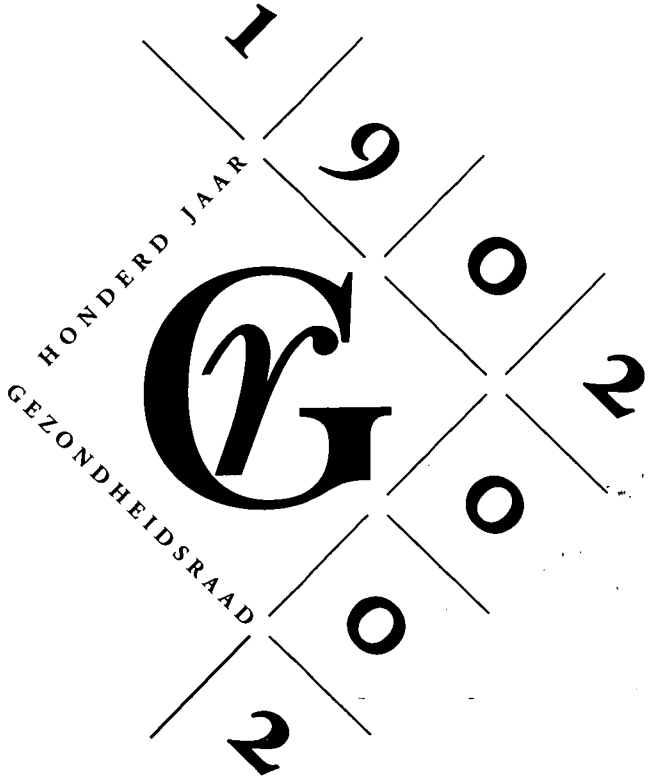
Docosahexaeenzuurrijke olie

Docosahexaenoic acid rich oil

Commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen
Committee on the Safety Assessment of Novel Foods



Gezondheidsraad

2002/03VNV

Docosahexaeenzuurrijke olie

Tweede beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten

Docosahexaenoic acid rich oil

Second opinion regarding consumer safety, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients

Gezondheidsraad:
Commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen (VNV)

Health Council of the Netherlands:
Committee on the Safety Assessment of Novel Foods

aan/to:

de Minister van Volksgezondheid, Welzijn en Sport/
the Minister of Health, Welfare and Sport

de Minister van Landbouw, Natuurbeheer en Visserij/
the Minister of Agriculture, Nature management and Fisheries

Nr. 2002/03VNV, Den Haag, 26 augustus 2002
No. 2002/03VNV, The Hague, August 26 , 2002

De Gezondheidsraad, ingesteld in 1902, is een adviesorgaan met als taak de regering en het parlement "voor te lichten over de stand der wetenschap ten aanzien van vraagstukken op het gebied van de volksgezondheid"(art. 21 Gezondheidswet).

De Gezondheidsraad ontvangt de meeste adviesaanvragen van de bewindslieden van Volksgezondheid, Welzijn & Sport; Volkshuisvesting, Ruimtelijke Ordening & Milieubeheer; Sociale Zaken & Werkgelegenheid en Landbouw, Natuurbeheer & Visserij. De Raad kan ook eigener beweging adviezen uitbrengen. Het gaat dan als regel om het signaleren van ontwikkelingen of trends die van belang kunnen zijn voor het overheidsbeleid.

De adviezen van de Gezondheidsraad zijn openbaar en worden in bijna alle gevallen opgesteld door multidisciplinair samengestelde commissies van -op persoonlijke titel benoemde- Nederlandse en soms buitenlandse deskundigen.

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect tot public health issues" (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport; Housing, Spatial Planning & the Environment; Social Affairs & Employment, and Agriculture, Nature management & Fisheries.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

Deze publicatie kan als volgt worden aangehaald:
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Ons kenmerk	: 2002/03VNV, U946/MR/al/622-BP
Datum	: 26 augustus 2002

Mijnheer de minister,

Dit schrijven dient ter beantwoording van de adviesaanvraag over de veiligheid van nieuwe voedingsmiddelen en nieuwe voedselingrediënten, die door u mede namens de Minister van Landbouw, Natuurbeheer en Visserij aan de Gezondheidsraad is voorgelegd. Aan de orde is een zogenoemde tweede beoordeling, conform de Europese verordening 258/97, van een docosahexaeenzuurrijke olie. De aanvrager, de firma OmegaTech, wil deze olie op de markt brengen onder de merknaam DHA Gold™ (DHA is de afkorting van *docosahexaenoic acid*). De olie wordt geproduceerd door de microalg *Schizochytrium* sp. Het nieuwe product komt niet direct beschikbaar voor de consument, maar het zal worden verwerkt in verschillende categorieën levensmiddelen. De beoordeling is verricht door de Commissie 'Veiligheidsbeoordeling nieuwe voedingsmiddelen' van de Gezondheidsraad (Commissie VNV).

De eerste beoordeling van de aanvraag voor markttoelating is verricht in het Verenigd Koninkrijk door het '*Advisory Committee on Novel Foods and Processes*' (ACNFP) van de Engelse voedselautoriteit '*Food Standard Agency*'. De ACNFP concludeert dat de DHA-rijke olie veilig is voor consumptie als voedselingrediënt in de toepassingen die in het dossier vermeld staan. Zij baseerde haar oordeel op de wetenschappelijke kennis van het vetzuurmetabolisme, bestaande blootstelling van de mens via de voeding en op door de aanvrager uitgevoerde veiligheidsonderzoeken. Volgens de ACNFP heeft de aanvrager, die de olie produceert, toegezegd de levensmiddelenfabrikanten die de olie in hun producten willen verwerken, voor te lichten over de aanbevolen dagelijks te consumeren hoeveelheid DHA. De consument moet van deze informatie op de hoogte worden gebracht door een goede etikettering van de DHA verrijkte eindproducten.

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Gezondheidsraad Vice-voorzitter
Health Council of the Netherlands

Onderwerp : Tweede beoordeling veiligheid Docosahexaeenzuurrijke olie
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De Commissie VNV baseert haar oordeel op het rapport van de eerste beoordeling door de ACNFP en op de informatie in het dossier. De Commissie VNV stemt grotendeels in met de Engelse beoordeling. De kwaliteit van het productieproces lijkt gewaarborgd. De olie bestaat voor 35 tot 45 % uit DHA en bevat geen ongewenste bestanddelen of microbiologische verontreinigingen.

Vanuit voedingskundig oogpunt is er geen bezwaar tegen het gebruik van de olie waarvan de geïdentificeerde bestanddelen al in bepaalde mate in onze gewone voeding aanwezig zijn. Met het oog op te behalen gezondheidswinst bevelen verschillende, nationale en internationale, wetenschappelijke organisaties en adviesraden een bepaalde dagelijkse inneming aan van de zogeheten visolievetzuren, eicosapentaeenzuur (EPA) en DHA. Niet alleen wereldwijd, maar ook binnen Europa varieert deze hoeveelheid flink, namelijk van 200 mg tot bijna 2000 mg per persoon per dag. De Commissie VNV constateert dat in Nederland een relatief lage inneming van visolievetzuren wordt aanbevolen. De Gezondheidsraad heeft vorig jaar het advies 'Voedingsnormen: energie, eiwitten, vetten en verteerbare koolhydraten' uitgebracht (GR01). Hierin wordt gesteld dat een gemiddelde dagelijkse inneming van 200 mg visolievetzuren voldoende is voor gunstige gezondheidseffecten, te weten een verminderde sterfte door hart en vaatziekten. Afzonderlijk normen voor EPA en DHA konden niet worden opgesteld.

De hoeveelheid DHA die de doorsnee Engelse consument via de gewone voeding binnenkrijgt is ongeveer 107 mg. Aanvullend op de eerste beoordeling stelt de Commissie VNV vast dat de innemingsgegevens van DHA voor inwoners van de andere EU-lidstaten onvoldoende onderbouwd zijn. Deze zijn namelijk gebaseerd op de Nationale voedselbalansen van de FAO die slechts aangeven hoeveel van een bepaald voedingsmiddel per hoofd van de bevolking beschikbaar is. De commissie vraagt zich dan ook af in hoeverre de Engelse consument, waarvan wel gedetailleerde en betrouwbare voedselconsumptie-informatie in het dossier wordt verstrekt, representatief is voor de Europese bevolking.

De aanvrager stelt de gewenste totale DHA inneming op 550 mg per dag. De hoeveelheid DHA in de gewone voeding zal dan met ongeveer 440 mg DHA afkomstig van de nieuwe olie kunnen worden aangevuld. Hiervoor zou één of twee porties van een product dat verrijkt is met de olie DHA Gold™ voldoende moeten zijn. Echter, dit innemingsniveau ligt dicht bij de bovengrens van DHA inneming die door de US FDA (1997) als veilig wordt beschouwd, te weten 1500 mg per dag. De ACNFP concludeert uit de resultaten van uitgebreid toxicologisch onderzoek bij proefdieren en veiligheidsonderzoek bij mensen, dat er geen aanwijzingen zijn dat een dergelijk

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hoog consumptieniveau zou leiden tot nadelige gezondheidseffecten. De Commissie VNV is het hiermee eens en heeft vanuit veiligheidsoverwegingen geen bezwaar tegen het op de markt brengen van het nieuwe product met de beoogde toepassing van een extra dagelijkse inneming van ongeveer 440 mg DHA. De Commissie VNV voorziet echter praktische problemen gezien de marge van een factor drie tussen de gewenste inneming en de veilige bovengrens. Een belangrijk punt van kritiek is dat de aanvrager het assortiment levensmiddelen, waarin de DHA-rijke olie verwerkt zal gaan worden, niet nader heeft gespecificeerd. Ook is zij niet overtuigd dat de consument zich zal beperken tot één van de DHA verrijkte producten per dag, zoals voorgesteld door de aanvrager. Daarnaast brengt de Commissie VNV onder de aandacht dat er in de Nederlandse supermarkten al producten met een verhoogd DHA-gehalte liggen.

De commissie vindt de toezegging aan de ACNFP, dat de producent-en-aanvrager OmegaTech de eindfabrikanten van het DHA verrijkte voedingsmiddel zal adviseren over het juiste gebruik van DHA Gold, te vaag geformuleerd. De Commissie VNV heeft grote twijfels dat het overschrijden van de veilige bovengrens hierdoor zou kunnen worden voorkomen. Het is volstrekt onduidelijk, hoe de aanvrager wordt geacht zicht te houden op alle mogelijke toepassingen van het nieuwe ingrediënt. De Commissie VNV stelt voor de nieuwe olie alleen toe te staan in een beperkt en goed gedefinieerd productassortiment. Zij is het eens met de ACNFP die nadere etiketteringseisen stelt zodat de consument geïnformeerd wordt over zowel de aanbevolen dagelijkse DHA inneming als de aanvaardbare bovengrens van deze inneming. De commissie wijst tevens op de mogelijkheid van postmarketing onderzoek om inzicht te krijgen in hoeverre de garanties voor inneming voldoen.

De Commissie VNV stelt vast dat er behoefte is aan een betere wetenschappelijke onderbouwing van het maximaal veilige innemingsniveau van de visolievetzuren om meer duidelijkheid te verschaffen over mogelijke langetermijneffecten. De vraag in hoeverre het, bij overschrijding van de bovengrens, gevonden effect op de bloedstolling zou kunnen leiden tot nadelige gezondheidseffecten, is met de huidige stand van wetenschap niet te beantwoorden.

