA and DHA sources to food products do not come close to the conservative mean estimates. Not all foods in the marketplace within those permitted food categories would contain menhaden oil or other sources of EPA and DHA omega-3 fatty acids that substitute for other edible fat or oil. Also, because not all foods that a consumer eats every day would contain menhaden or other EPA and DHA oil used as a substitute oil, the actual total daily intakes of EPA and DHA from menhaden or other EPA and DHA oil for an average person should be significantly below 3.0 g/p/d (67 FR 8744 at 8746; February 26, 2002).
>
> It is difficult to estimate the actual total consumption of EPA and DHA. The Continuing Survey of Food Intakes by Individuals (CSFII 1994-1996, 1998) estimated EPA and DHA intakes from conventional foods. The 50th percentile intake of EPA and DHA from the survey was between 0.06 g and 0.07 g for adult women and 0.07 g and 0.1 g for adult men. The 90th percentile intake was between 0.18 g and 0.22 g for women and between 0.20 g and 0.43 g for men. Thus, EPA and DHA consumption from conventional foods in the United States is low. FDA is not aware of any nationally representative consumption data on EPA and DHA from dietary supplements. In the October 31, 2000 letter, FDA expressed concern about the exposure to EPA and DHA omega-3 fatty acids potentially exceeding 3.0 g/p/d if a qualified health claim were to appear on dietary supplements. This concern was due to conventional foods containing omega-3 fatty acids that were on the market; the use of structure/function claims on products containing EPA and DHA omega-3 fatty acids, which may promote product purchase; and dietary supplements that FDA found in the marketplace that contained significant amounts of EPA and DHA.

In a September 8, 2004 letter to Martin Hahn of Hogan and Hartson, FDA extended the requested use of this qualified health claim and it is now extended to conventional foods.

The agency believes that there is likely to be some increased consumption of EPA and DHA omega-3 fatty acids based on conventional foods that bear the qualified health claim; however, the amounts of EPA and DHA that can be used and the foods in which such food ingredients can be safely used are limited. The agency has established specific limitations of use under its menhaden oil GRAS rule (62 FR 30751; June 5, 1997), proposed and tentatively finalized reallocation of the use of menhaden oil without changing total exposure levels (67 FR 8744; February 26, 2002, 69 FR 2313; January 15, 2004). (NOTE: the finalized allocation has since been published on March 23, 2005, 70FR Number 55 Part 184 at page 14530). Also, manufacturers that have submitted GRAS notifications for other sources, to which the agency has not objected, have established conditions of use similar to those in the menhaden oil GRAS rule.

In the October 31, 2000 letter, FDA stated that a consumer could consume nearly 1 gram of EPA and DHA per day in the diet from conventional foods. The agency is uncertain about how much consumers will increase their intake of EPA and DHA omega-3 fatty acids from EPA and DHA containing conventional foods and dietary supplements due to the extended use of the qualified health claim. In order to help consumers gauge their total intake of EPA and DHA and to provide them a way to keep their intake of EPA and DHA within 3 grams per day, FDA intends to consider, as a factor in the exercise of its enforcement discretion, that conventional foods and dietary supplements that bear an omega-3 fatty acid qualified health claim declare the amount of EPA and DHA per serving in the claim. FDA recommends that the information on EPA and DHA content for use in a qualified health claim for EPA and DHA omega-3 fatty acids and reduced risk of CHD be presented in a manner that is consistent with FDA's guidance entitled, "FDA Nutrition Labeling Manual--A Guide for Developing and Using Data Bases." The dietary supplement may declare the amount of EPA and DHA per serving in "Supplement Facts," instead of making the declaration in the claim. Also, to ensure further that consumers do not exceed a 3.0 g/p/d intake, FDA will educate consumers not to exceed 3.0 g/p/d from all food and dietary supplement sources through print and web outreach information.

Further, FDA intends to consider, as a factor in the exercise of its enforcement discretion, that dietary supplements not recommend or suggest in labeling that consumers ingest more than 2 grams of EPA and DHA per day. FDA encourages manufacturers to limit their dietary supplement products bearing the qualified health claim to products recommending or suggesting daily intake of 1 gram or less of EPA and DHA omega-3 fatty acids.

Based on the data and information that FDA considered, which includes data and information that FDA relied upon in reaching its conclusions about the safety of EPA and DHA omega-3 fatty acids in its GRAS affirmation of menhaden oil, the data and information in the 1991 proposed (56 FR 60663; November 27, 1991) and 1993 final rules (58 FR 2683; January 6, 1993), and its current scientific literature review for other possible safety concerns, FDA concludes that the use of EPA and DHA omega-3 fatty acids used as a GRAS ingredient, consistent with FDA's GRAS rule for menhaden oil and GRAS notifications to which FDA did not object, the use of omega-3 fatty acids as a

dietary supplement is safe and lawful under 21 CFR 101.14 provided that daily intakes of EPA and DHA omega-3 fatty acids from conventional food and dietary supplement sources do not exceed 3.0 g/p/d.

From the foregoing analysis and rulemaking decisions of FDA on the GRAS affirmation of menhaden oil and of EPA and DHA, as well as on the submitted GRAS Notices where the agency had no objection to the conclusions of being GRAS including a specific submission on an omega-3 fish oil concentrate, Twin Rivers Technologies Omega-3 Triglyceride Concentrate (N3-TG-3020) is considered GRAS for the proposed uses specified in the regulations under the conditions described and at the maximum use levels described in Table 7.

The EPA and DHA levels in the Omega-3 Triglyceride Concentrate (N3-TG-3020) are increased above those found in naturally occurring fish oils by a very selective process involving separation of different fatty acid chain lengths from fish oils via either methylation or ethylation into fatty methyl or ethyl esters followed by distilling the short chain, non-omega-3 methyl or ethyl esters away from the longer chain methyl or ethyl esters and then re-esterifying the longer chain methyl or ethyl esters with glycerine to form a triglyceride, whose chain lengths are now concentrated in omega-3 content. Analysis of the triglycerides that comprise the Omega-3 Triglyceride Concentrate (N3-TG-3020) indicate that these glycerides are structurally unchanged and their positional distributions within the glyceride are comparable to the starting fish oil. Based on this similarity, Omega-3 Triglyceride Concentrate (N3-TG-3020) would be expected to be metabolized by the body in a similar manner as naturally occurring fish oil constituents.

Other Recent Publications

In addition, several extensive searches of the scientific literature were conducted to determine if there were any new publications relating to the safety of EPA and DHA since FDA's final regulation on the GRAS affirmation of menhaden oil. No new safety issues were identified. A few pertinent current reviews were identified where the utility of fish oils were reviewed and their clinical usefulness and significance discussed. These are briefly discussed below.

As part of a review of marine n-3 PUFAs and coronary heart disease, Schmidt *et al.*, (2005) recently reviewed the safety of omega-3 fatty acids and reported that while there has been concern about an increased risk of bleeding, especially after consumption of large doses of fish oil concentrates, there is little clinical evidence in support of this even in patients treated with aspirin or anticoagulants. They noted that it has been claimed that n-3 PUFA may deteriorate glycemic control in patients with diabetes mellitus, but recent data have discarded this hypothesis. In addition, while immune responses can be modulated by n-3 PUFA, there is no evidence that intake of n-3 PUFA is associated with an increased risk of serious infections or cancer (Larsson *et al.*, 2004). In a separate review, Schmidt *et al.*, (2005) reviewed the results of clinical trials on n-3 PUFA on clinical endpoints and provided recommendations for the use of n-3 PUFA in the prevention and treatment of coronary heart disease in patients after a myocardial infarct as well as discussed other areas where further studies are needed in the areas of

reocclusion rates of venous grafts as well as in addressing the antiarrhythmic effects of marine n-3 PUFAs. These data are important because the n-3 PUFA possess multiple effects including anti-inflammatory, anti-arrhythmic and antiplatelet effects that may benefit patients both acutely and in the long-term, by reducing infarct size. Additionally, they noted the need for studies on patients with hypertriglyceridemia, including patients with metabolic syndrome.

Lombardo and Chicco (2005) provided a review of the effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. The human studies assessed data from both nondiabetic and diabetic patients. It is well known that hypertriglyceridemia and elevated low-density lipoprotein cholesterol (LDL-C) and lower HDL-C concentrations are important risk factors for coronary heart diseases. Human studies have shown that fish oil supplements and diets containing fish oil (enriched on EPA and DHA fatty acids) are useful to reduce plasma triglyceride and VLDL lipoprotein concentrations, especially in the postprandial state. The mechanism for the reduction of triglyceride rich lipoprotein involves both a decrease in VLDL synthesis and an increase in the fractional catabolic rate of VLDL (triglyceride clearance). Moreover, a higher rate of fatty acid oxidation was observed in humans fed PUFAs-rich diets.

Concerning middle-aged and elderly patients with increased risk of coronary heart disease, Zhengling *et al.* (2004) have recently shown that in a saturated fat/cholesterol-restricted diet, a high fish oil content favorably affects VLDL and HDL subspecies, shifting lipoproteins subfractions to a less atherogenic pattern. Patients with type IIb or IV hypertriglyceridemia, given a standardized test meal, showed that n-3 fatty acids suppress both hepatic and intestinal Apo B secretion/synthesis and altered triglyceride-rich lipoprotein metabolism (changing postprandial triglyceride, cholesterol, Apo B48 and increasing LDL particle size (Tinker *et al.,* 1999). Moreover, regarding blood cholesterol levels and human atherosclerosis, all these studies could represent additional mechanisms for the reduced heart disease risk associated with n-3 fish oil consumption.

Based on epidemiological findings, the intake of n-3 fatty acids in the diet has been associated with a reduced cardiovascular risk. The mechanisms of risk reduction have been widely explained, either as a consequence of the lipid /lipoprotein changes or as platelet/function and hemorrheological changes, including reduced thromboxane B2 formation and red cell aggregability and viscosity. Monocyte macrophage changes have also been observed, leading to reduced formation of interleukin-1 and platelet aggregation factors, resulting in inhibited growth factor formation and promoting nitric oxide-induced endothelial relaxation (Nestel, 2000). Furthermore, middle hypotensive and antiarrhythmic effects of n-3 fatty acids have been shown in cell cultures and human studies (Harper and Jacobson, 2001). The antihypertensive effect of n-3 fatty acids may depend on vascular effects with improved endothelial vasodilator function, reduced reactivity of vascular smooth muscle of resistant vessels and increased vascular compliance.

In diabetics it is known that fish oil intake delays the development of glucose intolerance (Feskens *et al.*, 1991). Interestingly, a recent work by Stene and Joner (2004), in a larger

nationwide case-control study in Norway, found a significant association between the use of cod liver oil during the first year of life and a lower risk of type 1 diabetes mellitus, perhaps through the antiinflammatory effects of long-chain n-3 fatty acids.

Concerning the effect of fish oil administration on the glycemic control in diabetic patients with type 2 and type 1 diabetes mellitus, conflicting results have been obtained. Some early studies on type 2 diabetes mellitus showed that fish oil may worsen glycemic control; however, the adverse effect of fish oil has almost invariably been established with large doses of omega-3 fatty acids (5.5–8 g/day) in studies lacking an appropriate control group and with a small number of subjects. Other authors found no changes or even improved insulin sensitivity in patients with type 2 diabetes mellitus.

A meta analysis (Friedberg *et al.,* 1998) of 26 different trials on the effect of fish oil administration on both glycemic control and lipid parameters in type 2 and type 1 diabetic subjects showed (i) a decrease in plasma triglyceride concentration and slight but significant increase in LDL-C, both findings being more prominent in type 2 diabetes mellitus; no changes in total cholesterol and HDL-C were observed, (ii) no significant changes in HbA_{1c} occurred in diabetic patients, (iii) fasting blood glucose levels increased with borderline significance in type 2 diabetic subjects and were significantly lower in type 1 diabetic subjects; (iv) besides, significantly, dose-response effects of EPA (g/day) on HbA_{1c} and triglycerides and DHA (g/day) on fasting blood glucose levels, HbA_{1c} and triglycerides, were demonstrated only in type 2 diabetic subjects.

Recently, Skurnick-Minot *et al.* (2004) demonstrated a decrease in whole-body adiposity and adipocyte size in type 2 diabetic insulin-resistant patients after 2 months of treatment with fish oil capsule (1.8 g of n-3 PUFA). In these patients, plasma adiponectin tended to increase without any deterioration or amelioration of insulin sensitivity at this stage. Moreover, the diabetes and nutrition study group of the Spanish Diabetes Association, in a 7-year prospective, population-based, multicenter study showed that normoalbuminuria and nephropathy regression in well-controlled diabetic patients with long-term diabetes (type 1 and 2) are associated with enhanced PUFAs and less saturated fatty acid consumption (Spanish Diabetes Association, 2004).

FDA Regulatory Status of Formulation Components of Omega-3 Triglyceride Concentrate (N3-TG-3020)

Constituent and amount added to the formulation of Omega-3 Triglyceride Concentrate (N#-TG-3020) are as follows:

Mixed Tocopherols 2mg/g

The review of the regulatory status of this formulation constituent which follows indicates that it is approved for direct addition to foods and the amounts used are not either in excess to that approved by regulation or above that necessary to achieve the intended physical or technical effect as an antioxidant, anticaking agent, etc.

Mixed Tocopherols

Mixed tocopherols are listed under §182.3890 as a generally recognized as safe food substance when used in accordance with GMPs. It is used in the formulation as an antioxidant or chemical preservative at 2000 ppm.

DISCUSSION

The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of triglycerides of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is known by the commercial name of N3-TG-3020. The intended applications of this omega-3 triglyceride concentrate will be for those uses in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). The maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36 % of that of menhaden oil. These proposed uses and levels of addition to various food types are presented in Table 5. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above including one for a marine oil concentrate. Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate.

The estimated mean intake of EPA and DHA combined from all dietary sources in the general population, excluding infants under the age of one year, has been addressed in earlier FDA rulemaking (menhaden oil final rule; 62 FR 30751; June 5, 1997) where FDA estimated that the mean exposure to EPA and DHA from the use of menhaden oil in all food categories would be 2.8 grams/person/day. It was also addressed as well in the subsequent GRAS Notices referenced above. The total cumulative estimated mean intake of EPA and DHA combined from the proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate listed in the foods in Table 5, as well as from all other dietary sources in this general population is also estimated to be 2.8 grams per person per day. This estimate reflects 100 percent market penetration of the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate that are listed in Table 5. Because 100 percent market penetration of this product is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower. Further, this cumulative intake of EPA and DHA combined is still less than the safe limit for EPA and DHA combined of 3 grams per person per day established by the agency.

The proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate will result in no incremental increase in the intake of EPA and DHA combined over that described for menhaden oil in the final rule Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005. [§184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day. (4) To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid].

In addition, the safety of consumption of Omega-3 Triglyceride Concentrate (N3-TG-3020) used as an ingredient in food is based on its similarity to currently marketed fish oil products that have been the subject of several GRAS Notices referenced in this document , as well as the safety of ingestion of its major constituents, EPA and DHA. The safety of consumption of the whole product was determined by evaluating the source of the product, the production process, the nature and quantity of impurities, product specifications, and the identity and positional distributions of EPA and DHA in the glycerides comprising the product. Appropriate product specifications have been established to ensure that the final product is food grade, and compositional analysis of the product supports the presumption that there is no toxicological concern from any product impurities. Further, as long as the Omega-3 Triglyceride Concentrate (N3-TG-3020) use is in the food categories identified above at a level of 36 % of the maximum permissible levels of menhaden oil, and the resulting mean potential intake is less than 3.0 grams per day of EPA and DHA combined, it is safe, and GRAS, for addition to food.

CONCLUSION

Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively concluded that Omega-3 Triglyceride Concentrate, (N3-TG-3020) meeting the specifications cited above, and concentrated as described, is GRAS when used as an ingredient in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation when used at a maximum level of 36 % of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020), when used as described, is GRAS based on scientific procedures.

Stanley M. Tarka, Jr., Ph.D. — Date 1/4/06

W. Gary Flamm, Ph.D., FACT, FATS — Date 1/3/06

James W. Barnett, Jr., Ph.D., DABT — Date 12/30/05

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Appendix I.

Confidential Flow Diagram of TRT Manufacturing Process for N3-TG-3020 Omega-3 Triglyceride Concentrate

Omega-3 Process
Concept 3 (Erik's chai

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DETERMINATION OF PCDD/PCDF LEVELS

Prepared for:
Twin Rivers Technologies
Attn: Marty Lake
4700 Este Avenue
Cincinnati, OH 45232

This report contains 11 pages.

The results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report.

Project: Chemical Analysis

Purchase Order Number: N004545

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REPORT OF LABORATORY ANALYSIS

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REPORT OF: CHEMICAL ANALYSES

PROJECT: **PCDD/PCDF ANALYSES**

DATE: December 1, 2005

ISSUED TO: Twin Rivers Technologies
Attn: Marty Lake
4700 Este Avenue
Cincinnati, OH 45232

REPORT NO: 05-1023564

INTRODUCTION

This report presents the results from the analyses performed on one sample submitted by a representative of Twin Rivers Technologies. The sample was analyzed for the presence or absence of polychlorodibenzo-p-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) using a modified version of USEPA Method 1613B.

SAMPLE IDENTIFICATION

Client ID	Sample Type	Date Received	Pace ID
N3TG-50406-5280	Solid	11/17/05	1023564001

RESULTS

The results are included in the following:

Appendix A – Chain of Custody Documentation
Appendix B – PCDD/PCDF Results

DISCUSSION

The recoveries of the isotopically-labeled PCDD/PCDF internal standards in the sample extract ranged from 61-156%. All of the labeled standard recoveries obtained for this project were within the target ranges specified in Method 1613B. Also, since the quantification of the native 2,3,7,8-substituted congeners was based on isotope dilution, the data were automatically corrected for variation in recovery and accurate values were obtained. It should be noted that the sample collection date was not indicated on the chain of custody documentation; therefore, the recommended one-year hold time could not be verified.

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PROJECT: PCDD/PCDF ANALYSES **DATE:** December 1, 2005

 REPORT NO: 05-1023564

DISCUSSION (Cont.)

In some cases, the presence of interfering substances impacted the determinations of selected congeners. The affected values were flagged "I" where incorrect isotope ratios were obtained.

A laboratory method blank was prepared and analyzed with the sample batch as part of our routine quality control procedures. The results, found at the beginning of Appendix B, show the blank to contain trace levels of selected congeners. These were below the calibration range of the method. Sample levels similar to the corresponding blank levels were flagged "B" on the results tables and may be, at least partially, attributed to the background. It should be noted that levels less than ten times the background are not generally considered to be statistically different from the background.

A laboratory spike sample was also prepared with the sample batch using clean sand that had been fortified with native standard materials. The results, found at the end of Appendix B, show that the spiked native compounds were recovered at 93-114%. This indicates a high degree of accuracy for these determinations.

REMARKS

The sample extract will be retained for a period of 15 days from the date of this report and then discarded unless other arrangements are made. The raw mass spectral data will be archived for a period of not less than one year. Questions regarding the data contained in this report may be directed to the author at the number provided below.

Pace Analytical Services, Inc.

Scott C. Unze
Project Manager, HRMS
(612) 607-6383

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TABLE 1. 2,3,7,8-TCDD Equivalency Factors (TEFs) for the Polychlorinated Dibenzo-p-dioxins and Dibenzofurans

Number	Compound(s)	TEF
1	2,3,7,8-TCDD	1.00
2	1,2,3,7,8-PeCDD	1.00
3	1,2,3,6,7,8-HxCDD	0.1
4	1,2,3,7,8,9-HxCDD	0.1
5	1,2,3,4,7,8-HxCDD	0.1
6	1,2,3,4,6,7,8-HpCDD	0.01
7	OCDD	0.0001
8	* Total - TCDD	0.0
9	* Total - PeCDD	0.0
10	* Total - HxCDD	0.0
11	* Total - HpCDD	0.0
12	2,3,7,8-TCDF	0.10
13	1,2,3,7,8-PeCDF	0.05
14	2,3,4,7,8-PeCDF	0.5
15	1,2,3,6,7,8-HxCDF	0.1
16	1,2,3,7,8,9-HxCDF	0.1
17	1,2,3,4,7,8-HxCDF	0.1
18	2,3,4,6,7,8-HxCDF	0.1
19	1,2,3,4,6,7,8-HpCDF	0.01
20	1,2,3,4,7,8,9-HpCDF	0.01
21	OCDF	0.0001
22	* Total - TCDF	0.0
23	* Total - PeCDF	0.0
24	* Total - HxCDF	0.0
25	* Total - HpCDF	0.0

*Excluding the 2,3,7,8-substituted congeners.

Reference: 1997 WHO TEFs

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APPENDIX A

REPORT OF LABORATORY ANALYSIS
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Twin Rivers Technologies Natural Ingredients

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(513) 482-8800 main
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(617) 472-9200

1023564

November 16th, 2005

Pace Analytical.
1700 Elm Street
Suite 200
Minneapolis MN 55414-2485
Attention: Analytical Services

Please perform the following analyses on sample #: N3TG-50406-5280 001

- PCDDs (Dioxins) and PCDFs (Furans) by method 1613

I prefer that the results be emailed to marty.lake@trtlp.com

Please bill to PO# N004545 and send the invoice to:

Marty Lake
Twin Rivers Technologies
4700 Este Ave
Cincinnati, OH 45232

If you have any questions, please call (513) 482-8823 or email me (marty.lake@trtlp.com).

