ANZFA
Australia New Zealand Food Authority
TE MANA WHAKARITE KAI MŌ ĀHITEREIRIA ME AOTEAROA

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FINAL ASSESSMENT REPORT
(INQUIRY – SECTION 17)

APPLICATION A428

DHA-RICH DRIED MARINE MICRO ALGAE (*SCHIZOCHYTRIUM* SP.) AND DHA-RICH OIL DERIVED FROM *SCHIZOCHYTRIUM* SP. AS NOVEL FOOD INGREDIENTS

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EXECUTIVE SUMMARY

The Australia New Zealand Food Authority (ANZFA) received an Application (A428) on 13 March 2001, from Omega Tech Inc., to amend Standards A19 and 1.5.1 (the Novel Foods Standards) of the *Food Standards Code* to permit the use of dried marine micro-algae (*Schizochytrium* sp.) which is rich in the omega-3 long chain polyunsaturated fatty acid DHA (docosahexaenoic acid) as a novel food ingredient in a limited range of foods. The Applicant subsequently amended their Application on 28 August 2001 to include DHA- rich oil derived from the same species for use as a novel food ingredient in a limited range of foods. The Applicant has also requested in his 28 January 2002 communication that minor changes be made to the specification proposed in the Draft Assessment Report (Full Assessment – section 15) for the oil derived from the micro-algae. The changes proposed do not affect the safety of the oil. The earlier specification has narrow limits for some tests and proposed changes reflect the normal process operation capability more appropriately.

Under Standards A19 and 1.5.1 of the *Food Standards* Code, novel food is defined as a sub-set of non-traditional food, as defined in the Standard. DHA-rich marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. are non-traditional foods because they do not have a history of significant human consumption by the broad community in Australia and New Zealand. They are considered to be novel foods for the purposes of the Standard because there is insufficient knowledge in the broad community to enable safe use of these foods in the form or context in which they are proposed to be presented.

Omega-3 long chain fatty acids, specifically, DHA have been identified as important dietary nutrients with specific roles in the developing foetus and pre-term infants. There are also recent reports that indicate that DHA, as one of the omega-3 fatty acids, may have an important role in cardiovascular health and beneficial effects on the immune system in the general population. DHA is also considered to be vital for the development and function of the brain and eyes.

ANZFA released a Draft Assessment Report in relation to this application on 12 December 2001, seeking public comment. The submission period ended on 6 February 2002. A total of five submissions were received.

Issues addressed

Proposed uses

The Application proposes to use dried marine algae in the following foods (use levels ranging between 200-300 mg per serving):

- bread and other baked goods such as crisp-spreads;
- breakfast foods;
- table spreads;
- dressings/mayonnaise;
- modified milk products; and
- special purpose foods such as Formulated Meal Replacements/Supplementary Foods but excluding infant foods.

The Application proposes to use DHA-rich oil derived from *Schizochytrium* sp. for use in the following foods (up to 150 mg per serving):

- liquid foods;
- beverages;
- fruit drinks;
- sports drinks;
- table spreads and dressings;
- dairy/non-dairy foods such as yoghurts, cheese products and ice creams;
- manufactured meat products and analogues; and
- infant food and infant formula.

Safety evaluation

Schizochytrium micro-algal species are widely distributed in marine habitats although there are no reports that they have been used for human consumption. There are no reports of toxins being found in this class of micro-algae. Detailed specifications for both the micro-algae and for the oil derived from the micro-algae have been provided.

The toxicological studies that support the safety of DHA-rich *Schizochytrium* sp. micro-algae indicate that the micro-algae has low toxicity, is not genotoxic or teratogenic and has no effect on reproductive parameters. The no-observable-effect level (NOEL) from a 13-week feeding study in rats was 8% in the diet (equivalent to 4000 mg/kg bw/day of micro-algae, which equates to approximately 430 mg/kg/day of DHA). This was the highest dose level tested in this study. There are no human toleration studies available on the micro-algae or on the oil derived from the micro-algae. However, the compositional analysis of DHA-rich micro-algae or DHA-rich oil derived from the micro-algae do not raise any particular concerns in relation to the safety of the components of these products. There are also numerous published studies available on the safety of DHA and other DHA-rich oils. None of the available studies indicate any toxicity associated with DHA, even at high levels of exposure.

The data from the available animal studies, taken together with the composition data and the data on known levels of exposure to DHA, do not indicate any potential for toxicity associated with dried micro-algae or the oil derived from this micro-algae.

Composition of DHA-rich oil extracted from Schizochytrium sp.