Tenslotte wijst de Commissie VNV erop dat, indien het nieuwe product verwerkt zal gaan worden in voeding voor zuigelingen, aanvullende toxicologische informatie vereist is. De beschikbare gegevens zijn onvoldoende om de veiligheid voor een dergelijke toepassing te kunnen beoordelen.

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Gezondheidsraad Vice-voorzitter
Health Council of the Netherlands

Onderwerp : Tweede beoordeling veiligheid
Docosahexaeenzuurrijke olie
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Resumerend heeft de Commissie VNV geen bezwaar tegen het op de Europese markt toelaten van het nieuwe ingrediënt DHA Gold, mits dit wordt verwerkt in een beperkt en goed omschreven levensmiddelenassortiment. Elk product moet worden voorzien van een duidelijk etiket onder vermelding van de aanbevolen dagelijkse hoeveelheid visolievetzuren waar DHA deel vanuit maakt. Uit voorzorg beveelt de commissie aan om ook het te consumeren maximale aantal porties per dag op het etiket te noemen, gezien de onduidelijkheid over de gevolgen bij overschrijding van de veilig geachte bovengrens van 1500 mg DHA.

Ik onderschrijf de conclusies en aanbevelingen van de Commissie VNV.

Hoogachtend,

prof. dr JGAJ Hautvast

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Letter to the Dutch Minister of Health, Welfare and Sport

On August 26, 2002, professor JGAJ Hautvast, Vice-president of the Health Council of the Netherlands wrote as follows to the Minister of Health, Welfare and Sport:

This letter has been prepared in reply to your request for advice regarding the safety of novel foods and food ingredients, also made on behalf of the Minister of Agriculture, Nature Management and Fisheries. The subject in question is a so-called second opinion, in accordance with European Regulation 258/97, concerning a docosahexaenoic-acid-rich oil. The applicant, the company OmegaTech, is going to market this oil under the trade name of DHA Gold™ (DHA: docosahexaenoic acid). The oil is being produced using the microalgae *Schizochytrium* sp. The new product will not be directly available for consumers, but will be incorporated in variety of foodstuffs. This assessment has been carried out by the 'Committee on Safety Assessment of Novel Foods' (VNV Committee) of the Health Council of the Netherlands.

The initial assessment of the application for market introduction was carried out in the United Kingdom by the Advisory Committee on Novel Foods and Processes (ACNFP) of the Food Standards Agency. The ACNFP concluded that the DHA-rich oil is safe for consumption as a food ingredient to be used as indicated in the dossier. The Committee based its view on scientific knowledge of fatty-acid metabolism, current human dietary exposure levels and safety studies carried out by the applicant. The ACNFP states that the applicant, who manufactures the oil, has agreed to inform those food manufacturers wishing to incorporate the oil into their products about the recommended daily dietary allowance of DHA. This information must be made available to consumers by means of appropriate labelling of the DHA-enriched final food products.

The VNV Committee based its views on the report of the initial assessment by the ACNFP and on the information contained in the dossier. The VNV Committee largely agrees with the British assessment. It appears that, in terms of quality, the production process incorporates the necessary safeguards. The DHA content of the oil is 35 to 45%, and it contains neither undesirable components nor microbiological contaminants.

From the nutritional point of view, there is no objection to the use of this oil whose identified ingredients are already present in our normal diet to some degree. Aiming at potential health benefits, various national and international scientific organisations and advisory councils recommend a daily intake of the so-called fish-oil fatty acids, eicosapentaenoic acid (EPA) and DHA. This recommended intake varies considerably, not just at the global level, but also within Europe itself. The values range from 200 mg to almost 2000 mg per individual per day. The VNV Committee notes that, in the Netherlands, a relatively low intake of fish-oil fatty acids is recommended. Last year, the Health Council has published an advisory report entitled 'Dietary reference intakes: energy, proteins, fats, and digestible carbohydrates' (GR01). One of the conclusions in this report is that an average daily intake of 200 mg of fish-oil fatty acids is sufficient to produce beneficial health effects, namely reduced mortality from cardiovascular diseases. It was not possible to draw up separate standards for EPA and DHA.

On average, UK adults currently consume 107 mg of DHA per day as part of their normal diet. In addition to the initial assessment, the VNV Committee concludes that the DHA intake data for inhabitants of the other EC member states is too poorly supported. This is because these are based on the FAO Food Balance Sheets, which only indicate how much of a given foodstuff is available per head of population. The Committee therefore wonders to what extent UK consumers, about whom the dossier does provide detailed and reliable food-consumption information, are representative of the European population as a whole.

The applicant proposes 550 mg per day as the intended total DHA intake value. The amount of DHA in the normal diet could thus be supplemented with about 440 mg of DHA from the new oil. To realize this, one or two portions of a product that has been enriched with DHA Gold oil should be sufficient. However, this level of intake is close to the upper DHA intake level that is considered to be safe by the United States FDA (1997), namely 1500 mg per day. Based on the results of extensive toxicological studies in experimental animals and safety studies in humans, the ACNFP concludes that there is no evidence that such high levels of consumption would lead to adverse health effects. The VNV Committee concurs with this view. On safety grounds, it has therefore no objections to the new product being placed on the market for the intended purpose of achieving an additional daily intake of about 440 mg of DHA. However, the VNV Committee does foresee practical problems given the margin of a factor of three between the intented intake and the safe upper level of intake. A major point of criticism is that the appli-

cant has failed to provide specific details of the range of foodstuffs into which the DHA-rich oil is actually going to be incorporated. Also, the Committee is not convinced that consumers would limit themselves to just one of the DHA-enriched products per day, as suggested by the applicant. In addition, the VNV Committee would like to point out that Dutch supermarkets already sell products with elevated DHA levels.

The applicant company OmegaTech, which produces the new oil, has agreed to advise end-producers of the DHA-enriched foodstuffs concerning appropriate use of DHA Gold. However, the VNV Committee feels that this promis is too vaguely worded. The VNV Committee very much doubts that this would prevent the safe upper intake level from being exceeded. It is by no means clear how the applicant can be expected to monitor all possible uses of the new ingredient. The VNV Committee proposes that the new oil should only be approved for use in a limited and well defined range of products. It agrees with the ACNFP, which has imposed additional labelling requirements to ensure that consumers are informed both about the recommended dietary allowance of DHA and the safe upper intake level. The Commission would also like to highlight the option of post marketing surveillance, to determine whether the intake guarantees can be met.

The VNV Committee has established that there is a need for improved scientific support for the maximum safe level of intake of fish-oil fatty acid, providing a better understanding of possible long-term effects. It has been found that exceeding the upper intake level has an effect on blood coagulation. Given the current level of scientific knowledge, it is not known to what extent this might result in adverse health effects.
Finally, the VNV Committee points out that additional toxicological information is required in case the DHA-rich oil will be used in infant formulae. The toxicological data provided in the dossier is insufficient for an evaluation of the safety of this specific application.

In summary, the VNV Committee does not object to authorisation of the new ingrkdient DHA GoldTM on the European market, provided that it is incorporated into a limited and well-defined range of foodstuffs. Each product must be clearly labelled, indicating the recommended daily dietary allowance for fish-oil fatty acids, the group of substances to which DHA belongs. By way of precaution, the Committee recommends that also a maximum daily number of servings should be indicated on the label, because it is not known for certain whether adverse health effects can be excluded by exceeding an intake of 1500 mg.

I endorse the conclusions and recommendations of the VNV Committee,

(signed) professor JGAJ Hautvast

Literatuur/Literature

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SCF99 Opinion concerning the scientific basis for determining whether food products, derived from genetically modified maize, could be included in a list of food products which do not require labelling because they do not contain (detectable) traces of DNA or protein. Brussels, Scientific Committee on Food of the EU 1999.

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A De adviesaanvraag/Request for advice

B De commissie/The committee

C EU-procedure/EU-procedure

D Samenvatting van het dossier/Executive summary of the dossier

E Eerste beoordeling/First assessment

Bijlagen/Annexes

Bijlage **A**

De Adviesaanvraag/Request for advice

Op 18 augustus 1999 schreef de Minister van Volksgezondheid, Welzijn en Sport aan de Voorzitter van de Gezondheidsraad (brief kenmerk GZB/VVB 993428):

Sinds mei 1997 is in de Europese Unie de Verordening (EG) 258/97 van kracht inzake nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Daarmee werd de veiligheidsbeoordeling onderdeel van een communautaire procedure.

Met u is reeds de mogelijkheid besproken de beoordeling door de Gezondheidsraad te laten uitvoeren. Ik verzoek u dan ook mede namens de Staatssecretaris van Landbouw, Natuurbeheer en Visserij, in deze eerste fase van uitvoering van de Europese Verordening (EG) 258/97 gedurende een aantal jaren, de veiligheidsbeoordeling gestalte te geven. Voor het onderbrengen bij de Gezondheidsraad pleit het experimentele karakter dat de beoordeling de eerste jaren zal hebben. Dit experimentele karakter komt voort uit het feit dat het een nieuw soort beoordeling betreft van deels nieuwe categorieën van voedingsmiddelen of voedselingrediënten. Het is namelijk een veiligheidsbeoordeling vóór het op de markt brengen van met name voedingsmiddelen van een genetisch gemodificeerde oorsprong en zogenaamd functional foods (nutriceutica). Daarnaast ga ik ervan uit dat de onafhankelijke wetenschappelijke advisering door de Gezondheidsraad het vertrouwen van de Europese Commissie en de andere lidstaten in het Nederlandse oordeel nog versterkt.

Mijn beleid is erop gericht een zo groot mogelijke openheid en transparantie te realiseren van de gevolgde procedure en de beoordeling om de consument vertrouwen te geven in de veiligheid van de nieuwe

voedingsmiddelen. Ik verzoek de Gezondheidsraad hieraan bij te dragen door bijvoorbeeld inzage te geven in de dossiers waarvoor een aanvraag wordt ingediend, waarbij uiteraard bedrijfsvertrouwelijke gegevens worden beschermd en door de criteria, waarop de veiligheid zal worden beoordeeld, te publiceren.

De Minister van Volksgezondheid, Welzijn en Sport,
w.g. dr E Borst-Eilers

English translation

On 18 August 1999, the Minister of Health, Welfare and Sport wrote as follows to the President of the Health Council of the Netherlands (under reference GZB/VVB 993428):

Since May 1997, Regulation (EC) 258/97 concerning novel foods and novel food ingredients has been in force in the European Union. Under the Regulation, the safety of novel foods has to be assessed as part of a community procedure.

Following discussions regarding the possibility of the Health Council making such assessments, the State Secretary for Agriculture, Nature Management and Fisheries and I wish the Council to take responsibility for safety assessment for a period of several years during the fist phase of implementation of European Regulation (EC) 258/97. It is considered appropriate that the Health Council should initially take on this role because the assessment activities will be of an experimental nature, involving both a new form of assessment (i.e. pre-marketing assessment) and, in many cases, new categories of foodstuff (primarily foodstuffs with a genetically modified basis and functional foods or nutraceuticals). We also feel that if assessments are made by a body with the Council's independent scientific status, this will support the validity of the Netherlands' opinion in the eyes of the European Committee and other member states.

My wish is to make the procedure and the assessment as open and transparent as possible, so as to enhance consumer trust in the safety of novel foods. I would like the Health Council to support this objective by, for example, allowing perusal of the application dossier (insofar as consistent with the need to protect the confidentiality of commercially sensitive information) and publishing the criteria upon which safety assessments are made.