Best Regards,

Marty Lake

Rec'd [illegible] - Pace 11/17/08 08:45
15°C

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Pace Analytical Services, Inc.
1700 Elm Street
Minneapolis, MN 55414
Phone: 612.607.1700
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APPENDIX B

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Pace Analytical Services, Inc.
1700 Elm Street - Suite 200
Minneapolis, MN 55414

Tel: 612-607-1700
Fax: 612- 607-6444

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Method 1613B Blank Analysis Results

Client - Twin Rivers Technologies

Lab Sample ID	BLANK-8347	Matrix	Oil
Filename	F51130A_05	Dilution	NA
Total Amount Extracted	10.1 g	Extracted	11/24/2005
ICAL Date	10/22/2005	Analyzed	11/30/2005 17:39
CCal Filename(s)	F51129B_15	Injected By	MRO

Native Isomers	Conc ng/Kg	EMPC ng/Kg	PRL ng/Kg		Internal Standards	ng's Added	Percent Recovery
2,3,7,8-TCDF	ND	-----	0.120		2,3,7,8-TCDF-13C	2.00	72
Total TCDF	ND	-----	-----		2,3,7,8-TCDD-13C	2.00	77
					1,2,3,7,8-PeCDF-13C	2.00	85
2,3,7,8-TCDD	ND	-----	0.100		2,3,4,7,8-PeCDF-13C	2.00	84
Total TCDD	ND	-----	-----		1,2,3,7,8-PeCDD-13C	2.00	93
					1,2,3,4,7,8-HxCDF-13C	2.00	86
1,2,3,7,8-PeCDF	ND	-----	0.100		1,2,3,6,7,8-HxCDF-13C	2.00	79
2,3,4,7,8-PeCDF	ND	-----	0.087		2,3,4,6,7,8-HxCDF-13C	2.00	75
Total PeCDF	ND	-----	-----		1,2,3,7,8,9-HxCDF-13C	2.00	78
					1,2,3,4,7,8-HxCDD-13C	2.00	82
1,2,3,7,8-PeCDD	ND	-----	0.140		1,2,3,6,7,8-HxCDD-13C	2.00	74
Total PeCDD	ND	-----	-----		1,2,3,4,6,7,8-HpCDF-13C	2.00	72
					1,2,3,4,7,8,9-HpCDF-13C	2.00	59
1,2,3,4,7,8-HxCDF	0.11	-----	0.099	J	1,2,3,4,6,7,8-HpCDD-13C	2.00	67
1,2,3,6,7,8-HxCDF	ND	-----	0.110		OCDD-13C	4.00	55
2,3,4,6,7,8-HxCDF	ND	-----	0.095				
1,2,3,7,8,9-HxCDF	ND	-----	0.097		1,2,3,4-TCDD-13C	2.00	NA
Total HxCDF	0.11	-----	-----	J	1,2,3,7,8,9-HxCDD-13C	2.00	NA
1,2,3,4,7,8-HxCDD	ND	-----	0.110		2,3,7,8-TCDD-37Cl4	0.20	78
1,2,3,6,7,8-HxCDD	ND	-----	0.120				
1,2,3,7,8,9-HxCDD	ND	-----	0.120				
Total HxCDD	0.52	-----	-----	J			
1,2,3,4,6,7,8-HpCDF	0.20	-----	0.057	J	Total 2,3,7,8-TCDD		
1,2,3,4,7,8,9-HpCDF	ND	-----	0.095		Equivalence: 0.40 ng/Kg		
Total HpCDF	0.85	-----	-----	J	(Using WHO Factors - Using PRL where ND)		
1,2,3,4,6,7,8-HpCDD	1.50	-----	0.150	J			
Total HpCDD	2.70	-----	-----	J			
OCDF	0.72	-----	0.065	J			
OCDD	8.90	-----	0.140	J			

Conc = Concentration (Totals include 2,3,7,8-substituted isomers).
EMPC = Estimated Maximum Possible Concentration
PRL = Pace Analytical Reporting Limit
A = Limit of Detection based on signal to noise
P = Recovery outside of method 1613 control limits
Nn = Value obtained from additional analysis

I = Interference
E = PCDE Interference
ND = Not Detected
NA = Not Applicable
NC = Not Calculated
J = Value below calibration range
* = See Discussion

Report No.....1023564

REPORT OF LABORATORY ANALYSIS

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Pace Analytical Services, Inc.
1700 Elm Street - Suite 200
Minneapolis, MN 55414

Tel: 612-607-1700
Fax: 612-607-6444

Pace Analytical™

Method 1613B Analysis Results

Client - Twin Rivers Technologies

Client's Sample ID	N3TG-50406-5280		
Lab Sample ID	1023564001		
Filename	F51129A_11		
Injected By	MRO		
Total Amount Extracted	10.1 g	Matrix	Oil
% Moisture	NA	Dilution	NA
Dry Weight Extracted	NA	Collected	NA
ICAL Date	10/22/2005	Received	11/17/2005
CCal Filename(s)	F51129A_02	Extracted	11/24/2005
Method Blank ID	BLANK-8347	Analyzed	11/29/2005 19:24

Native Isomers	Conc ng/Kg	EMPC ng/Kg	LOD ng/Kg		Internal Standards	ng's Added	Percent Recovery
2,3,7,8-TCDF	ND	-----	0.093		2,3,7,8-TCDF-13C	2.00	89
Total TCDF	0.21	-----	0.093	J	2,3,7,8-TCDD-13C	2.00	87
					1,2,3,7,8-PeCDF-13C	2.00	96
2,3,7,8-TCDD	ND	-----	0.100		2,3,4,7,8-PeCDF-13C	2.00	102
Total TCDD	ND	-----	0.100		1,2,3,7,8-PeCDD-13C	2.00	156
					1,2,3,4,7,8-HxCDF-13C	2.00	115
1,2,3,7,8-PeCDF	ND	-----	0.150		1,2,3,6,7,8-HxCDF-13C	2.00	105
2,3,4,7,8-PeCDF	ND	-----	0.150		2,3,4,6,7,8-HxCDF-13C	2.00	102
Total PeCDF	ND	-----	0.150		1,2,3,7,8,9-HxCDF-13C	2.00	90
					1,2,3,4,7,8-HxCDD-13C	2.00	111
1,2,3,7,8-PeCDD	-----	0.43	0.230	I	1,2,3,6,7,8-HxCDD-13C	2.00	86
Total PeCDD	0.42	-----	0.230	J	1,2,3,4,6,7,8-HpCDF-13C	2.00	74
					1,2,3,4,7,8,9-HpCDF-13C	2.00	61
1,2,3,4,7,8-HxCDF	ND	-----	0.070		1,2,3,4,6,7,8-HpCDD-13C	2.00	68
1,2,3,6,7,8-HxCDF	ND	-----	0.100		OCDD-13C	4.00	67
2,3,4,6,7,8-HxCDF	ND	-----	0.100				
1,2,3,7,8,9-HxCDF	ND	-----	0.100		1,2,3,4-TCDD-13C	2.00	NA
Total HxCDF	0.50	-----	0.094	BJ	1,2,3,7,8,9-HxCDD-13C	2.00	NA
1,2,3,4,7,8-HxCDD	ND	-----	0.140		2,3,7,8-TCDD-37Cl4	0.20	88
1,2,3,6,7,8-HxCDD	0.60	-----	0.150	J			
1,2,3,7,8,9-HxCDD	ND	-----	0.140				
Total HxCDD	2.10	-----	0.140	BJ			
1,2,3,4,6,7,8-HpCDF	-----	0.62	0.180	I	Total 2,3,7,8-TCDD		
1,2,3,4,7,8,9-HpCDF	ND	-----	0.250		Equivalence: 0.76 ng/Kg		
Total HpCDF	ND	-----	0.220		(Using WHO Factors - Using LOD where ND)		
1,2,3,4,6,7,8-HpCDD	17.00	-----	0.440				
Total HpCDD	29.00	-----	0.440				
OCDF	2.50	-----	0.170	BJ			
OCDD	350.00	-----	0.190				

Results reported on a total weight basis

Conc = Concentration (Totals include 2,3,7,8-substituted isomers).
EMPC = Estimated Maximum Possible Concentration
LOD = Limit of Detection. Totals are averages of individual isomer LODs.
D = Result obtained from analysis of diluted sample
B = Less than 10 times higher than method blank level
P = Recovery outside of method 1613 control limits
J = Concentration detected is below the calibration range
Nn = Value obtained from additional analysis

I = Interference
E = PCDE Interference
ND = Not Detected
NA = Not Applicable
NC = Not Calculated
* = See Discussion

Report No.....1023564

REPORT OF LABORATORY ANALYSIS

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Pace Analytical Services, Inc.
1700 Elm Street - Suite 200
Minneapolis, MN 55414

Tel: 612-607-1700
Fax: 612- 607-6444

Pace Analytical™

2,3,7,8-TCDD Toxic Equivalency (TEQ) Calculations

Twin Rivers Technologies

Client's Sample ID	N3TG-50406-5280		
Lab Sample ID	1023564001		
Filename	F51129A_11		
Injected By	MRO		
Total Amount Extracted	10.1 g	Matrix	Oil
% Moisture	NA	Dilution	NA
Dry Weight Extracted	NA	Collected	NA
ICAL Date	10/22/2005	Received	11/17/2005
CCal Filename(s)	F51129A_02	Extracted	11/24/2005
Method Blank ID	BLANK-8347	Analyzed	11/29/2005 19:24

Parameter	Conc ng/Kg	RL ng/Kg	WHO	LB	MB	UB
2,3,7,8-TCDF	ND	0.093	0.10000	0.0000	0.0046	0.0093
Total TCDF	0.21	0.093	0.00000	0.0000	0.0000	0.0000
2,3,7,8-TCDD	ND	0.10	1.00000	0.0000	0.0524	0.1048
Total TCDD	ND	0.10	0.00000	0.0000	0.0000	0.0000
1,2,3,7,8-PeCDF	ND	0.15	0.05000	0.0000	0.0036	0.0073
2,3,4,7,8-PeCDF	ND	0.15	0.50000	0.0000	0.0374	0.0748
Total PeCDF	ND	0.15	0.00000	0.0000	0.0000	0.0000
1,2,3,7,8-PeCDD	ND	0.23	1.00000	0.0000	0.1141	0.2283
Total PeCDD	0.42	0.23	0.00000	0.0000	0.0000	0.0000
1,2,3,4,7,8-HxCDF	ND	0.070	0.10000	0.0000	0.0035	0.0070
1,2,3,6,7,8-HxCDF	ND	0.10	0.10000	0.0000	0.0050	0.0100
2,3,4,6,7,8-HxCDF	ND	0.10	0.10000	0.0000	0.0052	0.0105
1,2,3,7,8,9-HxCDF	ND	0.10	0.10000	0.0000	0.0051	0.0102
Total HxCDF	0.50	0.094	0.00000	0.0000	0.0000	0.0000
1,2,3,4,7,8-HxCDD	ND	0.14	0.10000	0.0000	0.0068	0.0136
1,2,3,6,7,8-HxCDD	0.60	0.15	0.10000	0.0600	0.0600	0.0600
1,2,3,7,8,9-HxCDD	ND	0.14	0.10000	0.0000	0.0068	0.0137
Total HxCDD	2.10	0.14	0.00000	0.0000	0.0000	0.0000
1,2,3,4,6,7,8-HpCDF	ND	0.18	0.01000	0.0000	0.0009	0.0018
1,2,3,4,7,8,9-HpCDF	ND	0.25	0.01000	0.0000	0.0013	0.0025
Total HpCDF	ND	0.22	0.00000	0.0000	0.0000	0.0000
1,2,3,4,6,7,8-HpCDD	17.00	0.44	0.01000	0.1686	0.1686	0.1686
Total HpCDD	29.00	0.44	0.00000	0.0000	0.0000	0.0000
OCDF	2.50	0.17	0.00010	0.0002	0.0002	0.0002
OCDD	350.00	0.19	0.00010	0.0345	0.0345	0.0345
				0.26 ng/Kg	**0.51 ng/Kg**	**0.76 ng/Kg**

Final values are valid to only 2 significant figures
TEQs for Totals classes include contributions from non 2,3,7,8 isomers only
LB = Lower Bound, Where "ND", TEQ Conc = 0
MB = Medium Bound, Where "ND", TEQ Conc = (LOD/2) * (TEF Factor)
UB = Upper Bound, Where "ND", TEQ Conc = LOD * (TEF Factor)
RL = Reporting Limit

Report No.....1023564

REPORT OF LABORATORY ANALYSIS

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Pace Analytical Services, Inc.
1700 Elm Street - Suite 200
Minneapolis, MN 55414

Tel: 612-607-1700
Fax: 612-607-6444

Method 1613B Laboratory Control Spike Results

Client - Twin Rivers Technologies

Lab Sample ID	LCS-8348		
Filename	F51130A_02	Matrix	Oil
Total Amount Extracted	10.0 g	Dilution	NA
ICAL Date	10/22/2005	Extracted	11/24/2005
CCal Filename	F51129B_15	Analyzed	11/30/2005 15:11
Method Blank ID	BLANK-8347	Injected By	MRO

Compound	Cs	Cr	Lower Limit	Upper Limit	% Rec.
2,3,7,8-TCDF	10	10.4	7.5	15.8	104
2,3,7,8-TCDD	10	10.2	6.7	15.8	102
1,2,3,7,8-PeCDF	50	57.2	40.0	67.0	114
2,3,4,7,8-PeCDF	50	51.5	34.0	80.0	103
1,2,3,7,8-PeCDD	50	46.5	35.0	71.0	93
1,2,3,4,7,8-HxCDF	50	48.7	36.0	67.0	97
1,2,3,6,7,8-HxCDF	50	53.6	42.0	65.0	107
2,3,4,6,7,8-HxCDF	50	51.7	35.0	78.0	103
1,2,3,7,8,9-HxCDF	50	50.1	39.0	65.0	100
1,2,3,4,7,8-HxCDD	50	53.3	35.0	82.0	107
1,2,3,6,7,8-HxCDD	50	55.2	38.0	67.0	110
1,2,3,7,8,9-HxCDD	50	51.6	32.0	81.0	103
1,2,3,4,6,7,8-HpCDF	50	53.9	41.0	61.0	108
1,2,3,4,7,8,9-HpCDF	50	55.5	39.0	69.0	111
1,2,3,4,6,7,8-HpCDD	50	49.1	35.0	70.0	98
OCDF	100	103.7	63.0	170.0	104
OCDD	100	105.1	78.0	144.0	105
2,3,7,8-TCDD-37Cl4	10	9.7	3.1	19.1	97
2,3,7,8-TCDF-13C	100	88.4	22.0	152.0	88
2,3,7,8-TCDD-13C	100	95.7	20.0	175.0	96
1,2,3,7,8-PeCDF-13C	100	107.7	21.0	192.0	108
2,3,4,7,8-PeCDF-13C	100	100.1	13.0	328.0	100
1,2,3,7,8-PeCDD-13C	100	116.9	21.0	227.0	117
1,2,3,4,7,8-HxCDF-13C	100	101.3	19.0	202.0	101
1,2,3,6,7,8-HxCDF-13C	100	93.5	21.0	159.0	94
2,3,4,6,7,8-HxCDF-13C	100	91.5	22.0	176.0	92
1,2,3,7,8,9-HxCDF-13C	100	94.7	17.0	205.0	95
1,2,3,4,7,8-HxCDD-13C	100	98.0	21.0	193.0	98
1,2,3,6,7,8-HxCDD-13C	100	87.8	25.0	163.0	88
1,2,3,4,6,7,8-HpCDF-13C	100	87.2	21.0	158.0	87
1,2,3,4,7,8,9-HpCDF-13C	100	73.2	20.0	186.0	73
1,2,3,4,6,7,8-HpCDD-13C	100	85.5	26.0	166.0	85
OCDD-13C	200	142.4	26.0	397.0	71

Cs = Concentration Spiked (ng/mL)
Cr = Concentration Recovered (ng/mL)
Rec. = Recovery (Expressed as Percent)
Control Limit Reference: Method 1613, Table 6, 10/94 Revision
X = Background subtracted value
P = Recovery outside of control limits
Nn = Value obtained from additional analysis
* = See Discussion

Report No.....1023564

REPORT OF LABORATORY ANALYSIS

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D IVERSIFIED L ABORATORIES, I NC.

3810 CONCORDE PARKWAY * CHANTILLY, VIRGINIA 20151 * (703) 222-8700

Certificate of Analysis

Submitted by: TWIN RIVERS TECHNOLOGIES, INC. - CINCINNATI, OHIO
Sample: PO #N004546 N3TG-50406-5280 11/16/2005
Date received: 11/17/2005
Analyses requested: PESTICIDE/PCB SCREEN (ppm)

alpha-BHC:	N.D.	PCNB:	N.D.
beta-BHC:	N.D.	CHLOROBENSIDE:	N.D.
delta-BHC:	N.D.	CHLORTHALONIL:	N.D.
gamma-BHC:	N.D.	CHLORPYRIFOS:	N.D.
TOTAL BHC:	N.D.	METHYL CHLORPYRIFOS:	N.D.
p,p'-DDT:	N.D.	CHLORPYRIFOS METHYL OXYGEN ANALOG:	N.D.
p,p'-DDD	N.D.	CARBOPHENOTHION:	N.D.
p,p'-DDE:	N.D.	ETHION:	N.D.
o,p'-DDT:	N.D.	PARATHION:	N.D.
o,p'-DDD:	N.D.	RONNEL:	N.D.
o,p'-DDE:	N.D.	ENDOSULFAN I:	N.D.
TOTAL DDT:	N.D.	FONOPHOS:	N.D.
ALDRIN:	N.D.	alpha-CHLORDANE:	N.D.
DIELDRIN:	N.D.	alpha-CHLORDENE:	N.D.
HEXACHLOROBENZENE:	N.D.	cis-NONACHLOR:	N.D.
ENDRIN:	N.D.	gamma-CHLORDANE:	N.D.
ENDRIN ALDEHYDE:	N.D.	gamma-CHLORDENE:	N.D.
ENDRIN KETONE:	N.D.	trans-NONACHLOR:	N.D.
HEPTACHLOR:	N.D.	OXYCHLORDANE:	N.D.
HEPTACHLOR EPOXIDE:	N.D.	ENDOSULFAN II:	N.D.
TOXAPHENE:	N.D.	ENDOSULFAN SULFATE:	N.D.
STROBANE:	N.D.	CAPTAN:	N.D.
TOTAL CHLORDANE:	N.D.	o,p'-METHOXYCHLOR:	N.D.
METHOXYCHLOR:	N.D.	PERMETHRIN:	N.D.
MIREX:	N.D.	POLYCHORINATED BIPHENYLS (PCB's):	N.D.
PERTHANE:	N.D.		

17 ADDITIONAL CHLORINATED HYDROCARBONS, PARTIALLY OR TENTATIVELY IDENTIFIED N.D.

Date Sent: 11/18/2005

000070

Control Number 54145

Director of Laboratory Services

"Service Through Science"

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D IVERSIFIED L ABORATORIES, I NC.

3810 CONCORDE PARKWAY * CHANTILLY, VIRGINIA 20151 * (703) 222-8700

Certificate of Analysis

Submitted by: TWIN RIVERS TECHNOLOGIES, INC. - CINCINNATI, OHIO
Sample: PO #N004546 N3TG-50406-5280 11/16/2005
Date received: 11/17/2005
Analyses requested: ORGANO-PHOSPHATED PESTICIDE SCREEN (ppm)
(CALCULATED ON A WHOLE MATTER BASIS)

PHORATE:	N.D.	CARBOPHENOTHION:	N.D.
DISULFETON (DISYSTON):	N.D.	FONOPHOS (DYFONATE):	N.D.
MALATHION:	N.D.	METHYL CHLORPYRIFOS:	N.D.
DIAZINON:	N.D.	CHLORPYRIFOS (DURSBAN):	N.D.
METHYL PARATHION:	N.D.	CHLORPYRIFOS, METHYL OXYGEN ANALOG:	N.D.
RONNEL (FENCHLORPHOS):	N.D.	TETRACHLORVINPHOS:	N.D.
PARATHION:	N.D.	CHLORFENVINPHOS:	N.D.
ETHION:	N.D.	TOKUTHION:	N.D. MET
CHLORTHAL:	N.D.		

000072

Date Sent: 11/30/2005

Control Number 54145

Director of Laboratory Services

SUBMISSION END

AM

FAX Transmission

AAC CONSULTING GROUP, INC.
7361 Calhoun Place
Suite 500
Rockville, MD 20855-2765

TO:	Carrie Hendrickson	
COMPANY:	FDA	
FAX:	301.436.1202	
PHONE:		Pages: 2, including cover
FROM:	Kathie Trivelli	
CALL: FAX:	301-838-3120 x129 301-838-3182	
DATE:	June 13, 2006	
RE:	Submission of GRAS Notification – Omega-3 Triglycerides	

MESSAGE:

Dear Carrie:

Attached please find the attachment indicated on page 40 of the Expert Panel Statement, for the above-referenced Submission. The original will follow via regular 1st class mail.

If you have any questions, please feel free to contact me.

000078

Twin Rivers Technologies Omega-3 Process Concept

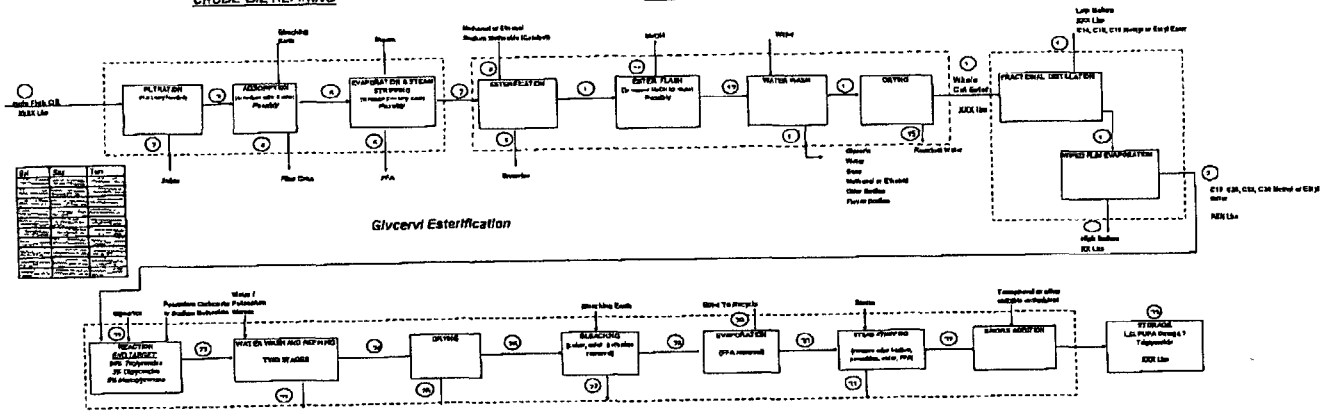
CONFIDENTIAL

CONFIDENTIAL

CRUDE OIL REFINING

ESTER - MAKING

Fractional Distillation



RECEIVED
2 / 2006
OFFICE OF FOOD ADDITIVE SAFETY

REVISED COVER LETTER #2

June 13, 2006

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

AAC
CONSULTING GROUP

7361 Calhoun Place,
Suite 500
Rockville, Maryland 20855-2765
301.838.3120
fax: 301.838.3182

RE: Submission of GRAS Notification – Omega-3 Triglyceride Concentrate ***(N-3-TG-3020)*****

***** PLEASE BE ADVISED THAT THE ORIGINAL COVER LETTER SUBMITTED ON May 26, 2006 contained a typographical error in the "RE:" line. It read (N-3-TG-2030). It should have read* (N-3-TG-3020). *A copy of the original date-stamped cover is enclosed. Also enclosed is a copy of this Revised Cover Letter. Please date-stamp the copy of this Revised Cover Letter and return in the enclosed, self-addressed envelope. Thank you.***

Dear Sir/Madame:

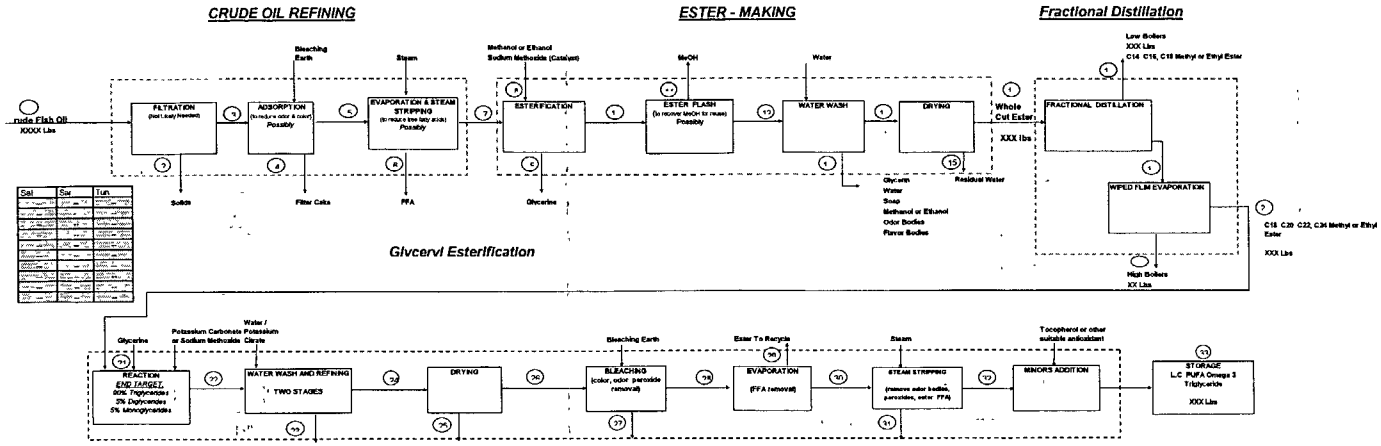
In accordance with proposed 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the agent to the notifier, Twin Rivers Technologies, a GRAS Notification of OMEGA-3 Triglyceride Concentrate (N-3-TG-3020). Also enclosed is a GRAS panel report setting forth the basis for the GRAS determination.