The compositional equivalence of this oil to other traditionally used DHA-rich oils available on the market has been examined. The results indicate that the fatty acid profile of DHA-rich oil is similar to those of other marine based oils. About 60% (w/w) of the micro-algae is made up of fatty acids of which DHA is the major component (35%) followed by palmitic acid (24%), DPA (13.6%) and myristic acid (10.1%).

Sterols make up about 3% of the total mass of the DHA-rich oil, all of which are present in the human food supply. Exposure to these sterols from the consumption of foods containing these DHA-rich products at the proposed levels would not exceed the current consumption of sterols in the general population from other food sources.

Estimated dietary exposure

The principal dietary sources of DHA are oily fish species such as salmon, tuna, sardines, and herrings which feed on the micro algae. Game meat is also a source of DHA. However, the consumption of fish/game meats in Australia and New Zealand is relatively low and therefore the normal exposure to DHA is low.

Using Australian nutrient composition data, ANZFA has estimated that mean exposure to DHA, from existing food sources, to be 100 mg per day for all respondents aged 2-100 years, with high consumers (95^{th} percentile) exposed to 480 mg per day. Estimates of diet-derived DHA exposure were not able to be determined for New Zealanders, as information on the levels of DHA in New Zealand foods was not available.

Dietary modelling has been conducted on the proposed uses of the DHA-rich micro-algae and its oil in various foods to determine the dietary intakes for the mean consumer and the 95^{th} percentile consumer. The mean total dietary exposure based on exposure to DHA solely from the use of micro-algae was determined to be 260 mg per day in Australia and 280 mg per day in New Zealand. The 95^{th} percentile total dietary exposure based on the same exposure data was determined to be 600 mg per day in Australia and 690 mg per day in New Zealand. Thus, exposure to DHA from all sources, even for the 95^{th} percentile consumer, would be under 1000 mg per day. Human breast milk contains low but significant levels of DHA and this source provides a daily intake of approximately 1.5 g of DHA for breast-milk fed infants.

Risk Analysis

The assessment of the safety of DHA-rich micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. is based on: (i) consideration of the safety of the source organism; (ii) the composition of the dried micro-algae and the oil derived from the micro-algae; (iii) toxicology studies conducted on the micro-algae; (iv) safety studies on DHA and DHA-rich oils; and (v) a history of human exposure to DHA in foods. Considered together, this data demonstrates that the DHA-rich micro-algae and the oil derived from it are safe. There is no evidence of toxicity associated with exposure to the micro-algae or to other sources of DHA at the anticipated levels of exposure. The compositional analysis of the micro-algae and the oil derived from the micro-algae indicates that the oil is comparable to other traditional sources of DHA and does not raise any safety concerns regarding other minor ingredients.

The dietary exposure assessment indicates the potential exposure to DHA from the micro-algae or the oil derived from the micro-algae is well within the levels shown to be safe from the animal studies conducted on the micro-algae, and from the animal and human studies conducted on DHA derived from other sources.

On the basis of the available data, it is proposed that there be no restriction on the level of use of DHA-rich micro-algae or DHA-rich oil derived from micro-algae as novel food ingredients. Both products are required to comply with the specifications proposed in the draft variations to Volumes 1 and 2 of the *Food Standards Code*. The Applicant requested that minor changes be made to the specification proposed in the Draft Assessment Report for the oil derived from the micro-algae. The changes proposed do not affect the outcome of the assessment and have been included in revised drafting.

The earlier specification has narrow limits for some tests and proposed changes reflect the normal process operation capability more appropriately.

The use of novel foods in Australia and New Zealand should be monitored in future in order to confirm their low risk nature and to review whether any additional regulatory action may be warranted in order to protect public health and safety.

Statement of Reasons

The proposed changes to Volume 1 and Volume 2 of the *Food Standards Code* are recommended for the following reasons:

- The available data on DHA-rich micro-algae (*Schizochytrium* sp.) and on DHA-rich oil derived from *Schizochytrium* sp. does not raise any safety concerns at the anticipated levels of exposure.

- The fatty acid composition of the *Schizochytrium* sp. micro-algae and the oil derived from *Schizochytrium* sp. are comparable to other traditional sources of DHA.

- *Schizochytrium* sp. micro-algae and oil derived from the micro-algae will provide an alternative source of omega-3 fatty acids in foods.

- The proposed changes to Volume 1 and Volume 2 of the *Food Standards Code* are consistent with the section 10 objectives of the ANZFA Act and the regulatory impact assessment.

1. INTRODUCTION

The Australia New Zealand Food Authority (ANZFA) is a bi-national statutory body responsible for developing draft food standards and draft variations of standards, to make recommendations to the Australia New Zealand Food Standards Council (ANZFSC) in relation to those drafts, and to review standards. ANZFSC may then decide to adopt the draft standards or draft variations of standards, which results in their incorporation into food laws of the Australian States and Territories, and New Zealand.