The Minister of Health, Welfare and Sport,
(signed) dr E Borst-Eilers

Bijlage **B**

De commissie/The committee

- Prof. dr LM Schoonhoven, *voorzitter/chairman*
  emeritus hoogleraar entomologie; Wageningen Universiteit en Researchcentrum/
  emeritus professor of entomology; Wageningen University and Research centre
- Prof. dr CAFM Bruijnzeel-Koomen
  hoogleraar dermatologie/allergologie; Academisch Ziekenhuis Utrecht/
  professor of dermatology/allergology; Academic Hospital Utrecht
- Ir EJ Kok
  toxicoloog; RIKILT-DLO Wageningen/toxicologist; State Institute for Quality Control of Agricultural Products, Wageningen
- Dr CF van Kreijl
  moleculair-bioloog; RIVM Bilthoven/molecular biologist; National Institute of Public Health and the Environment, Bilthoven
- Prof. dr P van der Laan
  hoogleraar statistiek; Technische Universiteit Eindhoven/
  professor of statistics; Technical University Eindhoven
- Dr B Loos, *adviseur/advisor*
  COGEM, Den Haag/Committee on Genetic Modification, The Hague
- Dr F Nagengast
  gastro-enteroloog; Academisch Ziekenhuis Nijmegen/
  gastro-enterologist; Academic Hospital Nijmegen

- Dr JMA van Raaij
  voedingsfysioloog; Wageningen Universiteit and Researchcentrum/
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- Prof. dr G Schaafsma
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Bijlage **C**

EU-procedure/EU-procedure

Als een fabrikant een nieuw voedingsmiddel op de markt brengt, dient de veiligheid voor de consument gewaarborgd te zijn. In 1997 werd de Europese verordening van kracht waarin de procedure is geregeld voor de goedkeuring voor marktintroductie van een nieuw voedingsmiddel (EC97). Bij deze procedure zijn verschillende actoren betrokken. De aanvrager moet beoordelen of het product werkelijk 'nieuw' is, dat wil zeggen dat het nog niet eerder in de Europese Unie in substantiële mate voor menselijke voeding is gebruikt en ook niet wezenlijk gelijkwaardig is aan een bestaand product. (Voor een wezenlijk gelijkwaardig product kan worden volstaan met een kennisgeving van de marktintroductie.) Ook moet het niet gaan om een levensmiddelenadditief, aroma of extractiemiddel, omdat deze producten op een andere wijze worden beoordeeld. Voor een nieuw voedingsmiddel in de zin van de Europese verordening moet de aanvrager een veiligheidsdossier overleggen volgens aanbevelingen van de Europese Commissie (EC97a). Deze aanbevelingen zijn gebaseerd op rapporten van verschillende instanties die zich met het onderwerp nieuwe voedingsmiddelen bezighouden, te weten de OECD (OECD93, OECD96) en de WHO/FAO (FAO96, WHO91). Ook de Gezondheidsraad heeft zich al eerder over dit onderwerp gebogen (GR92). Sinds het verschijnen van de aanbevelingen van de EU wordt in internationaal verband gewerkt aan explicitering en aanpassing aan de stand van de wetenschap (FAO01, OECD98, OECD00, SCF99, SSC99, WHO00).

De fabrikant levert het volgens de richtlijnen samengestelde dossier in bij het land waar het product het eerst op de markt zal komen. Daarop komt de nationale veiligheidsbeoordelingsautoriteit in actie. In Nederland is dat de Minister van Volksgezondheid, Welzijn en Sport. Zij heeft de Gezondheidsraad verzocht haar van advies te dienen. De

Voorzitter van de Gezondheidsraad heeft hiertoe de commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen (commissie VNV) ingesteld.

De commissie beoordeelt op basis van de huidige stand van de wetenschap of de door de fabrikant geleverde gegevens juist en volledig zijn en of zij het eens is met diens conclusies. Zij maakt een verslag van haar bevindingen — ook volgens de Europese aanbevelingen (EC97a, deel III) — en biedt dat de minister aan. De minister formuleert het Nederlandse oordeel over een voedingsmiddel en brengt dat in bij het Europese overleg in het Permanent Comité voor levensmiddelen. Alle Europese lidstaten worden uitgenodigd hun oordeel (de zogeheten tweede beoordeling) te geven over het dossier en over de eerste beoordeling alvorens genoemd Comité een eindoordeel velt. Als een dossier veel vragen oproept, gaat er een adviesvraag van de Europese Commissie naar het Wetenschappelijk Comité voor de menselijke voeding. Komt men dan nog niet tot overeenstemming dan beslist de Europese Ministerraad.

English translation

When manufacturers bring novel foodstuffs onto the market, consumer safety has to be ensured. In 1997, a European Regulation (EC97) came into force, laying down the procedure for approving the market introduction of novel foodstuffs. The procedure recognizes various actors. The applicant must decide whether a product is a novel foodstuff, i.e. a substance that has not previously been available for human consumption to any substantial extent within the European Union and is not substantially equivalent to any existing product. (If a foodstuff is substantially equivalent to any existing product, it is sufficient to inform the authorities of its market introduction). Food additives, aromas and extracts are excluded from the provisions of the directive, since they fall within the scope of an established assessment regime. Before marketing a novel foodstuff, the applicant must compile a safety dossier that complies with the Recommendations of the European Commission (EC97a). These Recommendations are based on reports by a number of bodies that have studied the issue of novel foodstuffs, in particular the OECD (OECD93, OECD96) and the WHO/FAO (FAO96, WHO91). The Health Council of the Netherlands has also considered the question earlier (GR92). Since publication of the EU recommendations, international efforts have been made to clarify and adapt the latest scientific knowledge in the field (FAO01, OECD98, OECD00, SCF99, SSC99, WHO00).

Having compiled a dossier in line with the guidelines, the manufacturer has to submit it to the competent authority in the country where the product is to be marketed first. This dossier is assessed by the national safety assessment authority. In the Netherlands, this is the Minister of Health, Welfare and Sport, who is advised by the Health Council. The President of the Health Council has created a Committee on the

Safety Assessment of Novel Foods (VNV Committee) to advise the minister on behalf of the Council.

On the basis of the scientific state of the art, the committee has to decide whether the information provided by the manufacturer is accurate and complete and whether the manufacturer's conclusions are sound. The committee then draws up a report on its findings for the minister; this report must also comply with the European Recommendation (EC97a, part III). After considering the report, the minister formulates the Netherlands' opinion regarding the foodstuff in question, which is discussed at European level in the Standing Committee on Foodstuffs. All other European member states are invited to express a 'second opinion' regarding the dossier and the first opinion. The Standing Committee then arrives at a final judgement. If a dossier is particularly contentious, the European Commission calls upon the Scientific Committee on Food for advice. If consensus still cannot be reached, the issue is referred to the European Council of Ministers.

Bijlage **D**

Samenvatting van het dossier/ Executive summary of the dossier

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Application for the Approval of DHA-rich Oil

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

SUMMARY DOCUMENT

I. Specification of the Novel Food.

Description of Substance

Common or Usual Name

DHA-rich Oil. DHA-rich oil is derived from the heterotrophically grown marine microalgae, *Schizochytrium* sp. The proposed trade name for DHA-rich Oil Derived from Dried Microalgae is DHA Gold™. Other synonymous names for this product include DHA oil or DHA-rich microalgal oil, when differentiating algal oil from fish oil.

Chemical Abstract Service (CAS) Registry Number

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

Empirical Formula

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

Quality Control Specifications and Methods

DHA-rich oil is described as a yellow to light orange-colored oil derived from the heterotrophically grown marine microalgae, *Schizochytrium* sp., intended for use as a nutritional food ingredient. The oil is winterized, refined, bleached and deodorized. Vitamin E is added for nutritional supplementation. Antioxidants and stabilisers are added in accordance with Directive 95/2/EC. Specifications for the nutritional food ingredient are given in Table I-1.

Five lots of DHA-rich oil were produced in a campaign designed to demonstrate a reproducible and representative process capable of meeting proposed product specifications. Results of quality control testing on the five demonstration lots of DHA-rich oil (referred to as DHALIPNS lots 97398, 97399, 97400, 97401, and 97402) are shown in the main dossier.

Table I-1. Product specifications for DHA-rich oil derived from dried microalgae

PHYSICAL AND CHEMICAL TESTS		
Test	**Specification**	**Test Method**
Color (Lovibond)	Passes test	AOCS Method Cc 13b-45
Acid Value	Not more than 0.5 mg KOH/g	AOCS Method Cd 3d-63
Peroxide Value (PV)	Not more 5.0 meq/kg oil	AOCS Method Cd 8-53
Moisture and Volatiles	Not more 0.05%	AOCS Method Ca 2d-25
Unsaponifiables	Not more than 4.5%	AOCS Method Ca 6b-53
Trans-fatty acids	Not more than 2%	AOCS Method Cd 14-61
DHA content	Not less than 32.0%	POS AS.SOP-104
Hexane	Not more than 10 mg/kg	AOCS Method Ca 3b-87
ELEMENTAL ANALYSIS		
Test	**Specification**	**Test Method**
Arsenic	Not more than 0.20 mg/kg	POS AS.SOP-103
Copper	Not more than 0.05 mg/kg	POS AS.SOP-103
Iron	Not more than 0.20 mg/kg	POS AS.SOP-103
Mercury	Not more than 0.20 mg/kg	POS AS.SOP-103
Lead	Not more than 0.20 mg/kg	POS AS.SOP-103

KOH : potassium hydroxide
Meq: milliequivalents

II. Effect of the production process applied to the Novel Food.

Overview of Process
DHA-rich oil is produced *via* an algal fermentation process using a microalgae from the genus *Schizochytrium.* The algae are grown *via* a pure culture heterotrophic fed-batch fermentation process. The organism used is an improved strain of the original wild-type culture, *Schizochytrium* sp. ATCC 20888. The improved strain was derived using a classical mutagenesis/screening program, which employed well-accepted techniques commonly used in industrial microbial strain improvement programs. The intermediate dried microalgae product is fermented, recovered and dried. The resulting dried microalgae cells are extracted to produce a crude oil product, which is further refined into the finished product using process operations commonly employed in the vegetable oil industry.

III. History of the Source Organism Used as the Novel Food

Taxonomic Classification of *Schizochytrium* sp.: The Organism

DHA-rich oil is extracted from dried algae produced via fermentation using *Schizochytrium* sp. The current taxonomic placement of the thraustochytrids, and *Schizochytrium* sp. specifically, is summarized below:

Kingdom:	Chromista (Stramenopilia)
Phylum:	Heterokonta
Class:	Thraustochytridae
Order:	Thraustochytriales
Family:	Thraustochytriaceae
Genus:	Schizochytrium
Species:	ATCC 20888

Based on existing published and unpublished scientific data, it is concluded that: 1) there have never been any published reports on toxic compounds produced by thraustochytrids; 2) most of the toxic compounds produced by microalgae are produced by bluegreen algae or dinoflagellates, and *Schizochytrium* sp. is in a separate kingdom from both of these types of microalgae; 3) the two toxic compounds known to be produced in the Chromista (to which *Schizochytrium* sp. belongs) are largely restricted to two genera (domoic acid in *Pseudonitzschia* and prymnesin in *Prymnesium* spp.) which are in a separate class and phylum, respectively, from the thraustochytrids; 4) chemical tests by scientists at OmegaTech and Monsanto Analytical Labs in St. Louis indicate that domoic acid is not present in *Schizochytrium* sp. microalgae; 5) biological assay for prymnesin toxin is negative; and 6) acute and subchronic dietary toxicity studies in rats and a battery of cytotoxicity/mutagenicity tests have been completed with no effects attributed to algal toxins (see Section XIII.B).