Please let me know if you have any questions.

Sincerely,

(b)(6)

Edward A. Steele
President



BEST ORIGINAL COPY

000081

Hendrickson, Carrie

From: Stanley Tarka [tarkagroup@comcast.net]
Sent: Wednesday, September 27, 2006 9:56 AM
To: Hendrickson, Carrie
Cc: Edwards, Alison; tarkagroup@comcast.net
Subject: RE: questions regarding GRAS notice (GRN 200)
Importance: High
Attachments: Twin Rivers Technologies GRAS Notice December 30 2005 Final Specs pp 14 Sept 25 2006.doc; Response to FDA Questions Regarding GRAS Notice 00200 Sept 27 2006.doc; EXPERT PANEL STATEMENT Omega-3-TG TRR Dec 30 2005 Specifications Revised Sept 27 2006.doc

AM

Dear Drs. Hendrickson and Edwards,

Please find attached to this email our response to each of the questions raised on GRN 00200. Also, I have attached revised specifications that reflect our latest learnings and to be competitive in the marketplace. These are noted on pages 12 and 14 of the Specifications and have also been reflect in the Expert Panel Statement. I have also removed Confidential from the manufacturing process.

Please feel free to contact me if you have any additional questions.

Thank you.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr. Ph.D.
Ph: 717-243-9216
Fax: 702-993-5458
Email: Tarkagroup@comcast.net

From: Hendrickson, Carrie [mailto:carrie.hendrickson@fda.hhs.gov]
Sent: Thursday, September 21, 2006 12:04 PM
To: 'tarkagroup@comcast.net'
Subject: FW: questions regarding GRAS notice (GRN 200)

Dr. Tarka -

Please confirm that you receive the email that I sent on Tuesday (September 19, 2006) requesting clarification/explanation of a few points in the Twin Rivers Technologies GRAS notice. I will be out of the office Friday and Monday and I want to be sure that you did get this information.

Thank you.

Carrie

Carrie Hendrickson, Ph.D.
Consumer Safety Officer

U.S. Food and Drug Administration
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review

9/27/2006

Contact information
tel: (301)436-1202
email: Carrie.Hendrickson@fda.hhs.gov

From: Hendrickson, Carrie
Sent: Tuesday, September 19, 2006 11:10 AM
To: 'tarkagroup@comcast.net'
Subject: questions regarding GRAS notice (GRN 200)

Dr. Tarka

We have a few questions/points of clarification regarding Twin Rivers Technologies GRAS notice for omega-3 triglyceride concentrate (which we have designated at GRN 000200):

1. In the notice (either on p. 10 or the confidential flow diagram), you describe the filtration, bleaching, and steam stripping of the crude fish oil as "potential" or "possible" steps. Please provide clarification. Does this mean that individual steps may be eliminated if not required or that crude oil may be used directly (without refining) as a starting material for the fatty acid esterification process?

2. In your notice, you describe use of methanol or ethanol in the first esterification step and the use of sodium methoxide in the first and second esterification steps. Please address the possibility of methanol ethanol residues as these relate to the safety of the finished, food grade concentrate. Is methanol detected in the final product? What is the limit of detection? Please describe the ability of the method of manufacture to remove methanol residues.

3. You provided a copy of your method of manufacture flow diagram; you indicate that the entire page is confidential. OFAS notes that much of the information in the process flow diagram is discussed in narrative form on pages 10-11 and 20-21. The information in the narrative is relevant to the GRAS determination, and confidentiality of this information would not be consistent with the GRAS program. Please clarify what details are specifically confidential, with consideration of your GRAS determination and the information an "expert" would need to know about the method of manufacture to make a determination of safety.

4. It's our understanding that all ingredients (including processing aids) used in the manufacture of your ingredient are suitable for food use. Is this correct? (A clear statement of such is relevant to both your GRAS determination and our ability to evaluate your determination.)

5. In the present notice, the acid value specification is given as NMT 1%. The acid value is generally expressed in units of mg KOH/g of oil. Does the specification in the notice refer to an acid value of 10 mg KOH/g of oil or a free fatty acid value of 1% (or 0.5% by calculation)? Note: Either value would be greater than the CRN specification (acid value maximum 3 mg KOH/g) for fish oils or the CODEX specification (acid value maximum 0.6 mg KOH/g fat) for refined oils. Please clarify the specification for acid value given in the notice.

**we suggest that you also review your notice for other areas where a clearer statement of the units of measure (e.g., percent *of/by what*; mg *of what*) would minimize the possibility of misinterpretation of the information provided in the notice.

Please contact me if you need any clarification.

Carrie Hendrickson, Ph.D.
Consumer Safety Officer

U.S. Food and Drug Administration
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review

Contact information
tel: (301)436-1202
email: Carrie.Hendrickson@fda.hhs.gov

Kendle

Dr.Carrie Hendrickson-Consumer Safety Officer 9/25/06
Dr. Alison Edwards-Chemistry Reviewer
U.S. Food and Drug Administration
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review
Phone: 301-436-1202

By E-mail to Carrie.Hendrickson@fda.hhs.gov

By Email: to Alison.Edwards@fda.hhs.gov

Reference: Questions Regarding GRAS Notice (GRN 200)

Dear Drs. Hendrickson and Edwards:

This letter is a follow-up to your email dated September 19, 2006 and the questions /points of clarification requested regarding Twin Rivers Technologies GRAS notice for omega-3 triglyceride concentrate (designated GRN 000200). I will address each question below as it has been presented.

1. In the notice (either on p. 10 or the confidential flow diagram), you describe the filtration, bleaching, and steam stripping of the crude fish oil as "potential" or "possible" steps. Please provide clarification. Does this mean that individual steps may be eliminated if not required or that crude oil may be used directly (without refining) as a starting material for the fatty acid esterification process?

RESPONSE: The use of the words "potential" or "possible" does mean that individual steps may be eliminated if not required in order to meet either our internal specifications or individual customer specifications that may evolve over time. In our manufacturing process, crude oil is able to be used as a starting material, without filtration, bleaching and stripping before further processing, because, by-default we subject the product stream to these refining steps at the end of the process, if not at the beginning. We will always perform these refining steps at the end of our process. Consequently, use of these refining steps at the beginning of the process would be additional to the use of refining steps at the end of the

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process. This would be done only if we find a need for such redundant processing for the purpose of commercial advantage that may provide market differentiation or to meet certain customer requirements.

2. In your notice, you describe use of methanol or ethanol in the first esterification step and the use of sodium methoxide in the first and second esterification steps. Please address the possibility of methanol ethanol residues as these relate to the safety of the finished, food grade concentrate. Is methanol detected in the final product? What is the limit of detection? Please describe the ability of the method of manufacture to remove methanol residues.

RESPONSE: The process, through wiped film evaporation and steam stripping after the glyceryl esterification reaction, intentionally removes methanol/ethanol residues to the low parts per million levels, far below amounts reported in many foodstuffs. For example, the methanol content of fresh canned fruit juices (principally orange and grapefruit juices) varies considerably with ranges from 10- 640 mg/liter with an average of 140 mg/liter. In an example, of a typical serving size for orange juice of 6 ounces (200 ml) and a methanol content of 500 mg/liter then the calculated consumption would be 100mg of methanol in a 6 ounce serving. Elevated levels of methanol from the cooking and canning of high pectin foods are also well documented. Fermented and distilled beverages can also contain higher levels of methanol with some neutral spirits having as much as 1.5 g/liter.

Specifications have been established to limit the presence of methanol and to non-objectionable levels. This employs the use of two analytical methods for the detection and quantification of methanol/ethanol residues in the finished product. The first test serves the purpose of measuring Low Level Methyl Ester (LLME) residues and is run on all batches. The finished product release limit is 1000 ppm maximum, LLME.

The second test measures total available methanol (TAME), including both free and bound methanol This test is run every 6 months or every 10th lot – whichever comes first. The limit for TAME is 300 ppm maximum.The Limit of Detection is 30 ppm and 50 ppm for the LLME method and TAME method, respectively.

3. You provided a copy of your method of manufacture flow diagram; you indicate that the entire page is confidential. OFAS notes that much of the information in the process flow diagram is discussed in narrative form on pages 10-11 and 20-21. The information in the narrative is relevant to the GRAS determination, and confidentiality of this information would not be consistent with the GRAS program. Please clarify what details are specifically confidential, with consideration of your GRAS determination and the information an "expert" would need to know about the method of manufacture to make a determination of safety.

RESPONSE: In order to provide consistency and ease of understanding, we have decided to remove **Confidential** from the entire page of the manufacture flow diagram.

4. It's our understanding that all ingredients (including processing aids) used in the manufacture of your ingredient are suitable for food use. Is this correct? (A clear statement of such is relevant to both your GRAS determination and our ability to evaluate your determination.)

RESPONSE: The FDA's understanding that all ingredients used in the manufacture of our product are suitable for food use is correct, with the possible exception of the crude fish oil raw material. As noted in the response to question #1 above, crude fish oil is converted in the process through use of several substantial purification (refining) steps into a refined oil, suitable for food use. This conversion from inedible "crude" oil into "edible" refined oil through use of physical and/or chemical oil refining steps is a well-established practice in the food oils and shortenings industries. A good example of such a refined oil can be found in the *Food Chemical Codex*, Fifth edition, for the monograph on Menhaden Oil, Refined. The description provided is as follows: Menhaden Oil, Refined, is prepared from fish of the genus *Brevoortia*, commonly known as menhaden, by cooking, pressing, and refining. Winterization may separate the oil and produce a solid fraction.

5. In the present notice, the acid value specification is given as NMT 1%. The acid value is generally expressed in units of mg KOH/g of oil. Does the specification in the notice refer to an acid value of 10 mg KOH/g of oil or a free fatty acid value of 1% (or 0.5% by calculation)? Note: Either

value would be greater than the CRN specification (acid value maximum 3 mg KOH/g) for fish oils or the CODEX specification (acid value maximum 0.6 mg KOH/g fat) for refined oils. Please clarify the specification for acid value given in the notice.

RESPONSE: Upon review of the above question we understand the inadvertent confusion in the presented Acid Value specification. Acid Value is never reported in units of measure (UOM) of "Percentages", and is always reported in UOM of mg KOH per gram of sample. Conversely, Free Fatty Acid results are always reported in UOM of "Percentage" FFA, and never in UOM of mg KOH per g sample. Our intent in the original text was to specify that our finished product conforms to the CRN specification of a maximum Acid Value of 3 mg KOH per gm. sample.

NOTE: Regarding your footnote: **we suggest that you also review your notice for other areas where a clearer statement of the units of measure (e.g., percent *of/by what*; mg *of what*) would minimize the possibility of misinterpretation of the information provided in the notice.

RESPONSE: We have examined all other units of measure and they are consistent with presentation of the respective information.

Also, I am attaching a revised Specification that more accurately reflects the most recent learnings from the marketplace in which we must compete. I have highlighted the changes made in red for ease in identifying what has changed. This is Page 14 of the GRAS Notification and Page 19 of the Expert Panel Statement.

I trust that this adequately addresses the questions posed and we look forward to the agency's decision on this GRAS notification. Should you have any additional questions, please feel free to contact me at you r earliest convenience at 717-243-9216 or via email at tarkagroup@comcast.net

Thank you.

000092

Sincerely,

Stanley M. Tarka, Jr., PhD.
Senior Consulting Toxicologist

000093

AAC
CONSULTING GROUP

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N-3-TG-3020)

I. GRAS Exemption Claim

A. Claim of Exemption From The Requirement for Premarket Approval Requirements Pursuant to Proposed CFR § 170.36(c)(1)

Omega-3 Triglyceride concentrate (N-3-TG-3020) has been determined to be generally recognized as safe, and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

_____ Date_____

Edward A. Steele

Agent for:

Twin Rivers Technologies
780 Washington Street
Quincy, MA 02169

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

B. Name and Address of Notifier

Edward A. Steele
President
AAC Consulting Group
7361 Calhoun Place, Suite 500
Rockville, MD 20855-2765
Telephone: 301-838-3182
Facsimile: 301-838-3182

C. Common or Usual Name of the Notified Substance and Identity of GRAS Substance

The subject that has been determined to be Generally Recognized as Safe (GRAS) and thus is the subject of this GRAS notification is a product derived from fish oil called "N3-TG-3020 Omega-3 Triglyceride Concentrate." This product is manufactured by Twin Rivers Technologies. N3-TG-3020 Omega-3 Triglyceride Concentrate is composed primarily of triglycerides, with lesser amounts of diglycerides. The glycerides making up N3-TG-3020 Omega-3 Triglyceride Concentrate contain a higher proportion of polyunsaturated fatty acids than the fish oil employed as the starting material, with the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) predominating at about 32 percent and 23 percent, respectively, yielding a combined EPA and DHA content of approximately 55 percent by weight. The fish oil employed as the starting material in the production of N3-TG-3020 Omega-3 Triglyceride Concentrate is extracted from multiple edible fish species including salmon, menhaden, anchovy, sardine, jack mackerel, mackerel and tuna.

N3-TG-3020 Omega-3 Triglyceride Concentrate is derived from fish oil by Twin Rivers Technologies employing a proprietary process for the production of a concentrated omega-3 fatty acid triglyceride containing substantially increased levels of EPA and DHA above those found in naturally occurring fish oils. It is described in detail in the section below titled Method of Manufacture. This process can be contrasted with other industrial fish-oil based practices for processing of marine oils into a refined fish oil concentrate.

D. Conditions of Use and Consumer Exposure

Twin Rivers Technologies intends to market N3-TG-3020 Omega-3 Triglyceride Concentrate for addition to food as a nutrient supplement to increase the dietary intake of the two omega-3 fatty acids, EPA and DHA, and as an alternative to other food-grade oils. N3-TG-3020 Omega-3 Triglyceride Concentrate will be added to the same categories of foods at concentrations providing the same combined EPA and DHA content as are permitted for menhaden under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). Further, N3-TG-3020 Omega-3 Triglyceride Concentrate will be used as the sole source of EPA and DHA in any given food category of this regulation and will not be combined or augmented with any other EPA/DHA-rich oil in making a food product.
The maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55

percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36 percent of that of menhaden oil. These proposed uses are presented in Table 1. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions (GRN Nos. 000102, 000105, 000109, 000138 and 000146) including one for a marine oil concentrate with similar increased EPA and DHA levels (GRN No. 000105). Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate.

The estimated mean intake of EPA and DHA combined from all dietary sources in the general population, excluding infants under the age of one year, has been addressed in earlier FDA rulemaking (menhaden oil final rule; 62 FR 30751; June 5, 1997) where FDA estimated that the mean exposure to EPA and DHA from the use of menhaden oil in all food categories would be 2.8 grams/person/day. It was also addressed as well in the subsequent GRAS Notices referenced above. The total cumulative estimated mean intake of EPA and DHA combined from the proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate listed in the foods in Table 1, as well as from all other dietary sources in this general population is also estimated to be 2.8 grams per person per day. This estimate reflects 100 percent market penetration of the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate that are listed in Table 1. Because 100 percent market penetration of this product is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower. Further, this cumulative intake of EPA and DHA combined is still less than the safe limit for EPA and DHA combined of 3 grams per person per day established by the agency. The proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate will result in no incremental increase in the intake of EPA and DHA combined over that described for menhaden oil in the final rule Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005. **[§184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day. (4) To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid].**

E. Basis for the GRAS Determination

The GRAS determination for N3-TG-3020 Omega-3 Triglyceride Concentrate under the proposed maximum use levels listed in Table 1 is based on scientific procedures as described under Title 21 of the Code of Federal Regulations (21CFR)§170.30(b). These scientific procedures have been used to demonstrate that the estimated intake of N3-TG-3020 Omega-3 Concentrate from the intended uses specified in Table 1, in conjunction with intakes of EPA and DHA combined from other dietary sources, is safe, and also GRAS under the Food, Drug, and Cosmetic Act (FDCA). FDA has already established that a combined intake of EPA and DHA of

less than 3 grams per person per day is a safe level of intake. In addition, this cumulative intake of EPA and DHA combined was also determined to be GRAS and the safety of this level of intake is generally recognized by experts qualified by both training and experience to evaluate the safety of substances directly or indirectly added to food, and is also based on generally available and accepted information.

Table 1. Proposed Maximum Use Levels in Food as Served of Omega-3 Triglyceride Concentrate (N3-TG-3020) Compared with Current Menhaden Oil Uses Permitted under 21CFR184.1472 and Final Rule Published in FR March 23, 2005(Volume 70, Number 55 PP14530-14532)

Category of food (and 21CFR §170.3(n) paragraph)	Maximum Level of Use of Menhaden Oil under 21CFR184.1472 and FR Vol 70 No 55 March 23 2005 Final Rule	Proposed Maximum Level of Use of N3-TG-3020 Omega-3 Triglyceride Concentrate[a]
Baked goods, baking mixes, §170.3(n)(1)	5.0 percent	1.8 percent
Cereals, §170.3(n)(4)	4.0 percent	1.4 percent
Cheese products, §170.3(n)(5)	5.0 percent	1.8 percent
Chewing gum, §170.3(n)(6)	3.0 percent	1.1 percent
Condiments, §170.3(n)(8)	5.0 percent	1.8 percent
Confections, frostings, §170.3(n)(9)	5.0 percent	1.8 percent
Dairy product analogs, §170.3(n)(10)	5.0 percent	1.8 percent
Egg products, §170.3(n)(11)	5.0 percent	1.8 percent
Fats, oils, §170.3(n)(12), but not in infant formula	12.0 percent	4.3 percent
Fish products, §170.3(n)(13)	5.0 percent	1.8 percent
Frozen desserts, §170.3(n)(20)	5.0 percent	1.8 percent
Gelatins, puddings, §170.3(n)(22)	1.0 percent	0.36 percent
Gravies, sauces, §170.3(n)(24)	5.0 percent	1.8 percent
Hard candy, §170.3(n)(25)	10.0 percent	3.6 percent
Jams, jellies, §170.3(n)(28)	7.0 percent	2.5 percent
Meat products, §170.3(n)(29)	5.0 percent	1.8 percent
Milk products, §170.3(n)(31)	5.0 percent	1.8 percent
Nonalcoholic beverages, §170.3(n)(3)	0.5 percent	0.18 percent
Nut products, §170.3(n)(32)	5.0 percent	1.8 percent
Pastas, §170.3(n)(23)	2.0 percent	0.7 percent

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

Table 1. Proposed Maximum Use Levels in Food as Served of Omega-3 Triglyceride Concentrate (N3-TG-3020) Compared with Current Menhaden Oil Uses Permitted under 21CFR184.1472 and Final Rule Published in FR March 23, 2005(Volume 70, Number 55 PP14530-14532)

Category of food (and 21CFR §170.3(n) paragraph)	Maximum Level of Use of Menhaden Oil under 21CFR184.1472 and FR Vol 70 No 55 March 23 2005 Final Rule	Proposed Maximum Level of Use of N3-TG-3020 Omega-3 Triglyceride Concentrate[a]
Plant protein products, §170.3(n)(33)	5.0 percent	1.8 percent
Poultry products, §170.3(n)(34)	3.0 percent	1.1 percent
Processed fruit juices, §170.3(n)(35)	1.0 percent	0.36 percent
Processed vegetable juices, §170.3(n)(36)	1.0 percent	0.36 percent
Snack foods, §170.3(n)(37)	5.0 percent	1.8 percent
Soft candy, §170.3(n)(38)	4.0 percent	1.4 percent
Soup mixes, §170.3(n)(40)	3.0 percent	1.1 percent
Sugar substitutes, §170.3(n)(42)	10.0 percent	3.6 percent
Sweet sauces, toppings, syrups, §170.3(n)(43)	5.0 percent	1.8 percent
White granulated sugar, §170.3(n)(41)	4.0 percent	1.4 percent

[a]Proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532) are set to provide the same concentrations of EPA+DHA as those provided by menhaden oil; based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate

The safety of consumption of N3-TG-3020 Omega-3 Concentrate for use as an ingredient in food is based on the similarity of this product's composition to other currently marketed fish-oil derived products including an Omega-3 Concentrate, as well as the safety of ingestion of its two major fatty acid constituents, EPA and DHA. Safety of consumption of the whole product was determined by evaluating the source of the product, the production process, the nature and quantity of impurities, product specifications, and the identity and positional distributions of EPA and DHA within the glycerides that comprise the final product. Appropriate specifications have been established to ensure that the final product is food grade, and compositional analysis of the product supports the presumption that there is no toxicological concern from the ingestion of any product impurities.

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

The EPA and DHA levels in the Omega-3 Triglyceride Concentrate (N3-TG-3020) are increased above those found in naturally occurring fish oils by a very selective process involving separation of different fatty acid chain lengths from fish oils via either methylation or ethylation into fatty methyl or ethyl esters followed by distilling the short chain, non-omega-3 methyl or ethyl esters away from the longer chain methyl or ethyl esters and then re-esterifying the longer chain methyl or ethyl esters with glycerine to form a triglyceride, whose chain lengths are now concentrated in omega-3 content. Analysis of the triglycerides that comprise the Omega-3 Triglyceride Concentrate (N3-TG-3020) is presented in Table 2 and compared to menhaden oil. The results indicate that these glycerides are structurally unchanged and their positional distributions within the glyceride are comparable to the starting fish oil. Based on this similarity, Omega-3 Triglyceride Concentrate (N3-TG-3020) would be expected to be metabolized by the body in a similar manner as naturally occurring fish oil.