Phenotypic characterization

OmegaTech developed an improved strain from the patented wild-type parent strain using a classical mutagenesis/screening program. This program utilized well-accepted techniques commonly used in industrial strain improvement programs. No recombinant DNA technology was employed. N230D was one of more than 1,000 randomly-chosen survivors of chemically mutagenised (NTG; 1-methyl-3-nitro-1-nitrosoguanidine) ATCC 20888 screened for variations in fatty acid content. This particular strain was valued for its improved DHA productivity.

NTG treatment-derived mutants sometimes acquire undesirable traits such as essential growth factor/vitamin requirements leading to growth retardation or altered morphology compared to the parent while attaining characteristics of interest. Therefore, laboratory studies were conducted to phenotypically characterize the mutant N230D and its parent ATCC 20888. These tests included morphological evaluation (light microscopy) throughout their growth cycle under standard growth and fermentation conditions as well as evaluations of differences in growth or substrate utilization patterns in a batch fermentation mode.

Under standard nutrient and environmental conditions for growth and DHA production, N230D performed equivalently to its parent in terms of overall growth and growth rate, carbon consumption and microscopic morphology. Cells can be characterized by a circular shape with varying sizes dependent on growth stage, and can acquire a highly vacuolar appearance later during fermentation and show lipid droplets or lipid bodies. Average cell size in both strains was ~6-8 microns. Glucose uptake, growth profiles and fermentation times were similar in triplicate fermentors for both strains. Moreover, microbial identification panels (Biolog Inc.) based on carbon source utilsation, though not designed for microalgae, showed a high degree of similarity. These results indicate that no adverse traits were produced in N230D due to mutagenesis. Superior performance of this strain could be due to enhanced carbon flow through the lipid production pathway.

IX Anticipated intake/extent of use of the Novel Food.

Anticipated Intake

It is proposed that DHA-rich oil derived from *Schizochytrium* sp. microalgae should be introduced into European countries as a novel food ingredient that could allow increased intakes of the long chain polyunsaturated fatty acid (LC PUFA) docosahexaenoic acid (DHA; 22:6n-3) by European consumers. European food and health authorities have recommended that individuals should increase their intakes of omega-3 fatty acids, including DHA, because raised intakes are associated with reduced risks of coronary heart disease. However, it is necessary to show that raising intakes of DHA so that consumers achieve these benefits does not introduce other risks either from DHA itself or from increased intakes of other components of the DHA-rich oil.

The average DHA intake for UK consumers is 107 mg/day and various estimates have suggested that this should be raised to between 200 and 800 mg per day in order to achieve benefits for coronary health. High level consumers at the 97.5th percentile only achieve 401 mg/day DHA but long term high level intake is probably better represented by 330 mg/day at the 95th percentile. For the purposes of this exercise it is assumed that a food manufacturer would wish to raise total daily mean intake target around 550 mg/day (for example 2 portions) DHA per major food group so that intakes of average consumers would be adequate to achieve health benefits without high level consumers being placed at any potential risk.

Although consumers are intended to use only one food source of DHA-enriched oils the effect of combining the two foods contributing the highest intakes was investigated. For bread and spreadable fats combined, the mean intake was 743 mg/day of DHA.

The use of DHA-rich oils as a food ingredient can raise intakes of DHA to potentially beneficial levels. High level intakes would not exceed 1.5g DHA at up to 350mg DHA per serving and such intakes are unlikely to be sustained in the long term. Intakes of cholesterol were marginally raised for high level consumers but phytosterol intakes were also raised by up to 17% in the same individuals.

At-risk Populations

Phytosterolemia or sitosterolemia

Phytosterolemia or sitosterolemia (with or without accompanying xanthomatosis) is a rare lipid storage disease inherited in an autosomal recessive pattern (Ling and Jones 1995 and Bhattacharyya et al. 1991). Phytosterolemia is characterized chemically by increased plant sterols and 5α-saturated stanols in plasma and tissue. Phytosterolemia is characterized pathologically by premature atherosclerosis, xanthomas, and some patients have developed hemolytic syndromes. The absorption rate of phytosterol is very high in these patients, and secretion into bile (of at least sitosterol) is less than that of cholesterol, yielding a sluggish turnover and excretion rate of phytosterols. In normal blood serum, the concentration of sitosterol is less than 1 mg/dl, and in untreated patients with phytosterolemia, serum sitosterol values in the range of 10 to 65 mg/dl have been reported. As of 1991, approximately 22 persons with this disease had been identified.

Diagnosis of phytosterolemia can be made in early childhood in patients who develop xanthomas. Treatment is largely palliative, excluding from the diet vegetable oils, shortening, margarine, nuts, seeds, chocolate, olives, avocados and cereal products with the germ remaining. Cholestyramine and neomycin are sometimes prescribed.

Bleeding Times

There was early recognition that bleeding times were longer in subjects consuming high amounts of LC, n-3 PUFA. It was also recognized that while Eskimos suffered less from CHD, they experienced a high incidence of cerebral hemorrhage. It appeared that the very factors which may be protective for CHD also may prolong bleeding. However, when bleeding time was investigated clinically with low to moderate doses of fish oil (0.5 to 2.0 g per day of n-3 FA), no significant increases were observed (Connor, 1994). The FDA has concluded that consumption of <3 g/d of EPA plus DHA should not cause increased bleeding times (Fed Reg 62:30751).

Glycemic Control

A few studies have reported deterioration of glycemic control in diabetics after fish oil supplementation (Stacpoole et al., 1989; Connor, 1994; Berdanier, 1994). In general no glycemic effects are observed in normal subjects (Berdanier, 1994) or in non-insulin dependent diabetic subjects (Morgan et al., 1995).

X. Information from previous human exposure to the Novel Food or its source.

Background

DHA-rich oil consists of approximately 35% docosahexaenoic acid (DHA; 22:6n-3), together with other fatty acids and traces of phytosterols as described in Section I and summarised in Table X-1. The fatty acids and sterols occur naturally in plant and animal products (Southgate et al., 1980, Morton et al., 1995) and it is therefore possible to use established techniques to estimate background levels of intake. One of the few sources of individual food consumption data are the UK National Diet and Nutrition Surveys (NDNS). However, since these data reflect only consumption in the UK it will be necessary to use supplementary data to ensure that the UK NDNS data are sufficiently representative of other European consumers. Only adult food consumption data have been used in this analysis because DHA-rich oil containing foods are aimed specifically at this age-group.

Intakes of EPA, DPA (n-6) and DHA by UK adults

Mean intakes of EPA, DHA and DPA(n-6) are 75 mg/day, 107 mg/day and 27 mg/day respectively (1.08 mg/kg bw/day, 1.54 mg/kg bw/day and 0.39 mg/kg bw/day) and range up to 303 mg/day, 401 mg/day and 83 mg/day (4.46 mg/kg bw/day, 5.85 mg/kg bw/day and 1.22 mg/kg bw/day) at the 97.5th percentile. The principal sources of EPA and DHA in the diet are fatty fish and for DPA(n-6) the main source is offal. Fatty fish are the principal source of EPA and DHA in the diet. However, only 35% of adults regularly consume fatty fish. In the absence of fatty fish in the diet average intakes of EPA and DHA would be 33 mg/day and 54 mg/day respectively, for the same group of consumers.

Intakes of sterols by UK adults

Intakes of individual plant sterols are reported in Table X-6 (of the main dossier) and of total phytosterols (excluding cholesterol) in Table X-7 (of the main dossier). Average cholesterol intakes for UK adults are 303 mg/person/day, which is consistent with the intakes of UK adults reported by Morton *et al.* Intakes from different food groups differ slightly from the Morton *et al.* paper because food groups have been categorised in different ways. Estimates based on UK adults are higher than those reported for 'Total Diet' studies reported by Morton *et al.* Morton *et al.* explain this as being due to the Total Diet survey including low consumers such as children and the elderly.
Intakes of phytosterols range from about 1 mg/day to almost 125 mg/day. The highest contributor to total phytosterol intakes is β-sitosterol (mean = 64 mg/day; 97.5th percentile = 123 mg/day), followed by campesterol (mean = 27 mg/day; 97.5th percentile = 55 mg/day). The principal sources of intake are from oils and fats, followed by bread and other cereals.
Average daily intakes are slightly lower that those reported by Morton *et al.* for Total Diet samples in 1991 (although similar to those reported for 1987). This discrepancy is mainly related to intakes from oils and fats with intakes from other sources being broadly consistent. The difference is probably due to difficulties in recording oil and fat consumption in the adults survey since they tend to be 'hidden' in other foods.
Intakes of total phytosterols for UK adults range from 113 mg/day (1.64 mg/kg bw/day) at the mean to 221 mg/day (3.31 mg/kg bw/day) at the 97.5th percentile. These intakes are comparable to national intakes reported in an opinion on the safety assessment of phytosterols by the EU Scientific Committee on Foods (Opinion 6th April 2000).

Potential intakes of omega fatty acids and sterols in other EU Member States

Levels of key omega fatty acids and sterols can be combined with the consumption of foods by UK adults to provide estimates of intake that concur with previous estimates published in the scientific literature. Average intakes predicted using FAO Food Balance Sheets tend to over-estimate intakes and this is believed to be associated with uncertainties in making reliable estimates of fat and oil consumption. However, on the basis of these data it can be observed that UK consumers provide a reasonable model for typical consumers in European countries.

XI. Nutritional Information on the Novel food

Nutritional Equivalence to Existing Foods

The sources in the diet of LC PUFA's and other components identified DHA-rich oil are discussed extensively in Sections IX, X and XIII of the main dossier. It has been shown that all the key components are present in varying amounts in the human food chain (this is discussed in detail in Section XIII). However, it can be said that DHA-rich oil will in many cases be used to provide DHA that would typically be provided by fish or fish oil. Whilst fish stocks are in decline and concern is raised about the levels of contaminants present (PCB, pesticides etc.), DHA-rich oil from algae represents a valuable alternative. Indeed algal oil derived from the species *Cryptocodinium cohnii* is already available on the European Market for use in infant foods and nutritional supplements, having been reviewed by the Netherlands and UK Voluntary Novel Foods Committees in 1996 (ACNFP Annual Report 1996).

It has been shown that the individual ratios of PUFA vary according to the food source and for DHA-rich oil the key points to note are the much lower levels of eicosapentaenoic acid (EPA 20:5n-3) and the increased content of docosapentaenoic acid (DPA 22:5n-6). DPA(n-6) is present in a wide variety of foods (see Sections IX, XI and XIII), but to specifically review the compositional ratio of DPA(n-6):DHA(n-3) in human breast milk, it is reported to range normally from 1:1 to 1:6 (Clandinin et al., 1981; Putnam et al., 1982; Carlson et al., 1986; Sanders et al., 1978; Sanders et al., 1979; Koletzko et al., 1992). The ratio of DPA(n-6) to DHA(n-3) in *Schizochytrium* and it's DHA-rich oil is 1:3, within the range reported for breast milk.

XII Microbiological Information on the Novel Food

DHA-rich oil is manufactured under the general guidelines of food chemical Good Manufacturing Practices (Food Chemical Codex pp xxvii, 4th edition). The incorporation of typical food borne microbes is inhibited by a combination of heat treatment applied to the cultured algal cells, the environmental conditions of the oil extraction and processing, and the extremely low water activity of the finished oil product.

XIII. Toxicological Information on the Novel Food.

The safety of DHA-rich oil intended for consumption as a nutritional food ingredient is established by the history of safe consumption of the components of the oil. The safety is further supported by published safety studies on a similar single-cell oil and by the historical safe use of fish oils of similar composition. The *Schizochytrium* sp. marine microalgae is the source of the oil. *Schizochytrium* sp. is known to be consumed by marine animals which, in turn, are consumed by humans. Neither of two toxins (domoic acid and prymnesium) found in other genera of the same kingdom of microorganisms are present in the source species. Finally, the safety of the oil is confirmed by safety studies utilising the intact, dried microalgae or the DHA-rich oil.

Summary of Confirmatory Safety Studies with Dried *Schizochytrium* sp. Microalgae and its Constituent DHA-rich Oil

Schizochytrium sp. microalgae contains oil rich in polyunsaturated fatty acids (PUFAs). DHA is the most abundant PUFA component of the oil (approx. 35% w/w). DHA-rich oil extracted from *Schizochytrium* sp. microalgae is currently used in the U.S. as a nutritional supplement, 1 gram of oil delivering approximately 350 mg DHA, and has been Generally Regarded as Safe as a nutritional food ingredient up to 1.5 g DHA per day. Assuming an average human body weight of 60 kg, ingestion of 1 gram of oil would result in a dosage of 16.6 mg oil/kg bodyweight. Fatty acid and sterol components of oil from *Schizochytrium* sp. microalgae have been characterized and identified as normal constituents of common human foods. Therefore, the components of *Schizochytrium* sp. microalgal oil have a history of safe consumption. Exposure to components of *Schizochytrium* sp. microalgal oil from its use as a nutritional food ingredient are within the normal range of exposures from consumption of foods with these components.

Several safety studies have been conducted with dried DHA-rich microalgae from *Schizochytrium* sp. These studies were performed according to 1982 FDA Redbook Guidelines (US FDA, 1982) and in compliance with FDA Good Laboratory Practice (GLP) regulations. For more detailed information on the safety studies, refer to Overview of Confirmatory Safety Studies with *Schizochytrium* sp. Dried Microalgae, Appendix D (of the main dossier). Draft manuscripts, accepted and pending publication in peer-reviewed journals, are available in Appendix E (of the main dossier). All of the safety studies provide confirmatory safety information on the source microalgae and the oil it contains since the fatty acid and sterol components already have a history of safe consumption in food. These confirmatory safety studies have been conducted on *Schizochytrium* sp. dried microalgae produced in three separate production campaigns (fermentation processes PB26, AS4, HD1). During these campaigns, process improvements were implemented to increase the production of oil and DHA content and add supplemental vitamin E acetate to the oil. Vitamin E acetate provides a supplemental nutritional source of antioxidant given the high PUFA content of oil. Compositional analysis found the same fatty acids and sterols present in oil produced during the three production campaigns. Process improvements did not result in the introduction of new fatty acids or sterols into the oil.

A battery of mutagenicity studies was carried out with: 1) intact *Schizochytrium* sp. microalgal cells (Ames, In Vitro Human Lymphocytes, Mouse lymphoma assays); 2) lysed *Schizochytrium* sp. microalgal cells (Ames, AS52/XPRT Gene Locus, Mouse Micronucleus assays); and 3) an Ames test with DHA-rich oil, extracted and refined as described in Section II, from *Schizochytrium* sp. microalgae.

A one-generation rat reproduction study was carried out with dried DHA-rich microalgae from *Schizochytrium* sp. administered at average dosages up to 17,800 mg/kg per day (males) and 20,700 mg/kg per day (females) when animals were fed up to 30% dried microalgae in the diet. Dietary teratology studies with the dried microalgae were conducted in the rat and the rabbit. Rats were fed up to 30% dried microalgae in the diet (up to 22,000 mg/kg per day); the rabbit dietary teratology study could not be completed due to technical problems. A rabbit gavage teratology study was conducted at dosages up to 1,800 mg/kg per day dried microalgae.

An acute gavage study in mice was conducted with DHA-rich oil, extracted and refined as described in Section II, from *Schizochytrium* sp. microalgae, administered at a single high dosage of 2,000 mg/kg.

Thirteen-week rat feeding studies were carried out with dried DHA-rich microalgae from *Schizochytrium* sp. in one study at dietary levels up to 30% (approximately 18,000 mg/kg per day) and in a second study at dosages up to 4,000 mg/kg per day.

Target animal safety trials were conducted with dried DHA-rich microalgae from *Schizochytrium* sp. , specifically in laying hens and broiler chickens. For the laying hens, dose levels of 165, 495 and 825 mg DHA/hen per day were administered in the form of dried microalgae. For the broiler chickens, treatments of 82, 240 and 408 mg DHA/broiler per day were administered in the form of dried microalgae.

Results of confirmatory safety studies establish that dried DHA-rich microalgae from *Schizochytrium* sp. and its component oils are not mutagenic in bacterial and mammalian test systems and are not teratogenic in a rat dietary teratology study and a rabbit gavage teratology study. There is no evidence that DHA-rich microalgae interfered with reproductive performance or progeny development in a rat one-generation dietary reproduction study. The algae was fed to rats for 13 weeks in two separate feeding studies. In the first study, the dietary levels of microalgae were too high and caused nutritional imbalances in test animals. In the second 13-week feeding study, lower dietary levels of the DHA-rich microalgae were fed. In this study, there was no evidence of toxicity, and the only findings were anticipated changes in clinical chemistry parameters and microscopic changes commonly observed in rats following consumption of diets high in PUFA's. In two target animal safety studies, namely, laying hens and broiler chickens, no evidence of any adverse effect on any parameters evaluated in either study were noted. DHA-rich oil, extracted and refined from *Schizochytrium* sp. microalgae, produced no effects when administered by gavage as a single high dose to mice. There were no adverse effects observed in confirmatory safety studies that could be attributed to algal toxins which supports the absence of detectable algal toxins from the microalgae (see Section III).

GRAS Determination in US

DHA-rich oil derived from *Schizochytrium* sp. microalgae has recently been determined by a panel of U.S. food safety experts to be GRAS (Generally Recognized as Safe) in food applications at a level providing 1.5 g/day of DHA and 0.5 g/day of DPA(n-6). This represents a daily DPA(n-6) intake (on a mg/kg basis) similar to that of breast feeding infants. While the panel recognised that the safety data indicated the oil was safe at higher intake levels than this, it is a principle of the GRAS process that ingredients should only be used in accordance with good manufacturing practices (cGMP's), a basic principle of cGMP is that ingredients added to the food can only be used at levels adequate to achieve their intended effect. DHA intake levels of 1.5 g/day are recognised by many experts as the level necessary to provide key cardiovascular and immune system health benefits.

Table XIII-7(from main dossier). Summary of the "No Observed Effect Level" (NOEL) for DPA(n-6) and DHA from the animal safety trials with dried* Schizochytrium *sp. microalgae and oil containing DPA(n-6) and DHA

		NOEL(mg/kg/day)		
Trial	Test article	Test Article	DPA(n-6)	DHA
	Acute Feeding			
Mouse - acute oral limit study	oil	2,000	284	693
	Sub-chronic Feeding			
Rat - 90 day[1]	algae	4,000	117	340
Laying Hen -112 day[2]	algae	3,040	177	532
Broiler chicken – whole life	algae	2,331	136	408
Developmental & Reproductive Toxicity				
Rat – developmental toxicity[3]	algae	22,000	645	1,868
Rat – single generation reproduction[4]	algae			
males		17,800	522	1,512
females		20,700	607	1,757
Rabbit – developmental toxicity[3]	algae	1,800	53	153

[1] *Hammond et al., 2001a.*
[2] *Abril et al., 2000.*
[3] *Hammond et al., 2001b*
[4] *Hammond et al., 2001c.*

Conclusions

The safety of fatty acids present in DHA-rich oil is based on four factors: 1) extensive knowledge of FA metabolism; 2) experience of use as a result of their abundant natural presence in food, and the small quantities expected to be consumed; 3) published literature on the safety of the fatty acid components and of comparable oils; and 4) confirmatory safety studies with the dried microalgal source of DHA-rich oil and DHA-rich oil.

As described in this Section and in Section IX, the FA's are expected to be consumed in relatively small quantities, at levels similar to current consumption from natural sources. Fatty acids are easily converted to one or another form as needed or simply metabolized as a source of energy. Pathological accumulation of a specific FA type producing the sequelae found in animal studies would only occur in humans in cases of extreme abuse.

The human experience with these FA has been extensive and has demonstrated that, when part of a balanced diet, supplemental FA are beneficial. The few pathological findings in animals following

supplementary FA administration are likely one or a combination of an inappropriate animal model or an excessive dose far beyond that anticipated for humans.

The safety of phytosterols found in DHA-rich oil has basis in five factors: 1) experience of use as a result of their abundant natural presence in food and the small quantities expected to be consumed; 2) extensive knowledge of the absorption, distribution, metabolism and excretion of phytosterols in mammalian species; 3) extensive safety information as the result of testing these and similar phytosterols; 4) easy identification of at-risk populations (i.e., sitosterolemia); and 5) results of confirmatory safety studies. In summary, these factors allow a conclusion that intake of phytosterols present in DHA-rich oil, taken as a nutritional food ingredient, is safe.

DHA-rich oil and the source of DHA-rich oil, *Schizochytrium* sp., a thraustochytrid and member of the kingdom Chromista (Stramenopilia), is safe based on published and unpublished scientific data and further corroborated by confirmatory safety studies. *Schizochytrium* sp. occurs widely in the marine environment and is an indirect component of the human food chain through indirect consumption of fish and other marine animals which feed on the microalgae. There have never been any reports of toxic compounds being produced by members of the thraustochytrids. Analytical tests indicate that the two toxins known to be produced by two other genera on the Chromista (in completely separate classes from the thraustochytrids) are not present in *Schizochytrium*.

Bijlage **E**

Eerste beoordeling/First assessment

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

UK/2002/001

Opinion on an application under the Novel Food Regulation from OmegaTech for clearance of DHA Gold™, a DHA rich oil.

Applicant: **OmegaTech**

Responsible person: **Mr Nigel Baldwin**

Novel Food: **DHA Gold™**

EC Classification: **2**

Introduction

1. An application was submitted by OmegaTech to the UK Competent Authority on 13th February 2001 for approval of DHA Gold™, a DHA - rich oil. The full version of this dossier was placed on the UK competent authority website on 14th February 2001. During the course of the evaluation the UK Competent Authority sought further information to clarify certain aspects of the dossier.

2. DHA (docosahexaenoic acid) - rich oil is produced via an algal fermentation process using microalgae from the genus *Schizochytrium*, a member of the kingdom Chromista, which includes the golden algae.

3. *Schizochytrium* sp. has previously been assessed in the United States and was given GRAS (Generally Recognised As Safe) clearance as a nutritional food ingredient. In the United States, a daily intake of up to 1.5g of DHA was recommended (DHA-rich oil contains 35-45% DHA). Prior to this, the microalgae achieved GRAS status to be used in chicken feed at levels of incorporation of up to 2.8% for broilers and 4.7% in layers). There is evidence to suggest that each of the components of the oil is already present to a significant degree in the human food chain.