Table 2. Representative Fatty Acid Profile for N3-TG-3020 Omega-3 Triglyceride Concentrate

	Menhaden oil	**N3-TG-3020**
Fatty Acid	**Amount (in relative area percent)**	
C12:0	0.107	
C14:0	8.704	0.304
C14:1		
C15:0		
C16:0	18.779	2.663
C16:1	12.011	1.454
C16:4N3		
C17:0	2.280	0.241
C17:1	0.760	0.274
C18:0	3.233	3.077
C18:1N11	6.977	4.463
C18:1N9	2.883	2.770
C18:2	1.249	1.543
C18:3N6	0.502	0.397
C19:0	0.371	0.238
C19:1	0.644	0.257
C18:3N3	1.148	0.824
C18:4N3	2.039	1.576
C20:0	0.269	1.017
C20:1	1.174	1.573
C20:2	0.161	0.286
C20:3N6	0.312	0.511
C20:4N6	1.423	4.838
C20:3N3	0.140	0.251
C20:4N3	1.292	1.929
C20:5N3 (EPA)	12.742	30.289

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

	Menhaden oil	N3-TG-3020
Fatty Acid	**Amount (in relative area percent)**	
C22:1N11	0.295	0.808
C22:1N9	0.236	2.311
C21:5N3	0.725	1.440
C22:4N6	0.393	0.778
C24	0.429	0.721
C22:5N3	2.822	4.709
C22:6N3 (DHA)	8.898	21.020
Unidentified	7.003	7.434

In affirming the GRAS status of menhaden oil under 21CFR§184.1472, the FDA established that a daily intake of EPA and DHA combined not exceeding 3 grams per person per day is safe. The scientific basis to support the establishment of this safe level of intake has been published by the FDA. In 1997, FDA affirmed, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use to ensure that the total daily intake of EPA and DHA would not exceed 3.0 grams per person per day (g/p/d) (62 FR 30751; June 5, 1997; 21 CFR184.1472). EPA and DHA are the major omega-3 fatty acids in fish oil and together comprise about 20 percent by weight of menhaden oil. FDA established maximum use levels of menhaden oil in certain foods because of concerns over possible adverse effects of fish oil consumption on bleeding time, glycemic control, and LDL cholesterol (62 FR 30751 at 30757; June 5, 1997). In 2002, FDA published a proposed rule to reallocate the uses of menhaden oil in conventional food, while maintaining the total daily intake of EPA and DHA from menhaden oil at a level not exceeding 3.0 g/p/d (67 FR 8744; February 26, 2002). FDA placed specific limitations, including the category of foods, the functional use of the ingredient, and the level of use, to ensure that the consumption of EPA and DHA from conventional food sources would not exceed 3.0 g/p/d. FDA then published a tentative final rule (69 FR 2313; January 15, 2004. **NOTE: the finalized allocation has since been published in a final rule on March 23, 2005, 70FR Number 55 Part 184 at page 14530**), to additionally require that menhaden oil not be used as an ingredient in foods in combination with other added oil that is a significant source of EPA and DHA to ensure that total intake from conventional food sources do not exceed 3.0 g/p/d.

In addition, FDA has not objected to certain GRAS notifications for additional sources of EPA and DHA as food ingredients (fish oils other than menhaden oil) (GRAS Notice Nos: GRN000102, GRN000105, GRN000109, GRN000138, GRN 000146). These GRAS notices proposed maximum use levels consistent with those specified in the tentative final rule affirming, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use.

FDA has also responded without objection to a GRAS notification on algal oil DHA from Martek Biosciences Corporation. Martek estimated that the use of algal oil in a number of food categories at the maximum proposed use levels would result in a mean exposure of no more than 1.5 grams of DHA per day (GRAS Notice No. GRN000137).

The mean exposure to EPA and DHA from menhaden oil in all conventional food categories is estimated to be 2.7 g/p/d (67 FR 8744 at 8746; February 26, 2002). This is a conservative estimate with substantial margin for safety, and the agency believes, consistent with its prior

[redacted]

decision on the use of a qualified health claim for DHA and EPA omega-3 fatty acids (October 31, 2000 letter) for dietary supplements, that the addition of menhaden oil to food products has not come close to this conservative mean estimate exposure. FDA further believes that the GRAS uses for which it received a GRAS notification for other sources of EPA and DHA omega-3 fatty acids also provide conservative estimates of exposure and that the addition of these EPA and DHA sources to food products do not come close to the conservative mean estimates. Not all foods in the marketplace within those permitted food categories would contain menhaden oil or other sources of EPA and DHA omega-3 fatty acids that substitute for other edible fat or oil. Also, because not all foods that a consumer eats every day would contain menhaden or other EPA and DHA oil used as a substitute oil, the actual total daily intakes of EPA and DHA from menhaden or other EPA and DHA oil for an average person should be significantly below 3.0 g/p/d (67 FR 8744 at 8746; February 26, 2002).

FDA noted that it is difficult to estimate the actual total consumption of EPA and DHA. The Continuing Survey of Food Intakes by Individuals (1994-1996, 1998) estimated EPA and DHA intakes from conventional foods. The 50th percentile intake of EPA and DHA from the survey was between 0.06 g and 0.07 g for adult women and 0.07 g and 0.1 g for adult men. The 90th percentile intake was between 0.18 g and 0.22 g for women and between 0.20 g and 0.43 g for men. These data agree closely with what has been reported by Jonnalagadda *et al.,* (1995), Allison *et al.,* (1999) and Kris-Etherton et al., (2000). Thus, EPA and DHA consumption from conventional foods in the United States is low. FDA is not aware of any nationally representative consumption data on EPA and DHA from dietary supplements. In the October 31, 2000 letter, FDA permitted a Qualified Health Claim for dietary supplements containing EPA but expressed concern about the exposure to EPA and DHA omega-3 fatty acids potentially exceeding 3.0 g/p/d if a qualified health claim were to appear on dietary supplements. This concern was due to conventional foods containing omega-3 fatty acids that were on the market; the use of structure/function claims on products containing EPA and DHA omega-3 fatty acids, which may promote product purchase; and dietary supplements that FDA found in the marketplace that contained significant amounts of EPA and DHA.

On September 8, 2004, the requested use of this qualified health claim for omega-3 fatty acids was extended to conventional foods. The agency believes that there is likely to be some increased consumption of EPA and DHA omega-3 fatty acids based on conventional foods that bear the qualified health claim; however, the amounts of EPA and DHA that can be used and the foods in which such food ingredients can be safely used are limited. The agency has established specific limitations of use under its menhaden oil GRAS rule (62 FR 30751; June 5, 1997), proposed and tentatively finalized reallocation of the use of menhaden oil without changing total exposure levels (67 FR 8744; February 26, 2002, 69 FR 2313; January 15, 2004). **NOTE: the finalized allocation has since been published as a final rule on March 23, 2005, 70FR Number 55 Part 184 at page 14530.** Also, manufacturers that have submitted GRAS notifications for other sources, to which the agency has not objected, have established conditions of use similar to those in the menhaden oil GRAS rule.

000101

In the October 31, 2000 letter, FDA stated that a consumer could consume nearly 1 gram of EPA and DHA per day in the diet from conventional foods. The agency is uncertain about how much consumers will increase their intake of EPA and DHA omega-3 fatty acids from EPA and DHA

[redacted]

containing conventional foods and dietary supplements due to the extended use of the qualified health claim. In order to help consumers gauge their total intake of EPA and DHA and to provide them a way to keep their intake of EPA and DHA within 3 grams per day, FDA intends to consider, as a factor in the exercise of its enforcement discretion, that conventional foods and dietary supplements that bear an omega-3 fatty acid qualified health claim declare the amount of EPA and DHA per serving in the claim. FDA recommended that the information on EPA and DHA content for use in a qualified health claim for EPA and DHA omega-3 fatty acids and reduced risk of CHD be presented in a manner that is consistent with FDA's guidance entitled, "FDA Nutrition Labeling Manual--A Guide for Developing and Using Data Bases." The dietary supplement may declare the amount of EPA and DHA per serving in "Supplement Facts," instead of making the declaration in the claim. Also, to ensure further that consumers do not exceed a 3.0 g/p/d intake, FDA stated that they will educate consumers not to exceed 3.0 g/p/d from all food and dietary supplement sources through print and web outreach information.

Further, FDA intends to consider, as a factor in the exercise of its enforcement discretion that dietary supplements not recommend or suggest in labeling that consumers ingest more than 2 grams of EPA and DHA per day. FDA encourages manufacturers to limit their dietary supplement products bearing the qualified health claim to products recommending or suggesting daily intake of 1 gram or less of EPA and DHA omega-3 fatty acids.

Based on the data and information that FDA considered, which includes data and information that FDA relied upon in reaching its conclusions about the safety of EPA and DHA omega-3 fatty acids in its GRAS affirmation of menhaden oil, the data and information in the 1991 proposed (56 FR 60663; November 27, 1991) and 1993 final rules (58 FR 2683; January 6, 1993), and its current scientific literature review for other possible safety concerns, FDA concluded that the use of EPA and DHA omega-3 fatty acids used as a GRAS ingredient, consistent with FDA's GRAS rule for menhaden oil and GRAS notifications to which FDA did not object, and the use as a dietary supplement is safe and lawful under 21 CFR 101.14 provided that daily intakes of EPA and DHA omega-3 fatty acids from conventional food and dietary supplement sources do not exceed 3.0 g/p/d.]

The N3-TG-3020 Omega-3 Triglyceride Concentrate that is the subject of this safety assessment is comprised primarily of triglycerides of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). It is known by the commercial name of N3-TG-3020. The intended applications of this omega-3 triglyceride concentrate will be for those uses in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). The maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36% of that of menhaden oil. These proposed uses are presented in Table 1. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above including one for a marine oil concentrate. Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil

as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate. Therefore, N3-TG-3020 Omega-3 Concentrate can be considered safe under its intended conditions of use.

A review of the scientific literature published since the final rule appeared in the Federal Register on March 23, 2005, (70FR Number 55 Part 184 at page 14530) affirming the GRAS status of menhaden oil, confirmed the conclusion reached by the FDA that safety of ingestion of up to 3 grams per person per day of EPA and DHA combined is consistent with current information regarding the safety of consumption of these two omega-3 fatty acids. No new safety issues apart from FDA's original 3 concerns on bleeding time, glycemic control effects in diabetes and elevated LDL levels in populations with hypertriglyceridemia or hypercholesterolemia were identified. Recent research reports focus on the clinical usefulness and efficacy of EPA and DHA.

From the foregoing analysis and rulemaking decisions of FDA on the GRAS affirmation of menhaden oil and of EPA and DHA, as well as the on submitted GRAS Notices where the agency had no objection to the conclusions of being GRAS including a specific submission on an omega-3 fish oil concentrate (GRN 000105), Twin Rivers Technologies Omega-3 Triglyceride Concentrate (N3-TG-3020) should also be GRAS for the proposed uses specified in the regulations under the conditions described and at the maximum use levels described in Table 1.

Determination of the safety and GRAS status of N3-TG-3020 Omega-3 Triglyceride Concentrate for direct addition to foods under its intended conditions of use has been made through the deliberations of an Expert Panel consisting of W. Gary Flamm, Ph.D., FACT, FATS, James W. Barnett, Ph.D., DABT, and Stanley M. Tarka, Jr., Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have critically reviewed and evaluated the publicly available information summarized in this document, including the potential human exposure to EPA and DHA resulting from the intended use of N3-TG-3020 Omega-3 Triglyceride Concentrate, and have individually and collectively concluded:

'Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members have individually and collectively concluded that Omega-3 Triglyceride Concentrate, (N3-TG-3020) meeting the specifications cited in the expert panel GRAS statement, and concentrated as described, is GRAS when used as an ingredient in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation when used at a maximum level of 36 % of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020), when used as described, is GRAS based on scientific procedures.

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)
Therefore, N3-TG-3020 Omega-3 Triglyceride Concentrate meeting the specifications described in AAC's GRAS review titled DETERMINATION OF THE *GRAS* STATUS OF A CONCENTRATED OMEGA-3 TRIGLYCERIDE (N3-TG-3020) FOR USE AS A DIRECT FOOD INGREDIENT IN THE SAME FOOD CATEGORIES AS THOSE CURRENTLY LISTED IN 21CFR§184.1472(a)(3) (MENHADEN OIL) AT MAXIMUM USE LEVELS THAT ARE 36 PERCENT OF THOSE SPECIFIED IN THIS REGULATION and to be used as a food ingredient in the food categories and at the use levels listed in Table 1, and resulting in a potential intake of less than 3.0 grams per person per day of EPA and DHA combined is safe, and also GRAS, for addition to food."

F. Availability of Information

The detailed data and information that serve as a basis for this GRAS determination will be provided to the FDA upon request, or are available for the Food and Drug Administration's review and copying during reasonable business hours at the offices of:

Edward A. Steele
President
AAC Consulting Group
7361 Calhoun Place, Suite 500
Rockville, MD 20855-2765
Telephone: 301-838-3182
Facsimile: 301-838-3182
Email: esteele@aacgroup.com

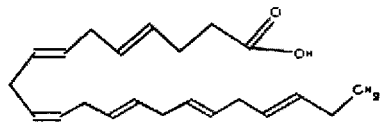
II. Detailed Information About the Identity of the Substance

A. Identity

The structures of EPA and DHA are represented by the following chemical formulae:

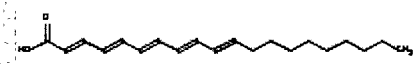
Figure 1- Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA)

NAME: Docosahexaenoic acid
RN: 25167-62-8
(22:6, n-3, DHA)



OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

NAME: Eicosapentaenoic acid
RN: 25378-27-2
(20:5, n-3, EPA)



The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of about 55 % EPA+DHA in triglyceride form. The chemical formula for Eicosapentaenoic acid is 20:5, n-3, EPA. The empirical formula for EPA is $C_{20}H_{30}O_2$ with a molecular weight of 302.45. The Chemical Abstracts Service (CAS) Number is 25378-27-2. The chemical formula for Docosahexaenoic acid is 22:6, n-3, DHA. The empirical formula for DHA is $C_{22}H_{32}O_2$ with a molecular weight of 328.49. The CAS Number is 25167-62-8.

B. Method of Manufacture

Twin Rivers Technologies manufacturing process for the production of a concentrated omega-3 fatty acid triglyceride containing substantially increased levels of EPA and DHA above those found in naturally occurring fish oils is described below. The process begins with a potential filtration treatment to remove solids from marine oils obtained from several potential sources of edible fish including salmon, menhaden, anchovy, sardine, jack mackerel, mackerel and tuna. This potential filtration step is followed by a potential adsorption step using bleaching earth to reduce odor and possible colors resulting in the production of a filtrate containing the fatty acids and a filter cake which is discarded. The next step involves the application of steam for stripping (optional) to reduce free fatty acids. This filtrate (when feed pretreatment via filtration and adsorption is employed) or otherwise unfiltered feedstock is then treated with either methanol or ethanol in the presence of a sodium methoxide catalyst resulting in transesterification of the glycerides and the free glycerine is removed. Next, the free unreacted methanol or ethanol are removed by flash evaporation and the resulting esterified product mix is treated with water wash to remove glycerin, soap, methanol or ethanol, as well as odor and flavor bodies. The water wash containing these components is removed and the esterified mass is then dried to remove any residual water. At this point, fractional distillation is utilized to remove the low boiling C14, C16, C18 methyl or ethyl esters and the remaining esterified mass is subjected to wiped film evaporation or other "Flash Distillation" in order distill the high boiling C18, C20, C22, C24 methyl or ethyl esters. Next, these distilled high-boiling esters are reacted with glycerin in the presence of either potassium carbonate or sodium methoxide to product a highly concentrated long chain triglyceride product (90%) with small amounts of diglycerides (5%) and

monoglycerides (5%). This reaction product is then treated with water and potassium citrate as a chelant in two stages to physically refine the product further and remove the soaps and potassium carbonate. The resulting long-chain polyunsaturated fatty acid triglyceride concentrate is dried to remove moisture, and treated with an adsorbent media to remove any color, odor or peroxides and this residual filter cake is discarded. The resulting concentrate in the form of the filtrate is then further evaporated to remove any residual esters, treated via steam stripping to remove any carry over odor bodies, peroxides, esters and free fatty acids. Finally, approved and acceptable antioxidants are added to retard oxidation and the long-chain polyunsaturated fatty acid omega-3 triglyceride concentrate is placed in storage under nitrogen. This process is similar in many respects to other industrial fish-oil based practices for processing of marine oils into refined fish oils and concentrates described in the GRAS Notices referred to earlier in this document, but also includes specific steps to produce a highly concentrated omega-3 triglyceride product. A schematic of the production process for Omega-3 Triglyceride Concentrate (N3-TG-3020) is shown in the confidential file provided in Appendix I of the GRAS document.

The production process for Omega-3 Concentrate (N3-TG-3020), summarized above, is a process that is similar in many respects to the standard industry practice for the processing of marine oils. Furthermore, this process is well characterized and can consistently yield a food-grade product that is safe for human consumption with the ongoing analytical testing and quality control procedures described below for potential contaminants.

Acceptable analytical methodology is also employed for Omega-3 materials that include measurement of Oxidative by-products (Acid value, Peroxide value, p-Anisidine Value, Totox), PCDDs and PCDFs, PCBs, and heavy metals including lead, cadmium, mercury, and arsenic. Twin Rivers Technologies will be sampling and analyzing this omega-3 product and process to ensure compliance with a voluntary monograph established in 2002 by the Council for Responsible Nutrition as an ongoing measure of quality control. In addition, they will be applying their internal release criteria to this omega-3 product, which also measures low level methyl or ethyl esters to ensure that the wiped film evaporator has suitably removed these constituents.

The members of the expert panel, individually and collectively, have critically evaluated the production process for Omega-3 Concentrate (N3-TG-3020) summarized above and have concluded that this process is similar in many respects to the standard industry practice for the processing of marine oils. Furthermore, the panel also concluded that this process is well characterized and can consistently yield a food-grade product that is safe for human consumption.

C. Food Grade Specifications for N3-TG-Omega-3 Triglyceride Concentrate

Twin Rivers Technologies has developed specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate to establish and maintain its food-grade status. These specifications are as follows:

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

Description:	Food-grade N3-TG-3020 Omega-3 Triglyceride Concentrate is composed predominantly of triglycerides (approximately 90%), with small amounts of diglyceride (about 8%) and monoglyceride (<2%), that are highly enriched in polyunsaturated fatty acids, primarily EPA and DHA. It is derived from fish oil extracted from multiple edible marine fish species (typically sardine, anchovy, menhaden, jack mackerel, mackerel, tuna and salmon) that is prepared in a multi-step proprietary process.
Appearance:	Yellow to light yellow at ambient temperature.
Triglycerides:	Not less than ("NLT") 90%.
Odor and taste:	At worst, a slightly fishy odor and taste.
EPA and DHA:	NLT 500 mg/g and not more than ("NMT") 600 mg/g.
EPA/DHA ratio:	NLT 1.5.
Lovibond Color:	5
Red, 5 ¼"	
Acid value:	NMT 3 mg KOH/g
Monoglyceride:	NMT 2%
Diglyceride:	NMT 10%.
Triglyceride:	NLT 90%.
Peroxide value:	NMT 5 meq/kg.
p-Anisidine	
value:	NMT 20.
Tottox value:	NMT 26 (Anisidine value +2x [peroxide value]
Iodine value:	NLT 284.4
Density:	930 to 950 mg/ml.
Tocopherols:	NLT 2.0 mg/g.
Moisture:	NMT 0.1%.
Unsaponifiables:	NMT 2.35 %.
Total PCBs:	NMT 0.09mg/kg.
PCDDs and	
PCDFs:	NMT 2.0 pg/g.
Heavy Metals	
Lead:	NMT 0.1 mg/kg.
Cadmium:	NMT 0.1 mg/kg.
Arsenic:	NMT 0.1 mg/kg.
Mercury:	NMT 0.1 mg/kg.
Delivery form:	Oil concentrate shipped under inert nitrogen atmosphere.

The members of an expert panel, individually and collectively, have critically evaluated the food-grade specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate summarized above, and have concluded that appropriate product specifications have been established to ensure that the final product is food grade and is safe for human consumption.

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

D. Testing for Contaminants

In addition to the testing specifications identified above for PCDD (dioxins), PCDFs (furans), total PCBs and heavy metals, Twin Rivers Technologies also examined potential levels of contaminants in the N3-TG-3020 Omega-3 Triglyceride Concentrate. Organochlorine and organophosphate pesticide levels were below the limit of detection (1, 5, 10, or 25 ng/g (i.e. ppb)). Levels of PCDD and PCDF were 0.40 and 0.76 pg/g respectively. A sample of N3-TG-3020 Omega-3 Concentrate as well as three potential marine oil feedstocks were also analyzed for polycyclic aromatic hydrocarbons. The detection limit for these compounds was 0.5 parts per billion. Fluorene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, total light PAHs, benzo(b)anthracene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h, i)perylene, total heavy PAHs, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, perylene, coronene and benzo(e)pyrene. Results for all four samples were "none detected" -for the aforementioned compounds. Twin Rivers Technologies will be screening each new lot manufactured from a different (geographically) sourced marine oil starting material for PAHs to ensure there are no outlier issues from the values reported here. The GRAS panel concluded that the analyses of the N3-TG-3020 Omega-3 Concentrate support the presumption that there is no toxicological concern from any product impurities.

III. Self-Limiting Levels of Use

Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate. Therefore, N3-TG-3020 Omega-3 Concentrate can be considered safe under its intended conditions of use.

IV. Detailed Summary of the Basis for the Notifier's Determination that N3-TG-3020 Omega-3 Concentrate is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by was requested by Twin River Technologies to determine the Generally Recognized As Safe (GRAS) status of a concentrated omega-3 triglyceride (N3-TG-3020) for use as a direct food ingredient in the same food categories as those currently listed in 21CFR§184.1472(a)(3) (Menhaden oil) at maximum levels that are 36 percent of those specified in this regulation. Specifically, these categories include the following: baked goods and baking mixes, cereals, cheese products, chewing gum, condiments, confections and frostings, dairy product analogs, egg products, fats and oils (but not in infant formula), fish products, frozen desserts, gelatins and puddings, gravies and sauces, hard candy, jams and jellies, meat products, milk products, nonalcoholic beverages, nut products, pastas, plant protein products, poultry products, processed fruit juices, snack foods, soft candy, soup mixes, sugar substitutes, sweet sauces, toppings and syrups, and white granulated sugar. The Expert Panel also evaluated previously filed GRAS

OMEGA-3 TRIGLYCERIDE CONCENTRATE (N3-TG-3020)

notifications on this subject with FDA as well as FDA's Tentative, Proposed and Final Rule affirming the Generally Recognized as Safe (GRAS) status of menhaden oil. A comprehensive search of the scientific literature was completed through October 13, 2005, along with an evaluation of FDA's database for safety and toxicity information submitted as part of several GRAS notices and a Citizen's Petition on fish oil, salmon oil, menhaden oil, an omega-3 concentrate (Marinol) and on tuna oil. The Expert Panel independently evaluated this information and other materials deemed necessary or appropriate. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the following decision:

> "Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members have individually and collectively concluded that Omega-3 Triglyceride Concentrate, (N3-TG-3020) meeting the specifications cited in the expert panel GRAS statement, and concentrated as described, is GRAS when used as an ingredient in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation when used at a maximum level of 36 % of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined.
>
> It is also the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, the Expert Panel concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020), when used as described, is GRAS based on scientific procedures." Because N3-TG-3020 Omega-3 Triglyceride Concentrate is GRAS for its proposed uses, it is excluded from the definition of a food additive, and thus may be marketed and sold for these uses in the U.S. without the promulgation of a food additive regulation under 21 CFR.

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3. Federal Register/62FR/ No.108/pp 30751-30757/ Thursday, June 5, 1997-21CFRPart 184 Substances Affirmed as Generally Recognized as Safe: Menhaden Oil. Final Rule.