4. The production strain of microalgae used for DHA Gold™ has been developed using conventional improvement techniques of the wild type strain and no recombinant DNA technology was used.

5. The application was prepared according to the European Commission's guidelines. DHA-Gold™ was identified as belonging to class 2.2 ("complex novel food from a non-GM source", "the source of the novel food has no history of use in the community"). The Committee's consideration of the data provided is presented according to these requirements.

I. Specification of the Novel Food

Information on this aspect is provided in section 1 of the application dossier. Supplementary information was supplied in March 2001.

6. DHA is a long chain polyunsaturated fatty acid, derived from heterotrophically grown microalgae. DHA Gold™, DHA-rich oil is described as a yellow to light orange-coloured oil derived from the heterotrophically grown marine microalga, *Schizochytrium* sp., intended for use as a nutritional food ingredient.

7. Quality control tests indicate that the production process is both reliable and reproducible and there is evidence to demonstrate that controls are in place to ensure individual batches meet manufacturing specifications.

8. Further information was sought regarding the compositional analysis of the oil. The Committee was concerned about the presence of components, particularly protein and carbohydrate, that may elicit an allergenic response.

9. Information was provided to demonstrate that the extraction process would not disproportionately concentrate any potentially toxic components.

Discussion

The Committee was satisfied that the oil consists only of lipid components already present in other existing dietary forms. The data provided (Appendix A of original application) on a number of batches show that a consistent and reliable end product is produced.

The producers were able to demonstrate that there is a very low level of residual protein (less than 0.1%) and carbohydrate in the final refined oil. This indicates that the oil is likely to elicit only a low risk of allergenicity.

II. Effect of the production process applied to the novel food

Information on this aspect is provided in section 2 of the application dossier.

10. The improvement of the *Schizochytrium* sp. was carried out using a classical mutagenesis/screening programme, which employs standard techniques commonly used in industrial microbial strain improvement (see section 2 of the application dossier). The production method is well defined, and a number of in-process monitoring steps are included in the manufacturing procedure to ensure the safety and quality of the oil is maintained. DHA-rich oil is manufactured under the general guidelines of the food chemical Good Manufacturing Practices (Food Chemical Codex pp xxvii, 4th edition).

Discussion

The Committee was satisfied that the production process is well controlled and that the in- process monitoring steps are appropriate to ensure a safe and consistent product.

III. History of source organism

Information on this aspect is provided in section 3 of the application dossier.

11. This class of microalga is primarily saprotrophic and is found throughout the world in estaurine and marine habitats. *Schizochytrium* sp. has a widespread distribution, and is consumed by a wide range of filter feeders. Although there are no reports of human consumption of *Schizochytrium*, the filter feeders (clams and mussels) that feed on this organism are part of the normal human diet.

12. *Schizochytrium* sp. belongs to the kingdom Chromista. This is not the same as the kingdom to which the bluegreen or dinoflagellate microalgae belong. This is significant since these two constitute the major known toxin producing microalgae, and most allergic responses to algal micro-organisms have been limited to exposure to these. Only two genera in the Kingdom Chromista are known to produce toxins, neither of which is in the same class as *Schizochytrium* sp. There have been no reports of toxins being found in this class.

13. The improved strain of *Schizochytrium* was developed from a patented wild-type parent strain, by using the standard chemical mutagen, NTG (1-methyl-3-nitro-1-nitrosoguanidine). Modified strains derived using this procedure sometimes acquire undesirable traits. Therefore, tests were conducted to characterise phenotypically the modified strain and its parent. The results indicate that the new strain performed equivalently to its parent and no adverse traits were observed due to the mutagenesis.

14. In addition, comparative compositional data of the oil from the parent (wildtype) and modified daughter strains demonstrated expected alterations in the balance in fatty acids, with the oil from the daughter strain having an increase in DHA content and a reduced level of palmitate. No unexpected fatty acids were found in the oil from the modified strain.

15. The sterol components of the parent and daughter strain oils were confirmed to be qualitatively constant when analysed. No unexpected sterols were identified in the daughter strain oil.

Discussion

The Committee was satisfied that the parent organism has no history of toxin production. The Committee was also content that no unexpected phenotypical changes had been introduced, and that the composition of the oil obtained from the daughter strain was similar to that from the parent apart from the desired increase in DHA content, and a compensatory decrease in palmitate levels.

IX. Anticipated intake/ extent of use.

Information on this aspect is in section 9 of the application dossier.

16. In 1994, the UK Committee on Medical Aspects on Food Nutrition Policy (COMA) recommended that individuals should increase their intake of omega-3 fatty acids, including DHA, since raised intakes are associated with a reduced risk of coronary heart disease. It must be shown, however, that by increasing levels, no detrimental affects are introduced, either from the DHA or from the other components of the oil.

17. OmegaTech are intending to market DHA Gold™ only as an ingredient to food manufacturers, it will not be sold directly to consumers. The DHA-rich oil is, however, suitable for use in a wide range of food products. These include dairy products, fine bakery wares, confectionery, sauces, breakfast cereals and cereal bars, spreads, potato crisps and pasta. However, the company has agreed to include labelling in relation to the recommended intake of DHA, so that consumers can select products as appropriate. See paragraph below.

18. The level of incorporation of DHA Gold™ into these products is dependent on the existing background DHA levels. It is estimated that UK adults currently consume 107mg/day of DHA, with the 97.5th percentile consuming 401mg/day. However, the aim would be that the combined background and incorporated level of DHA would equal a daily mean intake of 550mg.

19. Under article 2 of Directive 90/496/EEC, it will be compulsory for food manufacturers to define the quantity of DHA per serving or per 100g in the final food. OmegaTech will recommend to their customers in Europe, the food manufacturers, that daily consumption should not exceed 1.5g of DHA, as was stipulated in the US GRAS approval. Many EU Member states have their own recommendations. For example, AFFSA (France), The Health Council of the Netherlands, and COMA (UK), and so usage levels would need to be determined for particular food products and may require adjustments for different markets in various Member States. The Company has agreed to ensure that labelling meets different national requirements.

Discussion

The Committee was content with the information provided by the applicant. However, it was considered that the producers of the oil should provide information concerning "recommended intake" to manufacturers intending to use DHA-Gold™ as a food ingredient. This information should then be included on the labelling, which would accompany the final food product, and so passed on to consumers.

X. Information on previous human exposure.

Information on this aspect can be found in section 10 of the application dossier. Supplementary information was supplied in March 2001.

20. DHA-rich oil contains a range of fatty acids, including eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) as well as DHA, and traces of phytosterols. EPA and DPA both occur naturally in plant and animal products, and so estimates can be made to indicate current background levels of intake.

21. The principal sources of EPA and DHA in the diet are from oily fish. Only 35% of adults in the UK regularly consume oily fish, however. The absence of fatty fish in the diet greatly reduces the levels of DHA and EPA in the diet. The levels of fatty acid intake vary across Europe. This is probably due to the differences in trends of oily fish consumption. Most dietary DPA comes from offal.

22. Further information on previous human consumption was provided. The producers were able to give details of DHA being supplied to a customer who then sold the oil as a dietary supplement. The product has been on sale in the United States for over two years, and the information provided is in the form of a list of calls received from the after sales care helpline. Over 400 calls have been received by this helpline, none of which have recorded any adverse effects relating to this product.

Discussion

The Committee accepted the data provided on the background levels of consumption of the fatty acid and phytosterol components of DHA – rich oil already in the human diet. Although conscious that the information provided from the helpline call centre in the USA was anecdotal and not a formal structured study, the Committee noted that there have been no reported adverse effects in the two years that the product had been on sale.

XI. Nutritional information

Information provided on this aspect is in section XI of the application dossier.

23. DHA is considered to play an important role in maintaining a healthy heart. Several markers of the cardiovascular system are directly influenced by dietary DHA. These include triglyceride levels, platelet aggregation (may

lower the risk of heart attack or stroke), cardiac rhythmicity and haemodynamics.

24. DHA is also considered to be vital for the development and function of brain and eyes. DHA oil is supplemented with Vitamin E for nutritional purposes.

Discussion

The Committee accepted that nutritional advice is to increase intake of omega-3 fatty acids and was aware that this oil could improve the nutritional properties of foods to which it is added. The Committee agreed that foods containing DHA - Gold™ oil would have to be labelled to inform consumers of recommended intake levels. There is an increased nutritional need for vitamin E when increasing the intake of polyunsaturated fatty acids, and we note that the oil is supplemented with vitamin E to address this point.

XII. Microbiological information

Information provided on this aspect is in section 12 of the application dossier.

25. DHA Gold™ is manufactured under the general guidelines of food chemical Good Manufacturing practices. A combination of heat treatment, environmental conditions of oil extraction and processing and the extremely low water activity of the finished oil, contributes to the inhibition of typical food-borne microbes.

Discussion

The Committee was content with the information provided by the applicant and considered the production process, the quality control measures and the nature of the final product to be sufficient to ensure no unintentional microbiological contamination of the oil.

XIII. Toxicological information

Information on this aspect is provided in the application dossier in section 13. Additional data requested by the Committee was supplied in February 2002.

26. A range of safety studies has been conducted with dried microalgae of the genus *Schizochytrium*. These studies were conducted in accordance to the 1982 FDA Redbook Guidelines and in compliance with the FDA Good Laboratory Practice (GLP) regulations to support the GRAS petition in the US.

27. The studies included a) a subchronic feeding study where dried DHA-rich microalga was fed to rats for at least 13 weeks, b) developmental toxicity evaluation in rats and rabbits, c) a single generation rat reproduction study and d) a mutagenicity study. Also an acute gavage study was conducted with extracted and refined DHA-rich oil, and a laying hen and a chicken broiler study were also conducted. The actual material tested in each of

the studies is shown in the table attached below. The Company also presented the findings from a swine toxicity study carried out on the algal biomass, but the Committee did not feel this contributed any further information for the safety assessment of the oil for human consumption.

Summary of the test material used in the toxicology studies.

Trial	Test Article	Strain
Acute Feeding		
Mouse	oil	N230D (Daughter, production strain)
Sub-chronic Feeding		
Rat - 90 day[a]	algae	ATCC 20888 (Parental, wildtype strain)
Laying Hen -112 day[d]	algae	N230D
Broiler Chicken – whole life	algae	N230D
Developmental & Reproductive Toxicity		
Rat – developmental toxicity[b]		
maternal	algae	ATCC 20888
offspring	algae	ATCC 20888
Rat – single generation reproduction[c]		
males	algae	ATCC 20888
females	algae	ATCC 20888
Rabbit – developmental toxicity[b]		
maternal	algae	ATCC 20888
offspring	algae	ATCC 20888

[a] Hammond et al. (2001b); [b] Hammond et al. (2001a); [c] Hammond et al. (2001c); [d] Abril et al. (2000);

Summary of the genetic toxicity studies performed using dried algae of the genus *Schizochytrium* an DPA(n-6) and DHA.