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5. Federal Register - 70FR 14530 March 23, 2005: Substances Affirmed as Generally Recognized as Safe;menhaden oil http://www.cfsan.fda.gov/~lrd/fr050323.html

6. GRAS Notice No.GRN 000102 (September 3, 2002): *FDA/CFSAN/OPA*: Agency Response Letter to Jedwards International on small planktivorous pelagic fish body oil. http://www.cfsan.fda.gov/~rdb/opa-g102.html

7. GRAS Notice No.GRN 000105 (October 15, 2002): *FDA/CFSAN/OPA*: Agency Response Letter to Unilever United States, Inc. on Marinol omega-3 concentrate. http://www.cfsan.fda.gov/~rdb/opa-g105.html

8. GRAS Notice No.GRN 000109 (December 4, 2002): *FDA/CFSAN/OPA*: Agency Response Letter to Piper Rudnick on behalf of Clover Corporation on tuna oil. http://www.cfsan.fda.gov/~rdb/opa-g109.html

9. GRAS Notice No.GRN 000138 (April 20, 2004): *FDA/CFSAN/OPA*: Agency Response Letter to JHeimbach on behalf of Ocean Nutrition Canada, Ltd on fish oil from anchovy. http://www.cfsan.fda.gov/~rdb/opa-g138.html

10. GRAS Notice No. GRN 000102 (February 12, 2004): *FDA/CFSAN/OPA*: Agency Response Letter to Martek Biosciences Corporation on algal oil. http://www.cfsan.fda.gov/~rdb/opa-g137.html

11. GRAS Notice No.GRN 000146 (August 17, 2004): *FDA/CFSAN/OPA*: Agency Response Letter to Jedwards International on salmon oil. http://www.cfsan.fda.gov/~rdb/opa-g146.html

12. Jonnalagadda SS, Egan SK, Heimbach JT, Harris SS, Kris-Etherton PM. (1995). Fatty acid consumption patterns of Americans: 1987–1988 USDA Nationwide Food Consumption Survey. *Nutr Res 15:1767–81.*

13. Kris-Etherton, PM, Shaffer Taylor, D, Yu-Poth, S, Huth, P, Moriarty, K, Fishell, V, Hargrove, RL, Zhao, G, Etherton, TD. (2000). Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr 71(1): 179S-188S.*

14. Letter from Christine J. Lewis, Ph.D., FDA to Jonathan W. Emord, Esq., Emord & Associates, P.C., "Letter Regarding Dietary Supplement Health Claim for Omega-3 Fatty Acids and Coronary Heart Disease" (Docket No. 91N-0103), October 31, 2000. http://www.cfsan.fda.gov/~dms/ds-ltr11.html

15. Letter to Martin Hahn of Hogan & Hartson LLP "Letter Responding to Health Claim Petition Dated November 3, 2003 (Martek Petition): Omega-3 Fatty Acids and Reduced Risk of Coronary Heart Disease" (Docket No. 2003Q-0401), September 8, 2004 Authorizing Qualified Health Claim for Conventional Foods. http://www.cfsan.fda.gov/~dms/ds-ltr37.html

EXPERT PANEL STATEMENT

DETERMINATION OF THE GRAS STATUS OF A CONCENTRATED OMEGA-3 TRIGLYCERIDE (N3-TG-3020) FOR USE AS A FOOD INGREDIENT

The undersigned, an independent panel of recognized experts, qualified by scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Twin River Technologies to determine the Generally Recognized As Safe (GRAS) status of a concentrated omega-3 triglyceride (N3-TG-3020) for use as a direct food ingredient in the same food categories as those currently listed in 21 CFR §184.1472(a)(3) (Menhaden oil) at maximum levels that are 36 percent of those specified in this regulation. Specifically, these categories include the following: baked goods and baking mixes, cereals, cheese products, chewing gum, condiments, confections and frostings, dairy product analogs, egg products, fats and oils (but not in infant formula), fish products, frozen desserts, gelatins and puddings, gravies and sauces, hard candy, jams and jellies, meat products, milk products, nonalcoholic beverages, nut products, pastas, plant protein products, poultry products, processed fruit juices, snack foods, soft candy, soup mixes, sugar substitutes, sweet sauces, toppings and syrups, and white granulated sugar. The Expert Panel also evaluated previously filed GRAS notifications on this subject with FDA as well as FDA's Tentative, Proposed and Final Rules affirming the Generally Recognized as Safe (GRAS) status of menhaden oil. A comprehensive search of the scientific literature was completed through October 13, 2005, along with an evaluation of FDA's database for safety and toxicity information submitted as part of several GRAS notices and a Citizen's Petition on fish oil, salmon oil, menhaden oil, an omega-3 concentrate (Marinol) and on tuna oil. The Expert Panel independently evaluated this information and other materials deemed necessary or appropriate. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

INTRODUCTION

N-3 polyunsaturated fatty acids (PUFA) or omega-3 fatty acids belong to an essential fatty acid family characterized by their first double bond at carbon atom number 3, counted from the methyl or omega end of the carbon chain constituting the backbone of fatty acids. N-3 PUFA are chemically and biologically distinct from n-6 PUFA, where the first double bond is at carbon atom number 6 (Schmidt, 1997).

There are two subgroups of n-3 PUFA or omega-3 fatty acids, as they are often collectively termed. One subgroup, α-linolenic acid, is derived from plant oils such as canola oil, rapeseed oil and linseed oil and is composed of 18 carbon atoms with three double bonds (nomenclature; 18:3). The other group of n-3 PUFA is derived from seafood, and the major marine n-3 PUFAs are eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6). In humans, α-linolenic acid can, to a limited extent, be elongated and desaturated to EPA and DHA. Otherwise, EPA and DHA are only acquired from seafood.

In a recent review by Schmidt *et al.,* (2005), it was reported that the content of marine n-3 PUFA varies between fish species (Hepburn *et al.*, (1986), Soringeur *et al.,* (1997), Kris-Etherton *et al.*, (2002)) being high in fatty fish like mackerel and salmon and low in lean fish such as flounder and cod (Table 1). The content of n-3 PUFA in seafood varies considerably in relation to location, water temperature and the season of capture. Values given in Table 1 should therefore be viewed in this light.

Table 1. The approximate content of n-3 PUFA in seafood

Seafood	n-3 PUFA g/100 g
Mackerel	1.8–5.3
Herring	1.2–3.1
Salmon	1.0–2.0
Trout	0.5–1.6
Tuna	0.5–1.6
Halibut	0.5–1.0
Shrimp	0.2–0.4
Cod and flounder	0.2

The intake of marine n-3 PUFA differs considerably between populations, being very high in traditionally living Eskimos (10–14 g/day), low in most Western populations (<0.2 g/day) and intermediate in areas like Japan and Norway (1–3 g/day).

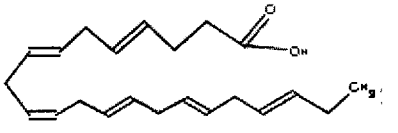
Uauy and Valenzuela (2000) reviewed the biochemical basis for essentiality of n-3 fatty acids and metabolism. They noted that the biochemical pathways for n-3 and n-6 fatty acid desaturation are only present in the chloroplasts of cells. Thus, only algae and some fungi are capable of forming these essential fatty acids (EFAs). Terrestrial and marine plants are the primary source of EFAs in the food chain; fish and other marine animals are able to elongate and desaturate the parent EFAs, forming the long-chain polyunsaturated fatty acids (LCPUFAs).(Simopoulos, 1991). Animal tissues, especially the liver, but also lung, heart, kidney, and brain, are capable of further elongating and desaturating the parent EFAs, generating a family of compounds for the respective families (n-3 and n-6 fatty acids). The competitive desaturation of the n-3, n-6, and n-9 series by ∂-6 desaturase is of major significance because this step is controlled by the interaction of hormones and diet, including the amount of linoleic acid (18:2, n-6, LA) and α-linolenic acid (18:3, n-3, LNA) in the diet. The controlling step in the metabolic pathway for both n-3 and n-6 EFA series is the ∂-6 desaturation of the respective precursor (LNA and LA, respectively). The uniqueness of the biological effects of feeding human milk or marine foods on EFA metabolism is based on the direct supply of

LCPUFA's bypassing the controlling step of the ∂-6 desaturase. Further, the conversion of parent EFAs to LCPUFAs is under active regulation. Therefore, the effects of providing EPA or DHA in the diet cannot be reproduced by providing the equivalent amount of LNA. Excess dietary LA associated with some vegetable oils, particularly safflower, sunflower, and corn oils consumed in most Western countries, may decrease the formation of DHA from LNA because the ∂-6 desaturase is inhibited by excess substrate (Galli and Marangoni, 1997). This review noted that other dietary factors such as overall energy and protein supply, and zinc and copper deficiency, affect EFA metabolism; for example, a zinc deficit is associated with decreased activity of ∂-6 desaturase, and thus too low arachidonic acid (AA) and DHA. Hormones such as insulin increase conversion of EFA to long-chain derivatives.

The inhibitory effect of EPA on ∂-5 desaturase activity has also been considered responsible for the lower AA observed when marine oil is consumed and this has been confirmed in molecular studies. Excess LA, as seen in enterally or parenterally fed patients receiving corn oil or safflower oil as the predominant fatty acid supply, will inhibit the elongation/desaturation of the parent EFAs, and thus will lower the LCPUFA supply necessary for membrane synthesis. Marine PUFAs provide minimal preformed AA and substantial amounts of preformed n-3 LCPUFA such as EPA and DHA.

The structures of these compounds are represented by the following chemical formulae:

NAME: Docosahexaenoic acid
RN: 25167-62-8
(22:6, n-3, DHA)



NAME: Eicosapentaenoic acid
RN: 25378-27-2
(20:5, n-3, EPA)

The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of 52.6% EPA+DHA in triglyceride form. As noted earlier, the only source of these omega-3 fatty acids is marine seafood. FDA affirmed the GRAS status of menhaden oil under 21 CFR §184.1472 and established that a daily intake of EPA and DHA combined not exceed 3 grams per person per day is safe. The scientific basis to support the establishment of this safe level of intake was published in the Federal Register at page 30751 (Volume 62, Number 108) on June 5, 1997, as part of the final rule on menhaden oil. As reported above, menhaden oil is one of the key edible fish sources that have a high concentration of EPA and DHA. Menhaden oil is a refined marine oil that is derived from menhaden fish (*Brevoortia* species) which are any of a general species of valuable Atlantic coastal fishes. It consists primarily of fatty acid triglycerides, with small amounts of monoglycerides and diglycerides. The triglycerides are esters of glycerol and fatty acids with chains of 14 to 22 carbon atoms. Menhaden oil differs from edible vegetable oils and animal fats in its high proportion of polyunsaturated fatty acids with 4, 5 and 6 double bonds (about 25 percent). The mean percentages for these polyunsaturated fatty acids in menhaden oil are C18:4 (2.3 percent), C20:4 (2.0 percent), C20:5 (13.1 percent), C22:5 (2.5 percent) and C22:6 (6.7 percent). Note that C20:5 and C22:6 are EPA and DHA, respectively, and are the major source of omega-3 fatty acids from fish oil. Menhaden oil also contains about 33 percent saturated fatty acids and about 31 percent monounsaturated fatty acids. In their review, FDA identified three potential safety issues of menhaden oil consumption above 3 grams per person per day: increased bleeding time by reducing platelet aggregability, a potential concern about glycemic control in non-insulin-dependent diabetics, and potential increases in LDL cholesterol.

An extensive review by the Agency of the available scientific information subsequently addressed these concerns and FDA stated the following:

Bleeding time: *"The totality of the scientific evidence demonstrates that when consumption of fish oils is limited to 3 g/p/d or less of EPA and DHA, there is no significant risk for increased bleeding time beyond the normal range."* A report from an industry-sponsored roundtable discussion on the topic of fish oils and bleeding time (CRN, 2000) also supports the conclusion that EPA and DHA are safe at intake levels at or below 3 g/p/d. On the other hand, ingestion of fish oils providing more than 3 g/d of

EPA and DHA have generally been found to produce increases in bleeding time that are statistically significant. At this time, there are insufficient data to evaluate the clinical significance of this finding. Because of the lack of data and because of the potential risk of excessive bleeding in some individuals with intakes at higher levels, FDA concludes that the safety of menhaden oil is generally recognized as safe only at levels that limit intake of EPA and DHA to 3 g/p/d.

Glycemic control in non-insulin dependent diabetics: "*Based on the available information, FDA concludes that consumption of EPA and DHA in fish oils at 3 g/p/d by diabetics has no clinically significant effect on glycemic control, although higher amounts of EPA and DHA (4.5 g/p/d and above) remain of concern. Therefore, FDA concludes that 3 g/p/d of EPA and DHA is a safe level with respect to glycemic control.*"

LDL Cholesterol: In the 1997 final rule, FDA noted that many studies on hypertriglyceridemic or hypercholesterolemic subjects, and some studies on normal subjects, reported an increase in LDL cholesterol or apo B (apolipoprotein B, a principal component of LDL) following fish oil supplementation (58 FR 2682 at 2705). Because increases in LDL cholesterol predict increased risk of coronary heart disease, FDA recently reevaluated those studies, as well as newer studies published since the health claim final rule, to address the question of whether 3 g/p/d of EPA and DHA derived from menhaden oil is generally recognized as a safe level with respect to its effect on LDL cholesterol. The agency considered the reported effects of fish oil on LDL cholesterol levels in healthy persons with normal cholesterol levels, as well as in persons with diabetes mellitus, hypertension, abnormal blood lipid levels, and cardiovascular disease.

As a result of its reevaluation, FDA found that although reported study results are variable, there appears to be a trend toward increased LDL cholesterol values with increased fish oil consumption in all population subgroups, with the magnitude of the increase appearing greater and more consistent in populations with abnormal blood lipid levels, hypertension, diabetes, and cardiovascular disease. They also noted that in the earlier 1993 health claims final rule, most reports of increased LDL were in studies where large amounts of fish oils were given (i.e., 5 g or more per day of EPA plus DHA), and any safety concern relating to changes in LDL cholesterol might be suitably addressed by restricting the intake of DHA and EPA (58 FR 2682 at 2705).

"*Based on these levels, FDA has determined that the mean intake of menhaden oil, if menhaden oil were to be used at the maximum allowable level in all permitted food categories, would be less than 3 g of DHA and EPA per day. Further, menhaden oil would substitute for other dietary fats, some of which have similar effects on LDL cholesterol. Based on its evaluation, the agency concludes that the petitioned levels of menhaden oil are safe with respect to the effect on LDL cholesterol.*"

On February 26, 2002, the National Fish Meal and Oil Association (NFMOA) amended its original 1986 filing for menhaden oil (GRASP 6G0316) to include suggested maximum use levels of menhaden oil for each food category in which menhaden oil can be used under 21 CFR §10.20 and §10.30 requesting that the agency amend §184.1472

Menhaden oil (21 CFR §184.1472) by reallocating the uses of menhaden oil in food, while maintaining the total daily intake of EPA and DHA from menhaden oil at a level not exceeding 3.0 g/p/d. FDA noted "The maximum limit of 3.0 g/p/d on the total daily intake of EPA and DHA has been considered a reasonable safeguard against the possible adverse effects stated above and to date no new information available has caused the agency to alter this limit." The reallocation is performed by: (1) reducing the maximum levels of use of menhaden oil in some of the currently listed food categories; (2) adding additional food categories along with assigning maximum levels of use in these new categories; and (3) eliminating the listing of subcategories, for example, cookies and crackers, breads and rolls, fruit pies and custard pies, and cakes, and including them under broader food categories, i.e., baked goods and baking mixes.

The NFMOA provided exposure analyses that contained estimates of EPA and DHA intake from menhaden oil for the revised uses of the currently listed food categories and the proposed uses of the new food categories. The NFMOA stated that the estimated daily exposure to EPA and DHA from those uses of menhaden oil is 2.7 g/p/d. NFMOA concluded that menhaden oil is GRAS for the revised uses of the currently listed food categories and the proposed uses of the new food categories, because the total daily intake of EPA and DHA from those uses of menhaden oil would not exceed 3.0 g/p/d, consistent with the June 1997 final rule.

In support of FDA's conclusions, on April 3, 2000 (Docket Number 91N-0103) a detailed review of the published literature was submitted on behalf of a joint task group of the Consumer Healthcare Products Association (CHPA), Council for Responsible Nutrition (CRN) and the National Fisheries Institute (NFI) on health claims for omega-3 fatty acids (EPA and DHA) in response to the FDA's request for scientific information and data in order to evaluate the evidence for the relationship between omega-3 fatty acids (EPA and DHA) and heart disease. As part of this review of all available clinical trials, the task group evaluated the three potential safety issues identified by the Agency and concluded that studies published since FDA's review in 1993 have been examined and no new information has become available to invalidate FDA's previous conclusions regarding safety of omega-3 fatty acids (EPA and DHA) up to 3g/day as stated in the GRAS affirmation of the menhaden oil petition.

During this interim period, the omega-3 fatty acids have also been the subject of several GRAS Notices submitted to FDA for use as products that would be substantially equivalent to menhaden oil and that would be substituted in its place in the approved food categories at levels equivalent to the EPA and DHA from menhaden oil. In each case, FDA responded that they had no questions on the proposed use and did not object to the GRAS notice. These submissions and FDA's responses are presented below.

1. GRAS Notice No. 000102: Response dated September 3, 2002 to a submission by Jedwards International on a small planktivorous pelagic fish body oil (SPPFBO) informing FDA of their view that this product is GRAS, through scientific procedures for use as a direct food ingredient in the same food categories as those

currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) at maximum use levels that are two thirds of those specified in that regulation.

As with the use of menhaden oil, the maximum levels of use of SPPFBO are designed to assure that the combined daily intake of two fatty acids that are components of SPPFBO (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In an amendment dated July 15, 2002, Jedwards noted that SPPFBO will be used as the sole added source of EPA and DHA in any given food category and is not to be combined or augmented with any other EPA/DHA-rich oil in making a food product.

Jedwards described generally available information about the identity and composition of SPPFBO. SPPFBO is derived from small planktivorous pelagic fish, primarily sea-harvested sardine (*Sardina pilchardus*) and anchovy (*Cetengraulis mysticetus*). Jedwards compared sardine and anchovy with menhaden and concluded that they are all plankton-eating fish that vary mainly in size. Because sardines and anchovies are commonly consumed fish, Jedwards considers that SPPFBO is already part of the diet.

Jedwards described SPPFBO as a complex mixture of glycerides, fatty acids, unsaponifiables and phospholipids. It is predominantly a mixture of triglycerides with small amounts of mono- and diglycerides. Like other fish oils, SPPFBO differs from edible vegetable oils and animal fats in its relatively high proportions of polyunsaturated fatty acids, such as EPA (which has five double bonds) and DHA (which has six double bonds). Jedwards also provided comparative fatty acid composition data of SPPFBO to that of menhaden oil and contrasted the fatty acid compositions of these two fish oils with that of a vegetable oil (i.e., soybean oil).

Jedwards described the method of manufacture of SPPFBO. The fish are cooked and pressed. The oil is separated from the expressed liquor and further processed using standard methods in accordance with current good manufacturing practice. The oil is refined by winterization, neutralization, bleaching, and deodorization. The methods and equipment used to process SPPFBO are standard in the processing of other edible oils and fats. Jedwards describes the manufacture of SPPFBO as similar to that of menhaden and other fish oils. Jedwards provided product specifications with limits on saponification value, iodine number, unsaponifiable matter, free fatty acids, peroxide value, lead, and mercury.

Jedwards acknowledged that FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil Final Rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR

§184.1472(a)(3)). Likewise, Jedwards concluded that there appear to be no risks associated with the consumption of SPPFBO, provided that consumption is limited to a combined total intake for EPA and DHA of 3 g/p/d, with SPPFBO as the sole source of added oils containing EPA and DHA. Because SPPFBO contains higher levels of EPA and DHA than menhaden oil, Jedwards intends to use SPPFBO at maximum levels that are two thirds of the maximum levels of use described in the regulation for menhaden oil.

Jedwards concluded that SPPFBO is GRAS because its manufacture, composition and intended use are similar to the manufacture, composition, and intended use of menhaden oil.

Based on the information provided by Jedwards, as well as other information available to FDA, the agency had no questions at that time regarding Jedwards' conclusion that SPPFBO is GRAS under the intended conditions of use, provided that the levels of use do not exceed two thirds of the levels of use described in 21 CFR §184.1472 and that a combined intake of EPA and DHA from all added sources does not exceed 3 g/p/d.

2. GRAS Notice No. GRN 000105: Response dated October 15, 2002 from FDA to a submission from Unilever United States on a fish oil concentrate (Marinol Omega-3 Concentrate) informing FDA of their view that this product is GRAS, through scientific procedures for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) at maximum use levels that are 57 percent of those specified in that regulation.

Further, Unilever also intended for their fish oil concentrate to be used in several additional food categories (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) at specified maximum use levels based on FDA's recently issued proposed rule (the menhaden oil proposal; 67FR 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 may be used. Unilever noted that any changes to 21 CFR §184.1472(a)(3) would also apply to fish oil concentrate, with maximum use levels that are 57 percent of those specified in that regulation.

As with the use of menhaden oil, the maximum levels of use of fish oil concentrate are designed to assure that the combined daily intake of two fatty acids that are components of fish oil concentrate (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In an amendment dated August 28, 2002, *Unilever noted that fish oil concentrate would be used as the sole added source of EPA and DHA in any given food category and would not be combined or augmented with any other EPA/DHA-rich oil in making a food product.*

As part of its notice, Unilever included a summary of conclusions of a panel of individuals (Unilever's GRAS panel) who evaluated the data and information that are the basis for Unilever's GRAS determination. Unilever's GRAS panel evaluated dietary

exposure, the method of manufacture, specifications, contaminant levels, and a literature review of toxicological studies. Unilever's GRAS panel concluded that fish oil concentrate, meeting food grade specifications, is GRAS for its intended use.

Unilever also described generally available information about the identity and composition of fish oil concentrate. Fish oil concentrate is a mixture that is predominantly triglycerides, with small amounts of mono- and diglycerides. Fish oils, including fish oil concentrate, differ from edible vegetable oils and animal fats in their relatively high proportions of polyunsaturated fatty acids, especially DHA and EPA. Unilever provides a representative fatty acid profile for fish oil concentrate, which shows that fish oil concentrate contains EPA at a level of approximately 20 percent (by weight) of total fatty acids and DHA at a level of approximately 18 percent of total fatty acids. By comparison, menhaden oil contains EPA at a level of approximately 12 percent and DHA at a level of approximately 8 percent.

Unilever also described the manufacture of fish oil concentrate. The product is manufactured using starting material extracted from edible marine fish species that normally include anchovy, sardine, jack mackerel and mackerel. The fish oil extract is bleached and filtered, followed by enzymatic hydrolysis using a lipase. The lipase selectively hydrolyzes saturated and monounsaturated fatty acids from the triglycerides that are components of the fish oil to give free fatty acids (predominantly saturated and monounsaturated) and glycerides enriched in polyunsaturated fatty acids (i.e., EPA and DHA). The glycerides and the free fatty acids are separated by short-path molecular distillation. The glycerides are further processed by adjusting the pH with a base, bleaching, adding antioxidants, filtering, and deodorizing to generate the fish oil concentrate final product. Unilever's GRAS panel evaluated the production process and concluded that the process is similar to the standard industry practice for processing marine oils. Unilever also provided product specifications for fish oil concentrate.

Unilever acknowledged that FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR §184.1472(a)(3)). In Unilever's view, a review of the literature published since FDA issued the menhaden oil final rule confirms FDA's conclusion that intake of EPA and DHA is safe as long as the combined daily intake does not exceed 3 g/p/d.