Trial	Test Article	Strain
Ames	oil (DHALIP-NS) [NB]	N230D
Ames[a]	intact algae	ATCC 20888
In Vitro Human Lymphocytes[a]	intact algae	ATCC 20888
Mouse Lymphoma[a]	intact algae	ATCC 20888
Ames[a]	lysed algae	ATCC 20888
AS52/XPRT Gene Locus[a]	lysed algae	ATCC 20888
Mouse Micronucleus[a]	lysed algae	ATCC 20888

[a] Hammond et al. (2001d)

NB. DHALIP-NS refers to the Company's internal product code for commercial article.

28. A human clinical study on the DHA-Gold™ oil was supplied in 2002.

29. It was noted that the toxicology studies were carried out on either the parental (ATCC 20888) or the production daughter (N230D)strains. Evidence was provided to demonstrate that the oil from the two strains were comparable, with the exception of the expected increases in DHA content. For further information, see section III, The History of the Source Organism.

30. Each of the studies is summarised below.

Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium*:
Part I: Sub-chronic Rat Feeding Study.

31. The purpose of this study was to determine the effects of DHA-rich (docosahexaenoic acid) -rich microalgae (DRM) of the genus *Schizochytrium*, administered in the diet of rats for 13 weeks.

32. A dried preparation of DRM was administered in the diet to groups of 20 male and 20 female Sprague-Dawley rats to provide an intake of 0, 400, 1500 and 4000 mg/kg/day for at least 13 weeks. Untreated controls received basal diet only. An additional group of 20 males and 20 females received rodent diet mixed with fish oil to provide a target dose of 1628 mg/kg bw/day. In view of DRM's high fat content (41%, mainly unsaturated fatty acids), vitamin E acetate had been added (during manufacture of DRM) to all test substance and fish oil-treated groups to provide supplementary dietary antioxidant.

33. The stability and homogeneity of the diets were checked regularly during treatment periods. Animals were observed twice daily for mortality and clinical signs. Ophthalmoscopic examinations were carried out prior to start of study and prior to killing the controls, fish oil treated rats and the high-dose DRM treated animals. Blood collected under halothane anaesthesia was collected from 10 rats/sex/group during week 6-7 and prior to killing; urine was also collected during the latter periods from the same rats. Haematological measurements were carried out on 13 relevant parameters (including thromboplastin-clotting time). Serum clinical chemistry measurements were carried out on 24 parameters. Urinalysis was carried out on at least 13 end points. At the end of the study, the animals were anaethesized by sodium pentobarbital and killed by exsanguination. Organ weight data were collected for the liver, spleen, heart, thymus, ovaries, testes, kidneys, adrenals, pituitary and brain. Complete sets of tissues were also collected for histopathology. All tissue slides from control, fish oil, and high dose DRM groups were examined microscopically. In addition, heart, kidneys and pituitary for males in all groups and liver from females in all groups were also examined microscopically.

34. Based on the results from a previous 13-week rat feeding study (CTBR study) with DRM, a Pathology Working Group was formed to review the heart slides from this study and CTBR study to resolve differences in terminology and severity scores between the two studies. The review also assessed the accuracy and consistency of the initial histopathological examinations of the hearts of male and female rats.

35. All animals survived during the study. There were no treatment-related clinical and ophthalmologic signs of toxicity. There were no treatment-related effects on body weight or food consumption compared with controls.

36. While there were a few significant intergroup differences in haematological parameters, these were not considered treatment-related, as there was no dose-response. The main clinical chemistry finding involved a drop in cholesterol and HDL levels in both sexes of the fish oil and the high-dose groups. A lowering in the latter two parameters was also noted in males receiving DRM at 1500 mg/kg bw/day.

37. At necropsy, no treatment-related effects on gross lesions and terminal body weights and absolute and relative organ weights were noted. Microscopic changes were mainly confined to the liver, kidneys and heart. In the liver, the incidence of periportal hepatocellular vacuolation was significantly increased in the female fish oil group (18/20) and all female treatment DRM groups (low dose, 16/20, mid-dose, 18/20 and high dose 19/20) when compared with the female untreated control group (8/20); there were no treatment-related differences in the severity of this observation. There was a significant increase in pelvic dilation of the kidneys in high dose DRM males (5/20) compared to control (0/20) and fish oil groups (2/20).

38. The nature of the histological changes affecting the heart (slight increases in the incidence and/or severity of inflammation and degenerative changes in the male rats) of animals from this study was similar to that reported in a previous study, although in this study, diets were not supplemented with vitamin E. A slight increase in the incidence, but not severity, of cardiomyopathy was observed in the 4000 mg/kg bw/day DRM dosed males in this study. The changes were characterised by small foci of mononuclear inflammatory cells and degeneration of myofibres, sometimes accompanied by fibrosis of the myocardium. The changes were reported to be identical to spontaneous "cardiomyopathy" associated with ageing. The incidence and severity of cardiomyopathy in this study were greater in male rats than females.

Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium*
Part II: Developmental Toxicity Evaluation in Rats and Rabbits

39. The developmental toxicity of DRM (supplemented with vitamin E during manufacture) was assessed in Sprague-Dawley rats (25/group, provided

with dried DRM in the diet at 0, 0.6, 6, and 30% on gestation days (GD) 6 - 15) and in New Zealand White Rabbits, 22/group, dosed with DRM at levels of 180, 600, and 1800 mg/kg bw/day by gavage (in 2 equal daily doses, 6 hrs apart for 13 consecutive days on GD 6-19). An additional group of 22 rabbits dosed with fish oil (also supplemented with vitamin E) was used as a negative control to provide an equivalent amount of fat to that received by the high-dose DRM rabbits. Control animals (22 per group) received the vehicle (0.5% carboxymethyl cellulose and 0.1% polysorbate 80).

40. Sperm-positive female animals were weighed and food consumption determined on GD 0, 6, 9, 12, 16, 18, and 20 (rats) and GD 0 through to GD 29 (rabbits). Animals were observed twice daily for clinical signs and mortality. All rats were killed on GD 20 by asphyxiation with carbon dioxide. Rabbits were killed on GD 29 by a lethal injection of sodium pentobarbital. A complete gross necropsy was conducted on all rats and rabbits. At necropsy, the uterus and ovaries were excised from each animal and the number of corpora lutea recorded. The uteri were examined for the location of foetus, resorptions and implantation sites. Live foetuses were dissected from their uterus, weighed and examined for morphologic and visceral abnormalities. All foetal carcasses were eviscerated and stained and examined for skeletal malformations.

Rats

41. No rats died during the course of the study. There were no treatment-related clinical signs. Animals in the 30% DRM group exhibited a reduction in weight gain from GD 16 to GD 18. Food consumption was also reduced in the latter group during GD 6 to GD 9 and between GD 16 and GD 18. Examination of the uteri confirmed that 88%, 88%, 92% and 80% of the mated animals in the control through to the high-dose DRM groups were pregnant and produced foetuses by GD 20. There were no treatment-related effects on corpora lutea, implantations, live foetuses, or in percent resorptions or late deaths. Statistical increases in the number of male foetuses and in the male sex ratio were noted in low- and mid-dose DRM groups (mainly due to a low percentage (39.1%) of male foetuses/litter in the control group). The incidence of foetuses with ossification centres in the first lumbar vertebrae (2%) was significantly lower in the high-dose-DRM group but was within the historical control range (1.5-15%). A statistically higher incidence of foetuses (but not litters) with reduced ossification of the ribs was seen in the mid- and high-dose DRM groups. This resulted from a single litter with a number of affected pups (mid-dose, 8 foetuses, high-dose, 5 foetuses). Treatment with DRM did not result in other skeletal and visceral anomalies in rats.

42. Since at the highest dietary concentration of DRM (30% - which equates to 22 g/kg/day) no treatment related clinical signs were observed, the authors proposed a NOEL to be the highest dose, 22 g/kg bw/day.

Rabbits

43. One animal in the 600 mg/kg bw/day DRM group died during GD 10 and a second was killed by an intubation error on GD 10 in the 1800 mg/kg/day DRM group. One female in the fish oil control group aborted on GD 23, and two females in the high-dose group aborted on GD days 25 and 26. No treatment-related clinical signs were reported in the DRM dosed groups. Reductions in body weight gain and food consumption were noted in the animals in the high-dose DRM group during GD 12-19 and when the entire treatment period was evaluated (there was reversal of this effect during the first half of the post-treatment period, GD 24-29). A similar loss in weight gain was noted in the fish oil-treated group.

44. Uteri examinations confirmed that 77%, 81%, 77%, 81%, and 89% of the artificially inseminated females in the control, fish oil and low through to high-dose DRM groups were pregnant when killed.

45. There were no significant differences between the DRM or fish oil treated groups and the control in mean number of corpa lutea, implantation sites, litter size, post implantation loss, and foetal body weight. Treatment with DRM did not result in skeletal and visceral anomalies in rabbits. The authors proposed a NOEL in rabbits of 600 mg/kg/ day of DRM for maternal toxicity and 1800 mg/kg bw /day of DRM for developmental toxicity.

Safety Assessment of DHA-Rich Microalgae of the genus *Schizochytrium*
Part Iii: Single generation rat reproduction study

46. The reproductive toxicity of dried DRM was examined in Sprague-Dawley rats (30/sex/group) in the diet at concentrations of 0, 0.6, 6 and 30% (equivalent to a dose of 400, 3900, and 17800 mg/kg bw/day for F0 males and 480, 4600, and 20700 mg/kg bw/day for F0 females respectively). Treatment in males continued throughout mating and until termination. Females were treated throughout gestation and through lactation day 21. Females were killed after raising their offspring to weaning at 21 days of age.

47. Animals were observed twice daily for clinical signs and mortality. Body weight and food consumption data were recorded weekly. For each F1 litter, the number of live and dead pups, gross abnormalities and individual weight of live pups were recorded at birth and on days 4, 7, 14 and 21 of lactation. Culled F1 animals (4/sex/litter) were subjected to gross necropsy. F0 animals were terminated (males, 3 weeks after the end of mating period and females on days 21, 22 or 23 *post partum*) by carbon dioxide asphyxiation and gross necropsies performed. The left testes from F0 males were used to assess sperm count, motility and morphology. Uteri and ovaries from F0 females were used for assessment of implantation sites and implantation loss. In addition, histopathological examinations

were conducted on epididymis, liver, ovaries, prostate, seminal vesicles, testis (right), uterus, vagina and tissues showing gross pathology.

F0 generation

48. Three male rats died during the study (a high-dose male died during week 5 and a control and two mid-dose DRM animals died, one during week 13 and one during week 14). These deaths were not related to treatment with DRM. Statistically non-significant increases in body weight in high dose males were noted from week 10 through to week 16. The body weight in female high-dose DRM group was increased significantly during pre-mating weeks 1 and 2 and throughout both gestation and lactation. Food consumption in the male DRM groups was reduced (only statistically significant in the high dose group during weeks 2-16). Food consumption was also reduced in high-dose DRM females during gestation. There were no treatment-related adverse effects in reproductive performance, or in the duration of gestation, mean litter size, mean pup weight, number of litters with dead pups, or post implantation loss. Apart from an increased hepatocellular vacuolation in the mid- and high-dose DRM female groups, there were no treatment related histopathological changes noted. Dietary DRM treatment had no effect on epididymal weights, sperm counts, percent motility, sperm morphology or spermatogenic cycle.

F1 generation

49. There were no treatment-related clinical signs apparent in the F1 pups. Pup viability and survival was similar in control and treated animals. DRM treatment had no effect on F1 body weights recorded on lactation days 0, 4, 7, 14, or 21. No treatment-related internal or external gross abnormalities were noted in pups that were born dead or in those that were subjected to necropsy at the termination of the trial.