Unilever also provided a per capita estimate of daily intake of EPA and DHA from the use of fish oil concentrate in each of the food categories for the initial intended use levels listed above. Unilever calculated these estimates using data submitted to FDA in Citizen Petition GRP 0Z0425. The data in GRP 0Z0425 were obtained from USDA's Continuing

Surveys of Food Intakes by Individuals, 1989-1992 3-day survey. By summing the intake of EPA and DHA from each of the food categories, Unilever estimated exposure to EPA and DHA from use of fish oil concentrate in the combined food categories at the initial intended use levels to be 2.8 g/person/day**. Unilever estimated daily intake of EPA and DHA from the use of fish oil concentrate from the future intended use levels (see additional products above) to be 2.7 g/person/day. *Because fish oil concentrate is intended to be used as an alternative to menhaden oil, Unilever concluded that there will be no increase in exposure to EPA and DHA from its use. FDA concurred with their own independent assessment (See ** Note below).*

Based on the information provided by Unilever, as well as other information available to FDA, the agency had no questions at the time regarding Unilever's conclusion that fish oil concentrate is GRAS under the intended conditions of use, provided that combined intake of EPA and DHA from all added sources does not exceed 3 g/p/d.

** **NOTE**: FDA normally considers exposure to an ingredient for the eaters only population (individuals in the survey who consumed at least one of the foods in which the ingredient is intended to be used during the survey period), rather than the entire per capita population (all individuals who participated in the survey). However, because of the number of food categories in which fish oil concentrate is intended to be used, the eaters only population is nearly identical to the per capita population; 98 percent of the individuals surveyed are eaters of at least one of the foods. FDA independently estimated intake of EPA and DHA from the proposed use of fish oil concentrate and concluded in this response that the additional food categories in Unilever's initial intended use not currently listed in 21 CFR §184.1472(a)(3) (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, non-dairy milk, imitation and soy milk) do not contribute significantly to exposure and that intake of EPA and DHA will remain below 3 g/person/day.

3. GRAS Notice No. 000109: Response dated December 4, 2002 to a submission by Anthony Young of Piper Rudnick, LLP on behalf of the Clover Corporation on tuna oil informing FDA of their view that this product is GRAS, through scientific procedures, for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) at maximum use levels that are 62 percent of those specified in that regulation.

As with the use of menhaden oil, the maximum levels of use of tuna oil are designed to assure that the combined daily intake of two fatty acids that are components of tuna oil (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In an amendment dated July 30, 2002, Clover noted that tuna oil would be used as the sole added source of EPA and DHA in any given food category and would not be combined or augmented with any other EPA/DHA-rich oil in making a food product.

In GRN 000097, Clover included the report of a panel of individuals (Clover's GRAS panel) who evaluated the data and information that are the basis for Clover's GRAS determination. Clover's GRAS panel reviewed data and information about tuna oil relating to the identity, manufacture, stability, conditions of use, estimated daily intake, and published toxicological studies conducted with fish oils. Clover's GRAS panel concluded that tuna oil is GRAS when meeting appropriate specifications and used to provide up to 4 g/p/d of EPA plus DHA. In GRN 000109, Clover specified lower maximum intended use levels to provide a maximum of 3 g/p/d of combined EPA and DHA and stated that it does not intend to market tuna oil for any other food ingredient uses. Clover concluded that tuna oil is GRAS for use in foods at levels proposed in this GRAS Notice.

Clover described generally available information about the identity and composition of tuna oil. Clover described fish oils as complex mixtures, which, like edible vegetable oils, consist of glycerides, fatty acids, phospholipids, and an unsaponifiable fraction. Clover indicated that tuna oil, like other fish oils, is similar to other edible vegetable oils and animal fats, differing principally in the higher proportion of long chain polyunsaturated fatty acids DHA (C22:6 n-3) and EPA (C20:5 n-3). Tuna oil mainly contains triglycerides, with small amounts of mono- and diglycerides. Tuna oil differs from other fish oils in the ratio of EPA to DHA, with a ratio of approximately 1:4 in tuna oil and 1:0.6 in menhaden oil.

Clover described the method of manufacture for tuna oil. Clover manufactures tuna oil from "raw tuna oil", a by-product of the edible tuna canning industry. Tuna species harvested for raw tuna oil production include: skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*), albacore (*Thunnus alulunga*), and bigeye (*Thunnus obesus*) from the South and Central Pacific Ocean. These species are all considered to be widely consumed by humans. Raw tuna oil is manufactured from tuna heads, off-cuts from filleting, skin, frame, red muscle meat, and belly flaps. These byproducts are ground and cooked, and the oil is separated from solids and water by centrifugation. Metal ions that could initiate oxidation of the oil are removed with the aqueous phase. The raw tuna oil is refined to make the final tuna oil products using some or all of the processing techniques of winterization, degumming, neutralization, bleaching and deodorization, processes that are standard in the edible oil industry. Clover describes the manufacture of several of its tuna oil-containing products.

Clover indicated that most non-triglyceride substances are removed during the various processing steps used to manufacture tuna oil. Clover also provided results from analyses of several lots of tuna oil that measured the concentrations of various environmental contaminants and pesticides in tuna oil. Clover concluded that the data show that the processing steps used to manufacture tuna oil remove or reduce all contaminants to levels that are either below the level of detection or are within "all known regulatory levels for these compounds." Clover also provided specifications for tuna oil.

Clover specified the intended maximum use levels of tuna oil and stated that the use levels were set using 22 percent as the average amount of EPA and DHA in menhaden oil

and 32.5 percent as the EPA and DHA content of tuna oil. Clover estimated combined intake of EPA and DHA from the proposed use of tuna oil would be 2.76 g/p/d, based on the mean content of EPA and DHA in tuna oil of 32.5 percent and the maximum proposed use levels.

Clover also acknowledged that FDA raised concerns about consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and increase blood glucose levels in non-insulin dependent diabetics (62 FR 30751). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. Clover cites a recent review article that concludes that "studies published since FDA's review in 1993 have been examined and no new information has become available to invalidate FDA's previous conclusions regarding safety of omega-3 fatty acids (EPA and DHA) up to 3g/p/d as stated in the GRAS affirmation of the menhaden oil petition."

Based on the information provided by Clover, as well as other information available to FDA, the agency had no questions at the time regarding Clover's conclusion that tuna oil is GRAS under the intended conditions of use, provided that the levels of use do not exceed 62 percent of the levels of use described in 21 CFR 184.1472 and that a combined intake of EPA and DHA from all added sources does not exceed 3 g/p/d.

4. GRAS Notice No. 000138: Response dated April 20, 2004 to a submission by Dr. James Heimbach of JHeimbach LLC. on behalf of Ocean Nutrition Canada, Ltd.(ONC) on a fish oil predominantly derived from anchovy informing FDA of their view that this product is GRAS, through scientific procedures, for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) and those projected in the tentative final rule that would amend 21 CFR §184.1472 (69 FR at page 2313; January 15, 2004).* ONC describes two formulations of its fish oil: an oil and a microencapsulated oil product. At the time, ONC intended for the oil to be used in the same food categories as those currently listed in 21 CFR §184.1472(a)(3) (menhaden oil) at levels that are 67 percent of the levels specified in that regulation. In the microencapsulated form of its product, fish oil constitutes approximately 55-60 percent (by weight) of the dry powder. ONC, therefore, intended for the microencapsulated oil product to be used in the same food categories as those currently listed in the menhaden oil regulation at levels that are 120 percent of the levels specified in that regulation.**

*** **NOTE**: Subsequent to receiving ONC's notice, FDA issued a tentative final rule that would amend 21 CFR §184.1472 (69 FR 2313; January 15, 2004).FDA issued this tentative final rule to amend its regulations by reallocating the uses of menhaden oil in food that were currently established at the time in §184.1472. *FDA also tentatively concluded that these uses of menhaden oil are generally recognized as safe (GRAS), but only when menhaden oil is not used in combination with other added oils that are*

significant sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Because FDA's proposed rule of February 26, 20002 did not include a condition of use for other added oils, FDA issued this tentative final rule to give interested persons the opportunity to comment on this limitation.

The Final Rule affirming menhaden oil as generally recognized as safe (GRAS) was published in the Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005 with no changes to the tentative rule. As noted here: §184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day:

Category of food	Maximum level of use in food (as served)
Baked goods, baking mixes, § 170.3(n)(1) of this chapter.	5.0 percent
Cereals, § 170.3(n)(4) of this chapter.	4.0 percent
Cheese products, § 170.3(n)(5) of this chapter.	5.0 percent
Chewing gum, § 170.3(n)(6) of this chapter.	3.0 percent
Condiments, § 170.3(n)(8) of this chapter.	5.0 percent
Confections, frostings, § 170.3(n)(9) of this chapter.	5.0 percent
Dairy product analogs, § 170.3(n)(10) of this chapter.	5.0 percent
Egg products, § 170.3(n)(11) of this chapter.	5.0 percent
Fats, oils, § 170.3(n)(12) of this chapter, but not in infant formula.	12.0 percent
Fish products, § 170.3(n)(13) of this chapter.	5.0 percent
Frozen dairy desserts, § 170.3(n)(20) of this chapter.	5.0 percent
Gelatins, puddings, § 170.3(n)(22) of this chapter.	1.0 percent
Gravies, sauces, § 170.3(n)(24) of this chapter.	5.0 percent
Hard candy, § 170.3(n)(25) of this chapter.	10.0 percent
Jams, jellies, § 170.3(n)(28) of this chapter.	7.0 percent
Meat products, § 170.3(n)(29) of this chapter.	5.0 percent
Milk products, § 170.3(n)(31) of this chapter.	5.0 percent
Nonalcoholic beverages, § 170.3(n)(3) of this chapter.	0.5 percent
Nut products, § 170.3(n)(32) of this chapter.	5.0 percent
Pastas, § 170.3(n)(23) of this chapter.	2.0 percent
Plant protein products, § 170.3(n)(33) of this chapter.	5.0 percent
Poultry products, § 170.3(n)(34) of this chapter.	3.0 percent
Processed fruit juices, § 170.3(n)(35) of this chapter.	1.0 percent
Processed vegetable juices, § 170.3(n)(36) of this chapter.	1.0 percent
Snack foods, § 170.3(n)(37) of this chapter.	5.0 percent
Soft candy, § 170.3(n)(38) of this chapter.	4.0 percent

Soup mixes, § 170.3(n)(40) of this chapter.	3.0 percent
Sugar substitutes, § 170.3(n)(42) of this chapter.	10.0 percent
Sweet sauces, toppings, syrups, § 170.3(n)(43) of this chapter.	5.0 percent
White granulated sugar, §170.3(n)(41) of this chapter.	4.0 percent

(4) To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid.

As with the use of menhaden oil, the maximum levels of use of ONC's fish oil are designed to assure that the combined daily intake of two fatty acids that are components of its fish oil and microencapsulated fish oil product (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) would not exceed 3 grams per person per day (g/p/d). In its notice dated September 26, 2003, ONC notes that its fish oil would be used as the sole added source of EPA and DHA in any given food category and that its fish oil would not be combined or augmented with any other EPA/DHA-rich oil in making a food product.

In GRN 000138, ONC included a summary of conclusions of a panel of individuals (ONC's GRAS panel) who evaluated the data and information that are the basis for ONC's GRAS determination. ONC's GRAS panel evaluated dietary exposure, source of the substance, method of manufacture, specifications, contaminant levels, and information from recent published toxicological and human studies. ONC's GRAS panel concluded that fish oil and microencapsulated fish oil product, meeting food grade specifications, are GRAS under the intended conditions of use.

ONC described the intended use of fish oil in foods and provided a table that lists the food categories and intended use level in each food category. ONC's fish oil is intended to be used as a direct food ingredient in food categories listed in 21 CFR §184.1472(a)(3) at maximum use levels that are 67 percent of the levels specified in that regulation. ONC intends for the microencapsulated fish oil product to be used in the same food categories as those currently listed in the menhaden oil regulation at levels that are 120 percent of the levels specified in that regulation. ONC estimates that the mean intake of EPA and DHA combined from the proposed uses of ONC's fish oil would not exceed 3 g/p/d.

ONC also described generally available information about the identity and composition of fish oil (predominantly anchovy). ONC's fish oil is a mixture of fatty acids, primarily in the form of triglycerides. ONC presented the fatty acid profiles for fish oil, herring oil, sardine oil, and salmon oil to show that its fish oil is similar to other edible fish oils. ONC's fish oil contains EPA at a level of approximately 18.5 percent (by weight) of total fatty acids and DHA at a level of approximately 11.8 percent (by weight) of total fatty acids, for a combined level of EPA and DHA of approximately 30 percent (by weight) of total fatty acids. By comparison, menhaden oil contains a combined level of EPA and DHA of approximately 21 to 22 percent of total fatty acids.

ONC also described the manufacture of its fish oil (predominantly anchovy). ONC's fish oil is extracted from multiple edible marine fish species caught off the coast of Peru, including anchovy (95-99 percent), sardine (1-5 percent), jack mackerel, Pacific mackerel, and other occasional species. Processing of the crude fish oil extract includes: 1) winterization, 2) deodorization; 3) treating with activated carbon (discussed in more detail below); 4) bleaching; and 5) blending with mixed tocopherols and other appropriate antioxidants. Optional steps include alkali refining (also called neutralization), and treatment with citric acid Microencapsulated fish oil product is manufactured by encapsulating it in a crosslinked gelatin/polyphosphate shell. ONC provides specifications for the crude oil, the food-grade oil, and the food-grade, mircroencapsulated oil product.

In a series of meetings between FDA and ONC on February 13, 2004, and March 31, 2004, FDA and ONC discussed the potential for contamination of ONC's product with polycyclic aromatic hydrocarbons (PAHs). At the latter meeting, and reiterated in an amendment from ONC dated April 2, 2004, ONC provided data to demonstrate that an activated carbon treatment, described in the original notice as 'optional,' successfully reduces the levels of PAH in oils, including its fish oil ONC stated that it is revising its standard procedure by removing the word 'optional' and requiring that activated carbon be used as a standard part of the production of the fish oil product that is the subject of GRN 000138.

ONC acknowledged that FDA has previously raised concerns about the consumption of high levels of two fatty acids (i e , DHA and EPA), which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30571; June 5, 1997) In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 grams per person per day (g/p/d). To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR §184.1472(a)(3)). In ONC's view, a review of the scientific literature published since FDA issued the menhaden oil final rule confirms FDA's conclusion that consumption of EPA and DHA is safe as long as the combined daily intake does not exceed 3 g/p/d

Based on the information provided by ONC, as well as other information available to FDA, the agency had no questions at the time regarding ONC's conclusion that fish oil (predominantly anchovy) is GRAS under the intended conditions of use.

5. GRAS Notice No. 000146: Response dated August 17, 2004 to a submission by Jedwards International on salmon oil informing FDA of their view that this product is GRAS, through scientific procedures for use as a direct food ingredient in the same food categories as those currently listed at the time in 21 CFR §184.1472(a)(3) (menhaden oil) and at the same maximum use levels when not combined or

augmented with any other food ingredient containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Jedwards International noted that FDA recently issued a proposed rule (the menhaden oil proposal; 67 FR 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 may be used. Jedwards asked in their notice that the agency apply any changes in 21 CFR 184.1472(a)(3) to the intended use of salmon oil. FDA issued a tentative final rule amending 21 CFR §184.1472 (69 FR 2313; January 15, 2004).

As with the use of menhaden oil, the maximum use levels of Jedwards' salmon oil are designed to assure that the combined daily intake of two fatty acids that are components of salmon oil (i.e., EPA and DHA) would not exceed 3 grams/person/day (g/p/d). FDA has affirmed the GRAS status of menhaden oil (21 CFR §184.1472 (a)(3)) provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. Similarly, Jedwards concluded that salmon oil is safe for use as a direct ingredient provided that the combined daily intake of EPA and DHA from consumption of salmon oil-containing foods does not exceed 3 g/p/d and salmon oil is added as the sole source of EPA and DHA in any given food category.

As part of its notice, Jedwards evaluated the data and information that are the basis for its GRAS determination. Jedwards discusses the source and chemical composition of salmon oil, the GRAS affirmed status of menhaden oil (21 CFR §184.1472), and the scientific literature on fish oils. According to Jedwards, salmon oil is manufactured in the same manner as menhaden oil and is virtually chemically identical in composition; hence salmon oil is a safe substitute for menhaden oil in various food categories listed in 21 CFR §184.1472 (a)(3) and subsequently updated in FDA's tentative final rule for menhaden oil (69 FR 2313; January 15, 2004).

Jedwards described generally available information about the identity and composition of salmon oil. Salmon oil is a complex mixture of glycerides, fatty acids, unsaponifiables, and phospholipids from the body of salmon, primarily *Salmo salar*. Salmon oil consists mainly of a mixture of triglycerides of various long chain (14-22 carbons) fatty acids, with small amounts of mono- and diglycerides. Jedwards noted that salmon oil, like menhaden oil, contains approximately 20 percent by weight of omega-3 fatty acids. According to Jedwards, the relative proportions of EPA and DHA, expressed on a weight percent basis, are typically 12 percent EPA and 8 percent DHA for menhaden oil and 8 percent EPA and 12 percent DHA for salmon oil. Jedwards also provided analytical results to show the EPA and DHA content of the salmon oil as well as the typical distributions of saturated (22%), monounsaturated (39%), and polyunsaturated (35%) fatty acids in the salmon oil ingredient. According to Jedwards, individual lots of salmon oil typically differ in the proportions of EPA and DHA, but lots are blended to achieve a standardized amount of 8 percent EPA and 12 percent DHA.

Jedwards stated that salmon oil is processed according to standardized procedures for fish oils. Fish are cooked and pressed, with the oil separated from the expressed liquor. The oil is further processed in accordance with current good manufacturing practice using standard refining methods employed in the processing of edible oils that include winterization, neutralization, bleaching, and deodorizing. Jedwards briefly described two purification processes used in the method of manufacture to remove persistent organochlorine pollutants and other contaminants potentially present in fish tissue. Salmon oil is filtered through clay to remove heavy metal contaminants and through activated charcoal to remove organic contaminants. Jedwards provided specifications for maximum levels of environmental contaminants in salmon oil.

Jedwards acknowledged that FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR 184.1472(a)(3)). Jedwards stated that, in accordance with the menhaden oil limitations, the proposed use of salmon oil would correspond with a dietary intake of EPA plus DHA that does not exceed 3 g/p/d. Further, salmon oil is not to be combined or augmented with any other food ingredient containing EPA and DHA.

Based on the information provided by Jedwards, as well as other information available to FDA, the agency had no questions at this time regarding Jedwards' conclusion that salmon oil is GRAS under the intended conditions of use.

IDENTITY, COMPOSITION AND SPECIFICATIONS

FATTY ACID COMPOSITION OF N3-TG-3020 OMEGA-3 TRIGLYCERIDE CONCENTRATE

Twin Rivers Technologies has generated fatty acid profiles for several batches of N3-TG-3020 Omega-3 Triglyceride Concentrate in order to show that its final product is comparable to other food-grade oils, and also to demonstrate that the production process does not structurally alter any of the component fatty acids, or generate any unique byproducts. These profiles were generated based on results from analyses employing AOCS Method Ce-1b-89 (Marine Oil Fatty Acid Composition by GLC). A representative fatty acid profile for N3-TG-Omega-3 Triglyceride Concentrate is displayed in Table 2 below and compared to menhaden oil.

Table 2. Representative Fatty Acid Profiles for N3-TG-3020 Omega-3 Triglyceride Concentrate Compared to Menhaden Oil

	Menhaden oil	N3-TG-3020
Fatty Acid	**Amount (in relative area percent)**	
C12:0	0.107	
C14:0	8.704	0.304
C14:1		
C15:0		
C16:0	18.779	2.663
C16:1	12.011	1.454
C16:4N3		
C17:0	2.280	0.241
C17:1	0.760	0.274
C18:0	3.233	3.077
C18:1N11	6.977	4.463
C18:1N9	2.883	2.770
C18:2	1.249	1.543
C18:3N6	0.502	0.397
C19:0	0.371	0.238
C19:1	0.644	0.257
C18:3N3	1.148	0.824
C18:4N3	2.039	1.576
C20:0	0.269	1.017
C20:1	1.174	1.573
C20:2	0.161	0.286
C20:3N6	0.312	0.511
C20:4N6	1.423	4.838
C20:3N3	0.140	0.251
C20:4N3	1.292	1.929
C20:5N3 (EPA)	**12.742**	**30.289**
C22:1N11	0.295	0.808
C22:1N9	0.236	2.311
C21:5N3	0.725	1.440
C22:4N6	0.393	0.778
C24	0.429	0.721
C22:5N3	2.822	4.709
C22:6N3 (DHA)	**8.898**	**21.020**
Unidentified	7.003	7.434

The fatty acid profile of N3-TG-3020 Omega-3 Triglyceride Concentrate, summarized above, is generally consistent with naturally occurring marine oils. The compositional analysis of the product supports the presumption that N3-TG-3020 Omega-3 Triglyceride Concentrate is metabolized by the body similarly to naturally occurring fish oil and poses no novel safety concerns.

SPECIFICATIONS

Specifications for concentrated omega-3 triglyceride (N3-TG-3020) are shown in Table 3.

Table 3. Food Grade Specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate

Twin Rivers Technologies has developed specifications for N3-TG-3020 Omega-3 Triglyceride Concentrate to establish and maintain its food-grade status. These specifications are as follows:

Description:	Food-grade N3-TG-3020 Omega-3 Triglyceride Concentrate is
	composed predominantly of triglycerides (approximately 90 %), with
	small amounts of diglyceride (about 8 %) and monoglyceride (<2 %),
	that are highly enriched in polyunsaturated fatty acids, primarily EPA
	and DHA. It is derived from fish oil extracted from multiple edible
	marine fish species (typically sardine, anchovy, menhaden, jack
	mackerel, mackerel, tuna and salmon) that is prepared in a multi-step
	proprietary process.
Appearance:	Yellow to light yellow at ambient temperature.
Triglycerides:	Not less than ("NLT") 90 %.
Odor and taste:	At worst, a slightly fishy odor and taste.
EPA and DHA:	NLT 500 mg/g and not more than ("NMT") 600 mg/g.
EPA/DHA ratio:	NLT 1.5.
Lovibond Color:	5
Red, 5 ¼"	
Acid value:	NMT 3 mg KOH/g
Monoglyceride:	2 %
Diglyceride:	NMT 8 %.
Triglyceride:	NLT 90 %.
Peroxide value:	NMT 5 meq/kg.
p-Anisidine	
value:	NMT 20.
Totox value:	NMT 26 (Anisidine value +2x [peroxide value]
Iodine value:	NLT 284.4
Density:	930 to 950 mg/ml.
Tocopherols:	NLT 2.0 mg/g.
Moisture:	NMT 0.1%.

Unsaponifiables:	NMT 2.35 %.
Total PCBs:	NMT 0.09mg/kg.
PCDDs and	
PCDFs:	NMT 2.0 pg/g.
Heavy Metals	
Lead:	NMT 0.1 mg/kg.
Cadmium:	NMT 0.1 mg/kg.
Arsenic:	NMT 0.1 mg/kg.
Mercury:	NMT 0.1 mg/kg.
Delivery form:	Oil concentrate shipped under inert nitrogen atmosphere.

The food-grade specifications for Omega-3 Triglyceride Concentrate (N3-TG-3020) summarized above are considered appropriate product specifications to ensure that the final product is safe for human consumption. Further, analyses of the product support the presumption that there is no toxicological concern from any product impurities.

MANUFACTURING PROCESS AND DESCRIPTION

Twin Rivers Technologies manufacturing process for the production of a concentrated omega-3 fatty acid triglyceride containing substantially increased levels of EPA and DHA above those found in naturally occurring fish oils is described below. The process begins with a potential filtration treatment to remove solids from marine oils obtained from several potential sources of edible fish including salmon, menhaden, anchovy, sardine, jack mackerel, mackerel and tuna. This potential filtration step is followed by a potential adsorption step using bleaching earth to reduce odor and possible colors resulting in the production of a filtrate containing the fatty acids and a filter cake which is discarded. The next step involves the application of steam for stripping to reduce free fatty acids. This filtrate (when feed pretreatment via filtration and adsorption is employed) or otherwise unfiltered feedstock is then treated with either methanol or ethanol in the presence of a sodium methoxide catalyst resulting in transesterification of the glycerides and the free glycerine is removed. Next, the free unreacted methanol or ethanol are removed by flash evaporation and the resulting esterified product mix is treated with water wash to remove glycerin, soap, methanol or ethanol, as well as odor and flavor bodies. The water wash containing these components is removed and the esterified mass is then dried to remove any residual water. At this point, fractional distillation is utilized to remove the low boiling C14, C16, C18 methyl or ethyl esters and the remaining esterified mass is subjected to wiped film evaporation or other "Flash Distillation" in order distill the high boiling C18, C20, C22, C24 methyl or ethyl esters. Next, these distilled high-boiling esters are reacted with glycerin in the presence of either potassium carbonate or sodium methoxide to product a highly concentrated long chain triglyceride product (90%) with small amounts of diglycerides (5%) and monoglycerides (5%). This reaction product is then treated with water and potassium citrate as a chelant in two stages to physically refine the product further and remove the soaps and potassium carbonate. The resulting long-chain polyunsaturated fatty acid triglyceride concentrate is dried to remove moisture, and treated with an adsorbent media to remove any color, odor or peroxides and this residual filter cake is discarded. The resulting concentrate in the form of the filtrate is then further evaporated to remove any residual esters, treated via steam stripping to remove any carry over odor bodies, peroxides, esters and free fatty

acids. Finally, approved and acceptable antioxidants are added to retard oxidation and the long-chain polyunsaturated fatty acid omega-3 triglyceride concentrate is placed in storage under nitrogen. This process is similar in many respects to other industrial fish-oil based practices for processing of marine oils into refined fish oils and concentrates described in the GRAS Notices referred to earlier in this document, but also includes specific steps to produce a highly concentrated omega-3 triglyceride product. A schematic of the production process for Omega-3 Triglyceride Concentrate (N3-TG-3020) is shown in the confidential file provided in Appendix I.

The production process for Omega-3 Concentrate (N3-TG-3020), summarized above, is a process that is similar in many respects to the standard industry practice for the processing of marine oils. Furthermore, this process is well characterized and can consistently yield a food-grade product that is safe for human consumption with the ongoing analytical testing and quality control procedures described below for potential contaminants.

Acceptable analytical methodology is employed for Omega-3 materials that include measurement of oxidative by-products (acid value, peroxide value, p-anisidine value, Totox), PCDDs and PCDFs, PCBs, and heavy metals including lead, cadmium, mercury, and arsenic. Twin Rivers Technologies will be sampling and analyzing this omega-3 product and process to ensure compliance with the specifications shown in Table 2 which also include those found in a voluntary monograph established in 2002 by the Council for Responsible Nutrition as an ongoing measure of quality control. In addition, they will be applying their internal release criteria to this omega-3 product, which also measures low level methyl or ethyl esters to ensure that the wiped film evaporator has suitably removed these constituents.

Potential Contaminants

Pesticides

A sample of a single lot of N3-TG-3020 Omega-3 Triglyceride Concentrate was screened for twenty-five organochlorine pesticides and their isomers, seventeen additional chlorinated hydrocarbons and eighteen organophosphate pesticides. None of these pesticides were found above the detection limit in any of the samples analyzed. The limits of detection achieved across these large categories of chemicals, depending upon the specific pesticide being considered were generally 1, 5, 10 or 25 ppb, while the limits of detection (LOD) for technical chlordane was 50-100 ppb and for PCBs, 250-500 ppb. These results are presented in Appendices II and III. Twin Rivers Technologies will be screening each new lot manufactured from a different (geographically) sourced marine oil starting material for these same pesticides to ensure there are no outlier issues from the values reported here.

Dioxins and Furans

A single lot of N3-TG-3020 Omega-3 Triglyceride Concentrate was analyzed for PCDDs (dioxins) and PCDFs (furans) using a modified version of USEPA Method 1613B. This lot yielded very low levels of PCDDs and PCDFs of 0.40 and 0.76 pg/g (WHO-TEQ),

assuming that any non-detected congeners were present at their respective limits of detection. These results are presented in Appendix I. Twin Rivers Technologies will be screening each new lot manufactured from a different (geographically) sourced marine oil starting material for dioxins and furans to ensure there are no outlier issues from the values reported here.

PAHs

A single lot of N3-TG-3020 Omega-3 Triglyceride Concentrate plus three potential feedstock marine oils were analyzed for polycyclic aromatic hydrocarbons (PAHs). The detection limit for these compounds was 0.5 parts per billion, including fluorene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, chrysene, total light PAHs, benzo(b)anthracene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h, i)perylene, total heavy PAHs, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, perylene, coronene and benzo(e)pyrene. Results for all four samples were "none detected" -for the aforementioned compounds. Twin Rivers Technologies will be screening each new lot manufactured from a different (geographically) sourced marine oil starting material for PAHs to ensure there are no outlier issues from the values reported here.

The analytical results for the contaminants summarized above support the presumption that there are no toxicological concerns from any product impurities.

USE AND EXPOSURE

Kris-Etherton *et al.,* (2000) analyzed polyunsaturated fatty acid intake in adults and compiled Table 4 which is presented below. It is clear from this data that omega-3 fatty acid consumption in the United States is between 0.1-0.2 g/day. To date, no official dietary recommendations have been made for n–3 fatty acids in the United States. Recommendations for total PUFA intake, however, have been made: 1–2% of energy from linoleic acid is required to prevent a fatty acid deficiency (Holman, 1970) and total PUFA intake should remain at 7% of energy (NRC Diet and Health, 1989) and not exceed 10% of energy (National Cholesterol Education Program, 1993)

TABLE 4. Polyunsaturated fatty acid intake of adults in the United States by sex and age[1]

	Total		18:2n–6		18:3n–3		18:4n–3 + 20:4n–6		20:5n–3 + 22:6n–3	
Sex and age (y)	1987–1988	1989–1991	1987–1988	1989–1991	1987–1988	1989–1991	1987–1988	1989–1991	1987–1988	1989–1991

					g/d					
Males										
12–19	16 ± 0.3	18 ± 1.1	14 ± 0.3	16 ± 0.9	2 ± 0.03	2 ± 0.07	0.1 ± 01	0.1 ± 01	0.1 ± 0.01	0.1 ± 0.07
20–39	17 ± 0.2		15 ± 0.2		2 ± 0.02		0.1 ± 01		0.1 ± 0.01	
20–49		18 ± 0.4		16 ± 0.4		2 ± 0.03		0.1 ± 01		0.2 ± 0.01
>40	16 ± 0.2		14 ± 0.2		2 ± 0.02		0.1 ± 01		0.1 ± 0.01	
50–69		16 ± 0.5		15 ± 0.4		1 ± 0.03		0.1 ± 01		0.2 ± 0.03
Females										
12–19	12 ± 0.3	13 ± 0.4	11 ± 0.2	12 ± 0.4	1 ± 0.03	1 ± 0.03	0.1 ± 0	0.1 ± 01	0.1 ± 0	0.1 ± 0.02
20–39	12 ± 0.2		11 ± 0.1		1 ± 0.01		0.1 ± 0		0.1 ± 0	
20–49		13 ± 0.2		11 ± 0.2		1 ± 0.02		0.1 ± 01		0.1 ± 0.01
>40	11 ± 0.1	12 ± 0.3	10 ± 0.1		1 ± 0.01		0.1 ± 0		0.1 ± 0	
50–69				11 ± 0.3		1 ± 0.02		0.1 ± 01		0.2 ± 0.02
Males and females										
>70		12 ± 0.3		11 ± 0.3		1 ± 0.02		0.1 ± 01		0.2 ± 0.00

[1] ± SE of 3-d intakes. Data for 1987–1988 adapted from Jonnalagadda *et al.,* (1995). Data for 1989–1991 adapted from Allison *et al.,* (1999).

The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of triglycerides of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is known by the commercial name of N3-TG-3020. The intended applications of this omega-3 triglyceride concentrate will be for the same uses in foods for which menhaden oil is permitted under 21 CFR §184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). The maximum level of use will be set to provide the same concentrations of EPA+DHA as those

provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36% of that of menhaden oil. These proposed uses are presented in Table 5. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above including one for a marine oil concentrate. Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate.

The estimated mean intake of EPA and DHA combined from all dietary sources in the general population, excluding infants under the age of one year, has been addressed in earlier FDA rulemaking (menhaden oil final rule; 62 FR 30751; June 5, 1997) where FDA estimated that the mean exposure to EPA and DHA from the use of menhaden oil in all food categories would be 2.8 grams/person/day. It was also addressed as well in the subsequent GRAS Notices referenced above. The total cumulative estimated mean intake of EPA and DHA combined from the proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate listed in the foods in Table 5, as well as from all other dietary sources in this general population is also estimated to be 2.8 grams per person per day. This estimate reflects 100 percent market penetration of the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate that are listed in Table 5. Because 100 percent market penetration of this product is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower. Further, this cumulative intake of EPA and DHA combined is still less than the safe limit for EPA and DHA combined of 3 grams per person per day established by the agency. The proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate will result in no incremental increase in the intake of EPA and DHA combined over that described for menhaden oil in the final rule Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005. [§184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day.(4) To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid].

Table 5. Proposed Maximum Use Levels in Food as Served of Omega-3 Triglyceride Concentrate (N3-TG-3020) Compared with Current Menhaden Oil Uses Permitted under 21§CFR184.1472 and Final Rule Published in FR March 23, 2005(Volume 70, Number 55 PP14530-14532

Category of food (and 21CFR §170.3(n) paragraph)	Maximum Level of Use of Menhaden Oil under 21CFR184.1472 and FR Vol 70 No 55 March 23 2005 Final Rule	Proposed Maximum Level of Use of N3-TG-3020 Omega-3 Triglyceride Concentrate[a]
Baked goods, baking mixes, §170.3(n)(1)	5.0 percent	1.8 percent
Cereals, §170.3(n)(4)	4.0 percent	1.4 percent

Table 5. Proposed Maximum Use Levels in Food as Served of Omega-3 Triglyceride Concentrate (N3-TG-3020) Compared with Current Menhaden Oil Uses Permitted under 21§CFR184.1472 and Final Rule Published in FR March 23, 2005(Volume 70, Number 55 PP14530-14532		
Category of food (and 21CFR §170.3(n) paragraph)	**Maximum Level of Use of Menhaden Oil under 21CFR184.1472 and FR Vol 70 No 55 March 23 2005 Final Rule**	**Proposed Maximum Level of Use of N3-TG-3020 Omega-3 Triglyceride Concentrate[a]**
Cheese products, §170.3(n)(5)	5.0 percent	1.8 percent
Chewing gum, §170.3(n)(6)	3.0 percent	1.1 percent
Condiments, §170.3(n)(8)	5.0 percent	1.8 percent
Confections, frostings, §170.3(n)(9)	5.0 percent	1.8 percent
Dairy product analogs, §170.3(n)(10)	5.0 percent	1.8 percent
Egg products, §170.3(n)(11)	5.0 percent	1.8 percent
Fats, oils, §170.3(n)(12), but not in infant formula	12.0 percent	4.3 percent
Fish products, §170.3(n)(13)	5.0 percent	1.8 percent
Frozen desserts, §170.3(n)(20)	5.0 percent	1.8 percent
Gelatins, puddings, §170.3(n)(22)	1.0 percent	0.36 percent
Gravies, sauces, §170.3(n)(24)	5.0 percent	1.8 percent
Hard candy, §170.3(n)(25)	10.0 percent	3.6 percent
Jams, jellies, §170.3(n)(28)	7.0 percent	2.5 percent
Meat products, §170.3(n)(29)	5.0 percent	1.8 percent
Milk products, §170.3(n)(31)	5.0 percent	1.8 percent
Nonalcoholic beverages, §170.3(n)(3)	0.5 percent	0.18 percent
Nut products, §170.3(n)(32)	5.0 percent	1.8 percent
Pastas, §170.3(n)(23)	2.0 percent	0.7 percent
Plant protein products, §170.3(n)(33)	5.0 percent	1.8 percent
Poultry products, §170.3(n)(34)	3.0 percent	1.1 percent
Processed fruit juices, §170.3(n)(35)	1.0 percent	0.36 percent
Processed vegetable juices, §170.3(n)(36)	1.0 percent	0.36 percent
Snack foods, §170.3(n)(37)	5.0 percent	1.8 percent
Soft candy, §170.3(n)(38)	4.0 percent	1.4 percent
Soup mixes, §170.3(n)(40)	3.0 percent	1.1 percent
Sugar substitutes, §170.3(n)(42)	10.0 percent	3.6 percent
Sweet sauces, toppings, syrups, §170.3(n)(43)	5.0 percent	1.8 percent
White granulated sugar, §170.3(n)(41)	4.0 percent	1.4 percent

[a]Proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532) are set to provide the same concentrations of EPA+DHA as those provided by menhaden oil; based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate

SAFETY STUDIES AND DISCUSSION

Menhaden Oil

There have been many published studies evaluating the safety of omega-3 fatty acids and these have all been evaluated by FDA in arriving at the determination that menhaden oil and its component fatty acids including EPA and DHA are GRAS as food ingredients subject to the limitations in 21 CFR §184.1472 and the final rule affirming as Generally Recognized as Safe (GRAS) menhaden oil published on March 23, 2005, 70 FR Number 55 Part 184 at page 14530. FDA also permitted the use of a Qualified Health Claim on dietary supplements containing EPA and DHA on October 31, 2000 as well as for conventional foods on September 8, 2004. In the October 31, 2000 letter, FDA concluded that the use of EPA and DHA omega-3 fatty acids as dietary supplements is safe and lawful under 21 CFR § 101.14, provided that daily intakes of EPA and DHA omega-3 fatty acids do not exceed 3 g/p/d from conventional food and dietary supplement sources. Further, FDA concluded that in order to help ensure that a consumer does not exceed an intake of 3 g/p/d of EPA and DHA omega-3 fatty acids from consumption of a dietary supplement with the qualified claim, an EPA and DHA omega-3 fatty acid dietary supplement bearing a qualified claim should not recommend or suggest in its labeling, or under ordinary conditions of use, a daily intake exceeding 2 grams EPA and DHA.

It is instructive to review FDA's rationale, discussion and conclusions on this to determine the safety of consumption of EPA and DHA in either supplement or conventional food forms. These are excerpted below and demonstrate that FDA is comfortable with the safety of the omega-3 fatty acids EPA and DHA as long as consumption is below 3 grams/person/day. This analysis is fully applicable to Twin Rivers Technologies Omega-3 Triglyceride Concentrate (N3-TG-3020) in terms of its inherent use and safety.

Safety Review Excerpted from FDA September 8, 2004 Letter Outlining How They Determined Safety of Omega-3 Fatty Acids to Permit a Qualified Health Claim for Conventional Foods

> Under 21 CFR §101.14(b)(3)(ii), if the substance is to be consumed at other than decreased dietary levels, the substance must be a food or a food ingredient or a component of a food ingredient whose use, at levels necessary to justify a claim, must be demonstrated by the proponent of the claim to FDA's satisfaction to be safe and lawful under applicable food safety provisions of the Federal Food, Drug, and Cosmetic Act.
>
> The Wellness petition (Docket No. 2003Q-0401) stated that omega-3 fatty acids, as EPA and DHA, have been a naturally occurring ingredient in foods consumed safely in the United States prior to January 1, 1958, and that there is no evidence that when consumed either in foods or as dietary supplements there is a cumulative effect in the diet that is unsafe. The Martek petition (Docket No. 2003Q-0401) stated that omega-3 fatty acids occur in conventional foods with a long history of safe use, such as fish, and are generally recognized as safe (GRAS) when used as direct food ingredients intended to increase omega-3 fatty acids. Some comments to the petition expressed an interest in using the omega-3 fatty acid qualified health claim for foods that contain EPA and DHA as a food ingredient from sources including fish oil and algal oil.

In order to meet the safe and lawful requirement for health claims (21 CFR §101.14(b)(3)(ii)), the use of EPA and DHA omega-3 fatty acid, when used in conventional food or as a dietary supplement at levels necessary to justify the claim, must be demonstrated, to FDA's satisfaction, to be safe and lawful. FDA evaluates whether the substance is "safe and lawful" under the applicable food safety provisions of the Act. For conventional foods, this evaluation involves considering whether the ingredient that is the source of the substance is GRAS, approved as a food additive, or authorized by a prior sanction issued by FDA (see 21 CFR §101.70(f)). Dietary ingredients in dietary supplements, however, are not subject to the food additive provisions of the act (see section 201(s)(6) of the Act (21 U.S.C. §321(s)(6)). Rather, they are subject to the adulteration provisions in section 402 of the Act (21 U.S.C. 342) and, if applicable, the new dietary ingredient provisions in section 413 of the Act (21 U.S.C. 350b), which pertain to dietary ingredients that were not marketed in the United States before October 15, 1994. The term "dietary ingredient" is defined in section 201(ff)(1) of the act and includes vitamins; minerals; herbs and other botanicals; dietary substances for use by man to supplement the diet by increasing the total daily intake; and concentrates, metabolites, constituents, extracts, and combinations of the preceding types of ingredients.

In 1997, FDA affirmed, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use to ensure that the total daily intake of EPA and DHA would not exceed 3.0 grams per person per day (g/p/d) (62 FR 30751; June 5, 1997; 21 CFR §184.1472). EPA and DHA are the major omega-3 fatty acids in fish oil and together comprise about 20 percent by weight of menhaden oil. FDA established maximum use levels of menhaden oil in certain foods because of concerns over possible adverse effects of fish oil consumption on bleeding time, glycemic control, and LDL cholesterol (62 FR 30751 at 30757; June 5, 1997). In 2002, FDA published a proposed rule to reallocate the uses of menhaden oil in conventional food, while maintaining the total daily intake of EPA and DHA from menhaden oil at a level not exceeding 3.0 g/p/d (67 FR 8744; February 26, 2002). FDA placed specific limitations, including the category of foods, the functional use of the ingredient, and the level of use, to ensure that the consumption of EPA and DHA from conventional food sources would not exceed 3.0 g/p/d. FDA then published a final rule on March 23, 2005, 70 FR Number 55 Part 184 at page 14530), to additionally require that menhaden oil not be used as an ingredient in foods in combination with other added oil that is a significant source of EPA and DHA to ensure that total intake from conventional food sources do not exceed 3.0 g/p/d.

In addition, FDA has not objected to certain GRAS notifications for additional sources of EPA and DHA as food ingredients (fish oils other than menhaden oil) (GRAS Notice Nos: GRN 000097, GRN 000102, GRN 000105, GRN 000109, GRN 000137, GRN 000138). These GRAS notices proposed maximum use levels consistent with those specified in the tentative final rule affirming, as GRAS, menhaden oil as a direct human food ingredient with specific limitations of use.

FDA has also responded without objection to a GRAS notification on algal oil DHA from Martek Biosciences Corporation. Martek estimated that the use of algal oil in a number of food categories at the maximum proposed use levels would result in a mean exposure of no more than 1.5 grams of DHA per day (GRAS Notice No. GRN 000137).

The mean exposure to EPA and DHA from menhaden oil in all conventional food categories is estimated to be 2.7 g/p/d (67 FR 8744 at 8746; February 26, 2002). This is a conservative estimate with substantial margin for safety, and the agency believes, consistent with its prior decision on

the use of a qualified health claim for DHA and EPA omega-3 fatty acids (October 31, 2000 letter), that the addition of menhaden oil to food products has not come close to this conservative mean estimate exposure. FDA further believes that the GRAS uses for which it received a GRAS notification for other sources of EPA and DHA omega-3 fatty acids also provide conservative estimates of exposure and that the addition of these EPA and DHA sources to food products do not come close to the conservative mean estimates. Not all foods in the marketplace within those permitted food categories would contain menhaden oil or other sources of EPA and DHA omega-3 fatty acids that substitute for other edible fat or oil. Also, because not all foods that a consumer eats every day would contain menhaden or other EPA and DHA oil used as a substitute oil, the actual total daily intakes of EPA and DHA from menhaden or other EPA and DHA oil for an average person should be significantly below 3.0 g/p/d (67 FR 8744 at 8746; February 26, 2002).

It is difficult to estimate the actual total consumption of EPA and DHA. The Continuing Survey of Food Intakes by Individuals (CSFII 1994-1996, 1998) estimated EPA and DHA intakes from conventional foods. The 50th percentile intake of EPA and DHA from the survey was between 0.06 g and 0.07 g for adult women and 0.07 g and 0.1 g for adult men. The 90th percentile intake was between 0.18 g and 0.22 g for women and between 0.20 g and 0.43 g for men. Thus, EPA and DHA consumption from conventional foods in the United States is low. FDA is not aware of any nationally representative consumption data on EPA and DHA from dietary supplements. In the October 31, 2000 letter, FDA expressed concern about the exposure to EPA and DHA omega-3 fatty acids potentially exceeding 3.0 g/p/d if a qualified health claim were to appear on dietary supplements. This concern was due to conventional foods containing omega-3 fatty acids that were on the market; the use of structure/function claims on products containing EPA and DHA omega-3 fatty acids, which may promote product purchase; and dietary supplements that FDA found in the marketplace that contained significant amounts of EPA and DHA.

In a September 8, 2004 letter to Martin Hahn of Hogan and Hartson, FDA extended the requested use of this qualified health claim and it is now extended to conventional foods. The agency believes that there is likely to be some increased consumption of EPA and DHA omega-3 fatty acids based on conventional foods that bear the qualified health claim; however, the amounts of EPA and DHA that can be used and the foods in which such food ingredients can be safely used are limited. The agency has established specific limitations of use under its menhaden oil GRAS rule (62 FR 30751; June 5, 1997), proposed and tentatively finalized reallocation of the use of menhaden oil without changing total exposure levels (67 FR 8744; February 26, 2002, 69 FR 2313; January 15, 2004). (NOTE: the finalized allocation has since been published on March 23, 2005, 70FR Number 55 Part 184 at page 14530). Also, manufacturers that have submitted GRAS notifications for other sources, to which the agency has not objected, have established conditions of use similar to those in the menhaden oil GRAS rule.

In the October 31, 2000 letter, FDA stated that a consumer could consume nearly 1 gram of EPA and DHA per day in the diet from conventional foods. The agency is uncertain about how much consumers will increase their intake of EPA and DHA omega-3 fatty acids from EPA and DHA containing conventional foods and dietary supplements due to the extended use of the qualified health claim. In order to help consumers gauge their total intake of EPA and DHA and to provide them a way to keep their intake of EPA and DHA within 3 grams per day, FDA intends to consider, as a factor in the exercise of its enforcement discretion, that conventional foods and dietary supplements that bear an omega-3 fatty acid qualified health claim declare the amount of EPA and DHA per serving in the claim. FDA recommends that the information on EPA and DHA content

for use in a qualified health claim for EPA and DHA omega-3 fatty acids and reduced risk of CHD be presented in a manner that is consistent with FDA's guidance entitled, "FDA Nutrition Labeling Manual--A Guide for Developing and Using Data Bases." The dietary supplement may declare the amount of EPA and DHA per serving in "Supplement Facts," instead of making the declaration in the claim. Also, to ensure further that consumers do not exceed a 3.0 g/p/d intake, FDA will educate consumers not to exceed 3.0 g/p/d from all food and dietary supplement sources through print and web outreach information.