An Evaluation Of The Mutagenic Potential Of DHALIP-NS In the Ames Salmonella/ Microsome Assay (EX 4709).

50. A precipitate was observed at doses of 500 and 1000 µg/plate. The plates dosed at 5000 µg/plate were not countable due to this precipitate. There was no toxicity observed at any test article concentrations. There appeared to be a two-fold increase in mean revertant colony numbers over that of the vehicle control (dimethyl sulfoxide) with strain TA98 at 500 and 1000 µg/plate without metabolic activation. This increase was neither dose-related nor reproducible in a repeat assay. There were no compound-related increases in the number of revertant colonies over the controls for the other 4 tester strains. The increases in the number of revertants as a result of treatment with the positive control compounds demonstrated the capability of the system to detect mutagens in this assay. It was concluded that DHALIP-NS is negative in the Ames test.

Exploratory Acute Oral Limit Study of DHALIP-NS in Mice

51. This (non-GLP) study was performed to assess the acute oral toxicity of DHALIP-NS (yellowish oil) in mice when administered as a single oral gavage dose. The compound (suspended in 0.5% carboxymethyl cellulose and 0.1% Tween 80 in distilled water) was dosed to 5 male and 5 female CD mice at 2000 mg/kg bw/day. The animals were observed for clinical signs at approximately 1, 2.5 and 4 hours after dosing and daily thereafter. Body weights were recorded before and after fasting on Day 0 and on post-dosing day 7. The animals were killed on day 7, post dosing.

52. There were no treatment-related deaths or clinical signs, or significant effects on body weight and gross necropsy related to administration of the test substance at a dose of 2000 mg/kg bw/day.

Laying Hen Study

53. A target animal safety trial with laying hens was conducted using dried DHA-rich microalgae of the genus *Schizochytrium* at three different dose levels: 82, 240, and 408 mg DHA/bird per day. Each treatment consisted of 64 laying hens divided into eight replicates per group with a total of 320 animals. Body weights, food conversion, egg production, egg weight, shell thickness and interior quality were measures at the end of the month for each of the four dosing periods. Eggs were also collected and analysed at the end of months 2 and 4 for their weight, shell thickness, interior egg quality and fatty acid profile.

54. At the end of the four-month period, two randomly selected hens from each dose level and replicate were killed and evaluated for haematological and histpathological changes. Blood clotting time was also determined, since it is known that fatty acids lead to a decrease in platelet reactivity. Gross necropsy was completed on all layers that died during the study or killed for scheduled evaluation. Weights were determined for most of the major organs and a range of tissues were studied for fatty acid content. The results of the experimental diets were determined via statistical analysis.

55. The results showed that there were no significant differences in any of the organ weights measured. No alterations were noted in the histopathological study, and there were no significant differences between treatments for any of the haematological analyses.

56. It was concluded, based on this study, that dried DHA-rich microalgae of the genus *Schizochytrium* is safe as a feed ingredient for laying hens at 3,040 mg/kg body weight/day dried microalgae delivering 532mg DHA/kg body weight/day.

Broiler Chicken Study

57. A similar target animal safety trial with broiler chickens was carried out using 2240 birds. They were sexed and randomly assigned to one of four dietary treatments, including three different levels of DHA-rich microalgae and one control group. 560 broilers were included in each group, and then divided in to eight replicates, with 70 birds in each, 35 each of both sex.

58. The same studies that were carried out with the laying hens were also used to analyse the diet affect on the broiler chickens.

59. The results indicated that there was no effect of treatment level on any of the evaluated broiler growth performance measures. No significant difference regarding weight gain, feed intake of feed conversion between treatment levels were noted. No histopathological or haematological differences were observed between the treatment groups.

60. Based on these results, it was concluded that dried DHA-rich microalga is safe as a feed ingredient for broiler chickens at 2331 mg/bird/day delivering approximately 408 mg DHA/bird/day.

Human Clinical Study

Following consideration of the animal data, the Committee requested further confirmatory information in humans, and this was subsequently provided in January 2002.

61. The aim of the study was to evaluate the effects of consuming 1.5g/day of DHA oil from DHA-Gold, considered to be in excess of estimated usage, on plasma lipids, haematology, biochemical markers of liver and cardiac functions, and certain haemostatic risk factors, which include plasma fibrinogen concentration, factor VII coagulant activity, C-reactive protein concentration, plasminogen activator inhibitor type 1 (PAL-1) activity and von Willebrand factor antigen concentrations (vWF). Platelet counts were also included in the study, since studies with fish oils have reported a fall in platelet count.

62. Seventy-nine individuals were divided randomly between two groups, the test group and the control, each containing approximately equal numbers of male and female subjects. Individuals in the test group consumed 4 capsules each containing 1000mg of oil per day, providing a daily total of 1.5g DHA from DHA Gold. The control group received an olive oil placebo, which has been shown to be inert.

63. Responses were assessed by measurements made on entry to the trial, day 1 and on days 28 and 29 of each treatment. Measurements included seated blood pressure and body weight. Fasting blood samples were collected on test days 1 and 28/29, and were used to determine blood counts, erythrocyte fatty acid composition, serum lipids, glucose, creatine kinase activity and liver function tests. Citrated samples were collected to determine PAL-1 activity, fibrinogen and factor VII coagulant activity. Further blood samples were also obtained for lipid analysis on these days.

64. Subjects recorded signs of illness, medication used, menstrual phase and deviations from the protocol in a diary. At the end of the study, subjects completed a questionnaire about their appreciation of the treatment, and any side effects experienced.

65. This study demonstrated that a consumption of 1.5g DHA daily as DHA Gold resulted in the expected changes in serum lipids within the normal ranges. A rise in plasma concentration of LDL cholesterol was noted, but this was accompanied by an increase in HDL cholesterol, and no overall net change in the LDL/HDL ratio was observed. DHA Gold was well tolerated among the test group, with no adverse effects on liver function, cardiac enzymes, glucose metabolism, and haematology markers of inflammation or haemostatic function being recorded, apart from the statistically significant increase in Factor VIIc. However, an increase is also seen with fish oils and is a result of the compensatory increase in clotting in response to the known effects of DHA on platelet vessel wall interactions. Therefore, it is of no toxicological concern.

66. **Discussion and Conclusion**

The safety of fatty acids present in DHA-rich oil is based on four factors:

i) Extensive knowledge of fatty acid metabolism.
ii) Extensive previous exposure due to their high level in background human diet and the small quantities expected to be present in foods using DHA-rich oils.
iii) Published literature on the safety of fatty acid components and comparable oils.
iv) Confirmatory safety studies.

The safety of phytosterols found in DHA-rich oil is based on five factors. These factors allow a conclusion that an intake of phytosterols present in DHA-rich oil is safe:

i) Experience of use due to their natural abundance in food and low levels expected to be used.
ii) Extensive knowledge of the absorption, distribution, metabolism and excretion of phytosterols in mammalian species.
iii) Extensive safety information as the result of testing these and similar phytosterols.
iv) Easy identification of at risk populations.
v) Confirmatory safety studies.

A number of confirmatory toxicological studies have been conducted with the DHA-rich microalgae, including 90-day rat feeding studies, teratology studies in the rat and rabbit and a single generation reproduction study in the rat. The NOEL for DHA in these animal studies ranged from 153 - 1,868 mg/kg/day, although in some cases these levels were the top tested and the actual NOEL could be higher.

The periportal vacuolation seen in the 90-day rat study is likely to be the result of the high fat level in the diet and therefore does not represent an adverse effect as such. There is, however, a slight increase in the incidence, but not in the severity, of cardiomyopathy in the high dose male rats in this study, which may be a result of treatment. However, it was not within the test protocol to histologically examine the heart in the single-generation rat reproduction study. The lesion seen was similar to that seen spontaneously in ageing rats of this strain. An expert panel in the United States reviewed the cardiomyopathy data. They concluded that the treatment related findings of the 13-week study had little relevance to the safety assessment of the use of DHA as a nutritional ingredient for humans. The ACNFP sought expert advice from an animal pathologist from the Committee on Toxicity. The same conclusion was drawn that the presence of heart lesions in the rat was of no significance in the safety evaluation of DHA for use in humans.

In the USA, DHA Gold™ has GRAS status at an intake level of 1.5g a day. This figure was based on the presence of DHA in human breast milk, although the safety data indicated the oil was safe at higher intake levels. The level of 1.5g a day is considered to be an adequate upper level of intake when considering individual Member States recommendations to achieve the intended effects, which include key cardiovascular and immune system health benefits.

The results of the 90 day rat feeding study indicates that the NOEL for DRM in the rat 90 day feeding study is at least 4000mg/kg/day (the highest dose tested), which is equivalent to an intake of 340mg/kg/day of DHA. No significant adverse effects were seen in either of the teratology studies or the single-generation reproduction study in the rat, other than slight reductions in food intake and body weight gain at high levels of incorporation of DRM in the diet. If the recommended dose for DHA were accepted to be 1500mg/person/day, this would equate to 25 mg/kg/day DHA for a 60-kg person, which would give a safety factor of 13.5 in relation to the rat study.

Published and unpublished scientific data relating to Schizochytrium sp., the species from which DHA-rich oil is derived, have not shown any adverse effects that are relevant to the safety of this oil for humans. There have been no reports of toxic compounds being produced by Schizochytrium sp., it occurs widely in the marine environment and is an indirect component of the human food chain.

The Committee agreed that a formal study in humans to confirm the safety of the oil was needed. The study provided by the Company was able to demonstrate that the inclusion of DHA Gold in the diet of human volunteers, at a level considered to be in excess of expected usage, had no effect on target parameters.

The study tested the Null Hypothesis that the mean values did not differ between treatments, since the aim of the safety testing is to demonstrate toxicity rather than to demonstrate equivalence. The study was designed to detect 1SD unit change in all the variables of interest, since a value of less

than 1 unit is considered unlikely to be of clinical significance. A sample size of 32 subjects has the power to detect a 0.7 SD unit change, and so the study recruited 40 individuals to allow for drop outs. This Committee was satisfied with the statistical power of the study.

OVERALL DISCUSSION

67. The application dossier contains good product specification data and a detailed description of the production process. The process is well monitored, with quality control and safety measures in place. The product is manufactured using a standard method, which has been shown to be both reliable and reproducible.

68. No nutritional concerns have been raised, since the entire product components are already present to some degree, as background, in human diets, and there is a recommendation generally for an increase in the level of DHA in the diet.

69. The product has undergone toxicological testing, and there are data from animal and other studies to support the safety of the derived oil. The inclusion of a human clinical trial provided further reassurance as to the safety of the DHA-Gold™ ingredient.

70. If this product is approved, the Applicant Company will make recommendations to food manufacturers regarding appropriate intake levels for DHA and appropriate labelling for the final food product. The Applicant Company has agreed to make recommendations to food manufacturers as described in the application dossier. Final products will need to be labelled with the ingredient name and the prescribed nutritional labelling. Since the oil is to be used as a nutritional ingredient, any claims made on the food due to the inclusion of the oil must comply with the general criteria for making nutrient content claims. Also, any health claims made will have to comply with the appropriate legislation in this area, regardless of any nutritional claim made.

Conclusion

The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Omegatech that DHA Gold™ is safe for use as a nutritional food ingredient, for the types of uses as described in the application dossier, subject to the labelling requirements described above.