Further, FDA intends to consider, as a factor in the exercise of its enforcement discretion, that dietary supplements not recommend or suggest in labeling that consumers ingest more than 2 grams of EPA and DHA per day. FDA encourages manufacturers to limit their dietary supplement products bearing the qualified health claim to products recommending or suggesting daily intake of 1 gram or less of EPA and DHA omega-3 fatty acids.

Based on the data and information that FDA considered, which includes data and information that FDA relied upon in reaching its conclusions about the safety of EPA and DHA omega-3 fatty acids in its GRAS affirmation of menhaden oil, the data and information in the 1991 proposed (56 FR 60663; November 27, 1991) and 1993 final rules (58 FR 2683; January 6, 1993), and its current scientific literature review for other possible safety concerns, FDA concludes that the use of EPA and DHA omega-3 fatty acids used as a GRAS ingredient, consistent with FDA's GRAS rule for menhaden oil and GRAS notifications to which FDA did not object, the use of omega-3 fatty acids as a dietary supplement is safe and lawful under 21 CFR 101.14 provided that daily intakes of EPA and DHA omega-3 fatty acids from conventional food and dietary supplement sources do not exceed 3.0 g/p/d.

From the foregoing analysis and rulemaking decisions of FDA on the GRAS affirmation of menhaden oil and of EPA and DHA, as well as on the submitted GRAS Notices where the agency had no objection to the conclusions of being GRAS including a specific submission on an omega-3 fish oil concentrate, Twin Rivers Technologies Omega-3 Triglyceride Concentrate (N3-TG-3020) is considered GRAS for the proposed uses specified in the regulations under the conditions described and at the maximum use levels described in Table 7.

The EPA and DHA levels in the Omega-3 Triglyceride Concentrate (N3-TG-3020) are increased above those found in naturally occurring fish oils by a very selective process involving separation of different fatty acid chain lengths from fish oils via either methylation or ethylation into fatty methyl or ethyl esters followed by distilling the short chain, non-omega-3 methyl or ethyl esters away from the longer chain methyl or ethyl esters and then re-esterifying the longer chain methyl or ethyl esters with glycerine to form a triglyceride, whose chain lengths are now concentrated in omega-3 content. Analysis of the triglycerides that comprise the Omega-3 Triglyceride Concentrate (N3-TG-3020) indicate that these glycerides are structurally unchanged and their positional distributions within the glyceride are comparable to the starting fish oil. Based on this

similarity, Omega-3 Triglyceride Concentrate (N3-TG-3020) would be expected to be metabolized by the body in a similar manner as naturally occurring fish oil constituents.

Other Recent Publications

In addition, several extensive searches of the scientific literature were conducted to determine if there were any new publications relating to the safety of EPA and DHA since FDA's final regulation on the GRAS affirmation of menhaden oil. No new safety issues were identified. A few pertinent current reviews were identified where the utility of fish oils were reviewed and their clinical usefulness and significance discussed. These are briefly discussed below.

As part of a review of marine n-3 PUFAs and coronary heart disease, Schmidt *et al.,* (2005) recently reviewed the safety of omega-3 fatty acids and reported that while there has been concern about an increased risk of bleeding, especially after consumption of large doses of fish oil concentrates, there is little clinical evidence in support of this even in patients treated with aspirin or anticoagulants. They noted that it has been claimed that n-3 PUFA may deteriorate glycemic control in patients with diabetes mellitus, but recent data have discarded this hypothesis. In addition, while immune responses can be modulated by n-3 PUFA, there is no evidence that intake of n-3 PUFA is associated with an increased risk of serious infections or cancer (Larsson *et al.*, 2004). In a separate review, Schmidt *et al.,* (2005) reviewed the results of clinical trials on n-3 PUFA on clinical endpoints and provided recommendations for the use of n-3 PUFA in the prevention and treatment of coronary heart disease in patients after a myocardial infarct as well as discussed other areas where further studies are needed in the areas of reocclusion rates of venous grafts as well as in addressing the antiarrhythmic effects of marine n-3 PUFAs. These data are important because the n-3 PUFA possess multiple effects including anti-inflammatory, anti-arrhythmic and antiplatelet effects that may benefit patients both acutely and in the long-term, by reducing infarct size. Additionally, they noted the need for studies on patients with hypertriglyceridemia, including patients with metabolic syndrome.

Lombardo and Chicco (2005) provided a review of the effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. The human studies assessed data from both nondiabetic and diabetic patients. It is well known that hypertriglyceridemia and elevated low-density lipoprotein cholesterol (LDL-C) and lower HDL-C concentrations are important risk factors for coronary heart diseases. Human studies have shown that fish oil supplements and diets containing fish oil (enriched on EPA and DHA fatty acids) are useful to reduce plasma triglyceride and VLDL lipoprotein concentrations, especially in the postprandial state. The mechanism for the reduction of triglyceride rich lipoprotein involves both a decrease in VLDL synthesis and an increase in the fractional catabolic rate of VLDL (triglyceride clearance). Moreover, a higher rate of fatty acid oxidation was observed in humans fed PUFAs-rich diets.

Concerning middle-aged and elderly patients with increased risk of coronary heart disease, Zhengling *et al.* (2004) have recently shown that in a saturated fat/cholesterol-restricted diet, a high fish oil content favorably affects VLDL and HDL subspecies,

shifting lipoproteins subfractions to a less atherogenic pattern. Patients with type IIb or IV hypertriglyceridemia, given a standardized test meal, showed that n-3 fatty acids suppress both hepatic and intestinal Apo B secretion/synthesis and altered triglyceride-rich lipoprotein metabolism (changing postprandial triglyceride, cholesterol, Apo B48 and increasing LDL particle size (Tinker *et al.,* 1999). Moreover, regarding blood cholesterol levels and human atherosclerosis, all these studies could represent additional mechanisms for the reduced heart disease risk associated with n-3 fish oil consumption.

Based on epidemiological findings, the intake of n-3 fatty acids in the diet has been associated with a reduced cardiovascular risk. The mechanisms of risk reduction have been widely explained, either as a consequence of the lipid /lipoprotein changes or as platelet/function and hemorrheological changes, including reduced thromboxane B2 formation and red cell aggregability and viscosity. Monocyte macrophage changes have also been observed, leading to reduced formation of interleukin-1 and platelet aggregation factors, resulting in inhibited growth factor formation and promoting nitric oxide-induced endothelial relaxation (Nestel, 2000). Furthermore, middle hypotensive and antiarrhythmic effects of n-3 fatty acids have been shown in cell cultures and human studies (Harper and Jacobson, 2001). The antihypertensive effect of n-3 fatty acids may depend on vascular effects with improved endothelial vasodilator function, reduced reactivity of vascular smooth muscle of resistant vessels and increased vascular compliance.

In diabetics it is known that fish oil intake delays the development of glucose intolerance (Feskens *et al.*, 1991). Interestingly, a recent work by Stene and Joner (2004), in a larger nationwide case-control study in Norway, found a significant association between the use of cod liver oil during the first year of life and a lower risk of type 1 diabetes mellitus, perhaps through the antiinflammatory effects of long-chain n-3 fatty acids.

Concerning the effect of fish oil administration on the glycemic control in diabetic patients with type 2 and type 1 diabetes mellitus, conflicting results have been obtained. Some early studies on type 2 diabetes mellitus showed that fish oil may worsen glycemic control; however, the adverse effect of fish oil has almost invariably been established with large doses of omega-3 fatty acids (5.5–8 g/day) in studies lacking an appropriate control group and with a small number of subjects. Other authors found no changes or even improved insulin sensitivity in patients with type 2 diabetes mellitus.

A meta analysis (Friedberg *et al.,* 1998) of 26 different trials on the effect of fish oil administration on both glycemic control and lipid parameters in type 2 and type 1 diabetic subjects showed (i) a decrease in plasma triglyceride concentration and slight but significant increase in LDL-C, both findings being more prominent in type 2 diabetes mellitus; no changes in total cholesterol and HDL-C were observed, (ii) no significant changes in HbA_{1c} occurred in diabetic patients, (iii) fasting blood glucose levels increased with borderline significance in type 2 diabetic subjects and were significantly lower in type 1 diabetic subjects; (iv) besides, significantly, dose-response effects of EPA (g/day) on HbA_{1c} and triglycerides and DHA (g/day) on fasting blood glucose levels, HbA_{1c} and triglycerides, were demonstrated only in type 2 diabetic subjects.

Recently, Skurnick-Minot *et al.* (2004) demonstrated a decrease in whole-body adiposity and adipocyte size in type 2 diabetic insulin-resistant patients after 2 months of treatment with fish oil capsule (1.8 g of n-3 PUFA). In these patients, plasma adiponectin tended to increase without any deterioration or amelioration of insulin sensitivity at this stage. Moreover, the diabetes and nutrition study group of the Spanish Diabetes Association, in a 7-year prospective, population-based, multicenter study showed that normoalbuminuria and nephropathy regression in well-controlled diabetic patients with long-term diabetes (type 1 and 2) are associated with enhanced PUFAs and less saturated fatty acid consumption (Spanish Diabetes Association, 2004).

FDA Regulatory Status of Formulation Components of Omega-3 Triglyceride Concentrate (N3-TG-3020)

Constituent and amount added to the formulation of Omega-3 Triglyceride Concentrate (N#-TG-3020) are as follows:

Mixed Tocopherols	2mg/g

The review of the regulatory status of this formulation constituent which follows indicates that it is approved for direct addition to foods and the amounts used are not either in excess to that approved by regulation or above that necessary to achieve the intended physical or technical effect as an antioxidant, anticaking agent, etc.

Mixed Tocopherols

Mixed tocopherols are listed under §182.3890 as a generally recognized as safe food substance when used in accordance with GMPs. It is used in the formulation as an antioxidant or chemical preservative at 2000 ppm.

DISCUSSION

The omega-3 triglyceride concentrate that is the subject of this safety assessment is comprised primarily of triglycerides of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is known by the commercial name of N3-TG-3020. The intended applications of this omega-3 triglyceride concentrate will be for those uses in foods for which menhaden oil is permitted under 21CFR§184.1472 and as noted in the Final Rule FR March 23, 2005(Volume 70, Number 55 PP14530-14532). The maximum level of use will be set to provide the same concentrations of EPA+DHA as those provided by menhaden oil. Based on EPA+DHA content of 20 percent in menhaden oil and 55 percent in the N3-TG-3020 Omega-3 Triglyceride Concentrate, the corresponding maximum proposed use levels are reduced accordingly to 36 % of that of menhaden oil. These proposed uses and levels of addition to various food types are presented in Table 5. These uses have been recognized by FDA as GRAS and have also been recognized in several earlier GRAS Notice submissions referenced above including one for a marine oil concentrate. Because the combined EPA and DHA content of foods to which N3-TG-3020 Omega-3 Triglyceride Concentrate will be added is identical to that permitted for menhaden oil under the March 23, 2005 final rule, N3-TG-3020 Omega-3 Triglyceride Concentrate will merely provide an alternative to menhaden oil as a source of EPA and DHA in the diet. Thus, no incremental increase in potential intake of EPA and DHA

combined will result from the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate.

The estimated mean intake of EPA and DHA combined from all dietary sources in the general population, excluding infants under the age of one year, has been addressed in earlier FDA rulemaking (menhaden oil final rule; 62 FR 30751; June 5, 1997) where FDA estimated that the mean exposure to EPA and DHA from the use of menhaden oil in all food categories would be 2.8 grams/person/day. It was also addressed as well in the subsequent GRAS Notices referenced above. The total cumulative estimated mean intake of EPA and DHA combined from the proposed maximum use levels of N3-TG-3020 Omega-3 Triglyceride Concentrate listed in the foods in Table 5, as well as from all other dietary sources in this general population is also estimated to be 2.8 grams per person per day. This estimate reflects 100 percent market penetration of the proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate that are listed in Table 5. Because 100 percent market penetration of this product is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower. Further, this cumulative intake of EPA and DHA combined is still less than the safe limit for EPA and DHA combined of 3 grams per person per day established by the agency.

The proposed uses of N3-TG-3020 Omega-3 Triglyceride Concentrate will result in no incremental increase in the intake of EPA and DHA combined over that described for menhaden oil in the final rule Federal Register/Volume 70, Number 55 at page 14531 on March 23, 2005. [§184.1472 Menhaden oil- (a) (3) In accordance with §184.1(b)(2) the ingredient may be used in food only within the following specific limitations to ensure that total intake of eicosopentaenoic acid or docosohexaenoic acid does not exceed 3.0 grams/person/day. (4) To ensure safe use of the substance, menhaden oil shall not be used in combination with any other added oil that is a significant source of eicosapentaenoic acid or docosahexaenoic acid].

In addition, the safety of consumption of Omega-3 Triglyceride Concentrate (N3-TG-3020) used as an ingredient in food is based on its similarity to currently marketed fish oil products that have been the subject of several GRAS Notices referenced in this document , as well as the safety of ingestion of its major constituents, EPA and DHA. The safety of consumption of the whole product was determined by evaluating the source of the product, the production process, the nature and quantity of impurities, product specifications, and the identity and positional distributions of EPA and DHA in the glycerides comprising the product. Appropriate product specifications have been established to ensure that the final product is food grade, and compositional analysis of the product supports the presumption that there is no toxicological concern from any product impurities. Further, as long as the Omega-3 Triglyceride Concentrate (N3-TG-3020) use is in the food categories identified above at a level of 36 % of the maximum permissible levels of menhaden oil, and the resulting mean potential intake is less than 3.0 grams per day of EPA and DHA combined, it is safe, and GRAS, for addition to food.

CONCLUSION

Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively concluded that Omega-3 Triglyceride Concentrate, (N3-TG-3020) meeting the specifications cited above, and concentrated as described, is GRAS when used as an ingredient in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation when used at a maximum level of 36 % of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020), when used as described, is GRAS based on scientific procedures.

Stanley M. Tarka, Jr., Ph.D.	Date
W. Gary Flamm, Ph.D., FACT, FATS	Date
James W. Barnett, Jr., Ph.D., DABT	Date

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Appendix I.

Flow Diagram of TRT Manufacturing Process for N3-TG-3020 Omega-3 Triglyceride Concentrate

Omega-3 Process
Concept 3 (Erik's chai

Hendrickson, Carrie

From: Stanley Tarka [tarkagroup@comcast.net]
Sent: Wednesday, October 18, 2006 3:34 PM
To: Hendrickson, Carrie; Edwards, Alison
Subject: RE: GRAS Notice No. GRN 000200/signature pages and Chemistry Questions from October 17 2006 Conference Call
Importance: High
Attachments: Final Signature Page Expert Panel Assessment TRR October 17 2006.pdf

AM

Dear Carrie and Alison,
Thank you for taking the time yesterday to discuss GRN 000200 and the remaining points that you needed clarified. I am attaching a PDF file with the signatures of the Expert Panel reflecting their concurrence with the new specifications.

Also, I would like to clarify and respond to the following questions raised:

As a reference point on page 19 of the Expert Panel Statement (page 14 of the GRAS Notification) for the specifications you asked the following questions:

1. We state on Page 21 under Potential Contaminants for PCBs: The limits of detection achieved across these large categories of chemicals, depending upon the specific pesticide being considered were generally 1, 5, 10 or 25 ppb, while the limits of detection (LOD) for technical chlordane was 50-100 ppb and for **PCBs, 250-500 ppb**. This number differs from the specifications reported in the Table.

Response: The laboratory that is we use for PCB testing uses the CRN Monograph methodology (noted in the reference) which has a LOD low enough to assure we meet the NMT 0.09 mg/kg specification. The sentence on page 21 under Pesticides should read: "The limits of detection achieved across these large categories of chemicals, depending upon the specific pesticide being considered were generally 1, 5, 10 or 25 ppb, while the limits of detection (LOD) for technical chlordane was 50-100 ppb and for PCBs, the limit of detection was 0.005 mg/kg (5ppb)."

2. On the Specifications page 14 or 19, for the EPA Specs we state:
EPA and DHA: NLT 500 mg/g and not more than ("NMT") 600 mg/g.
FDA wants us to clarify if this is per gram of the ingredient or per gram of fat or lipid (terminology)

Response: The answer to this second question is that this is per gram of our ingredient, which is nearly 100% fat.

I hope that this answers all remaining questions. Please confirm that you have received and feel free to contact me if you have any additional questions.

Thanks.

Stanley

Stanley M. Tarka, Jr. Ph.D.
President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17013

10/19/2006

Ph: 717-243-9216
Fax: 702-993-5458
Email: Tarkagroup@comcast.net

From: Stanley Tarka [mailto:tarkagroup@comcast.net]
Sent: Monday, October 16, 2006 1:20 PM
To: 'Hendrickson, Carrie'
Subject: RE: GRAS Notice No. GRN 000200/signature pages
Importance: High

Hi Carrie,
Attached is a pdf with the signature pages that you requested for GRN 000200.

Please confirm that you received these and that they are acceptable.

Thanks in advance.

Stanley

Stanley M. Tarka, Jr. Ph.D.
Ph: 717-243-9216
Fax: 702-993-5458
Email: Tarkagroup@comcast.net

From: Hendrickson, Carrie [mailto:carrie.hendrickson@fda.hhs.gov]
Sent: Friday, October 13, 2006 5:42 PM
To: Stanley Tarka
Subject: GRAS Notice No. GRN 000200/signature pages

Stanley –

Regarding: GRN 200 (omega-3 triglyceride concentrate); signature pages

I am working to wrap up our review of the GRAS notice (omega-3 triglyceride concentrate) and it was brought to my attention that, in the materials you submitted by email on September 27, 2006, two pages were included that had unsigned signature lines. One page is the GRAS exemption claim, which should have Edward Steele's signature; the other page is the GRAS panel conclusion, which should have your signature along with those of W. Gary Flamm and James Barnett.

Given that certain specifications for the final food-grade product were changed in the September 27 documents, signatures on these pages would indicate that the GRAS exemption claim and the GRAS panel's conclusions apply to the finished product as now specified in these most recent documents. Could you send copies of the signed signature pages?

If you have any questions, please do not hesitate to give me a call.

Carrie

Carrie Hendrickson, Ph.D.
Consumer Safety Officer

U.S. Food and Drug Administration
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review

Contact information
tel: (301)436-1202
email: Carrie.Hendrickson@fda.hhs.gov

From: Stanley Tarka [mailto:tarkagroup@comcast.net]
Sent: Wednesday, September 27, 2006 9:56 AM
To: Hendrickson, Carrie
Cc: Edwards, Alison; tarkagroup@comcast.net
Subject: RE: questions regarding GRAS notice (GRN 200)
Importance: High

Dear Drs. Hendrickson and Edwards,

Please find attached to this email our response to each of the questions raised on GRN 00200. Also, I have attached revised specifications that reflect our latest learnings and to be competitive in the marketplace. These are noted on pages 12 and 14 of the Specifications and have also been reflect in the Expert Panel Statement. I have also removed Confidential from the manufacturing process.

Please feel free to contact me if you have any additional questions.

Thank you.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr. Ph.D.
Ph: 717-243-9216
Fax: 702-993-5458
Email: Tarkagroup@comcast.net

From: Hendrickson, Carrie [mailto:carrie.hendrickson@fda.hhs.gov]
Sent: Thursday, September 21, 2006 12:04 PM
To: 'tarkagroup@comcast.net'
Subject: FW: questions regarding GRAS notice (GRN 200)

Dr. Tarka -

Please confirm that you receive the email that I sent on Tuesday (September 19, 2006) requesting clarification/explanation of a few points in the Twin Rivers Technologies GRAS notice. I will be out of the office Friday and Monday and I want to be sure that you did get this information.

Thank you.

Carrie

Carrie Hendrickson, Ph.D.
Consumer Safety Officer

U.S. Food and Drug Administration
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review

Contact information
tel: (301)436-1202
email: Carrie.Hendrickson@fda.hhs.gov

From: Hendrickson, Carrie
Sent: Tuesday, September 19, 2006 11:10 AM
To: 'tarkagroup@comcast.net'
Subject: questions regarding GRAS notice (GRN 200)

Dr. Tarka

We have a few questions/points of clarification regarding Twin Rivers Technologies GRAS notice for omega-3 triglyceride concentrate (which we have designated at GRN 000200):

1. In the notice (either on p. 10 or the confidential flow diagram), you describe the filtration, bleaching, and steam stripping of the crude fish oil as "potential" or "possible" steps. Please provide clarification. Does this mean that individual steps may be eliminated if not required or that crude oil may be used directly (without refining) as a starting material for the fatty acid esterification process?

2. In your notice, you describe use of methanol or ethanol in the first esterification step and the use of sodium methoxide in the first and second esterification steps. Please address the possibility of methanol ethanol residues as these relate to the safety of the finished, food grade concentrate. Is methanol detected in the final product? What is the limit of detection? Please describe the ability of the method of manufacture to remove methanol residues.

3. You provided a copy of your method of manufacture flow diagram; you indicate that the entire page is confidential. OFAS notes that much of the information in the process flow diagram is discussed in narrative form on pages 10-11 and 20-21. The information in the narrative is relevant to the GRAS determination, and confidentiality of this information would not be consistent with the GRAS program. Please clarify what details are specifically confidential, with consideration of your GRAS determination and the information an "expert" would need to know about the method of manufacture to make a determination of safety.

4. It's our understanding that all ingredients (including processing aids) used in the manufacture of your ingredient are suitable for food use. Is this correct? (A clear statement of such is relevant to both your GRAS determination and our ability to evaluate your determination.)

5. In the present notice, the acid value specification is given as NMT 1%. The acid value is generally expressed in units of mg KOH/g of oil. Does the specification in the notice refer to an acid value of 10 mg KOH/g of oil or a free fatty acid value of 1% (or 0.5% by calculation)? Note: Either value would be greater than the CRN specification (acid value maximum 3 mg KOH/g) for fish oils or the CODEX specification (acid value maximum 0.6 mg KOH/g fat) for refined oils. Please clarify the specification for acid value given in the notice.

**we suggest that you also review your notice for other areas where a clearer statement of the units of measure (e.g., percent *of/by what*; mg *of what*) would minimize the possibility of misinterpretation of the information provided in the notice.

Please contact me if you need any clarification.

Carrie Hendrickson, Ph.D.

10/19/2006

Consumer Safety Officer

U.S. Food and Drug Administration
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review

Contact information
tel: (301)436-1202
email: Carrie.Hendrickson@fda.hhs.gov

Attachment: Final Signature Page
Expert Panel Assessment TRR...

CONCLUSION

Based on a critical evaluation of the publicly available data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively concluded that Omega-3 Triglyceride Concentrate, (N3-TG-3020) meeting the specifications cited above, and concentrated as described, is GRAS when used as an ingredient in the manufacture of food in the categories identified in the menhaden oil GRAS Affirmation regulation when used at a maximum level of 36 % of menhaden oil, and resulting in a mean potential intake of no more than 3.0 grams per day of EPA and DHA combined.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Omega-3 Triglyceride Concentrate (N3-TG-3020), when used as described, is GRAS based on scientific procedures.

(b)(6)

Stanley M. Tárka, Jr., Ph.D.

10/17/06
Date

(b)(6)

W. Gary Flamm, Ph.D., FACT, FATS

10/17/06
Date