On 24 November 2000, ANZFSC adopted the *Australia New Zealand Food Standards Code* (known as Volume 2 of the *Food Standards Code*) that will apply in both Australia and New Zealand. A two-year transitional period has been implemented at the conclusion of which Volume 2 of the *Food Standards Code* will be the sole code for both countries. In the interim, for the majority of the food standards, there is a system of dual standards operating in both Australia and New Zealand.

Standard A19 – Novel Foods, was gazetted on 16 December 1999 and came into effect on 16 June 2001 following an 18-month implementation period. The Novel Food Standard is incorporated in both Volume 1 (as Standard A19) and Volume 2 (as Standard 1.5.1) of the *Food Standards Code*. Standard A19 and Standard 1.5.1 prohibit a novel food being sold by way of retail sale as food, or for use as a food ingredient, unless it is listed in the Table to clause 2 of the Standard, and complies with any special conditions specified in that Table. This Draft Assessment Report includes proposed draft variations for both Volume 1 and Volume 2 of the *Food Standards Code*.

The purpose of Standard A19 and Standard 1.5.1 is to ensure that non-traditional foods which have features or characteristics that may raise safety concerns will undergo a risk-based safety assessment before they are offered for retail for consumption in Australia or New Zealand. Because the Standards have a definition of a novel food that is based on the level of knowledge about the safe use of a food in the community, a preliminary assessment of this level of knowledge for a particular non-traditional food is needed in order to assess whether an application to amend the Standards is necessary. The Standards provide some assistance in this regard by indicating the factors to be taken into account in this decision-making process. Guidelines for assessing the novelty of a non-traditional food are provided in the ANZFA document *Guidelines for amending the Food Standards Code: Standard A19/Standard 1.5.1 – Novel Foods*. A decision in this regard is made in consultation with the Senior Food Officers in each of the States, Territories and New Zealand.

2. PROBLEM

Under the current food regulations, novel foods are required to undergo a pre-market safety assessment, as per Standard A19/1.5.1 – Novel Foods.

Novel food is defined in the Standard as:

> A non-traditional food or food ingredient for which there is insufficient knowledge in the broad community to enable safe use in the form or context in which it is presented, taking into account-
>
> (a) the composition or structure of the product;
> (b) levels of undesirable substances in the product;

(c) the potential for adverse effects in humans;
(d) traditional preparation and cooking methods; or
(d) patterns and levels of consumption of the product;

DHA-rich marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. are novel foods because there is insufficient knowledge in the broad community to ensure safe use in the form in which it is presented. *Schizochytrium* sp. is not a traditional food source and its safety needs to be assessed before it can be marketed. Similarly the DHA-rich oil derived from *Schizochytrium* sp. may contain unknown components from this new food source and its safety needs to be assessed before it can be marketed.

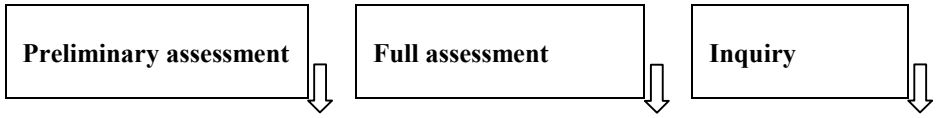
3. OBJECTIVE

The objective of this Application is to determine whether the food regulations can be amended to approve the use of dried marine micro-algae (*Schizochytrium* sp.) which is rich in the omega-3 long chain polyunsaturated fatty acid DHA (docosahexaenoic acid) as a novel food ingredient in a wide range of foods. Such an amendment to the *Food Standards Code* will need to be consistent with the section 10 objectives of ANZFA Act. The three primary objectives of the Authority are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

3.1 Background

ANZFA received an Application from Omega Tech Inc. on 13 March 2001 to amend Standards A19 and 1.5.1 of the *Food Standards Code* to permit the use of dried marine micro-algae (*Schizochytrium* sp.) which is rich in the omega-3 long chain polyunsaturated fatty acid DHA (docosahexaenoic acid; 22:6n-3) as a novel food ingredient in a limited range of foods. In the Initial Assessment (previously referred to as the Preliminary Assessment), the Application considered both the dried marine micro-algae as well as the oil derived from the micro-algae, following informal communication with the Applicant. The Applicant formally amended their Application on 28 August 2001, to include DHA-rich oil derived from the same species for use as a novel food ingredient in a limited range of foods. Accordingly, the Draft Assessment and this Final Assessment have considered both the dried micro-algae and the DHA-rich oil derived from the same species.



REPORTS	Initial assessment	Draft assessment	Final assessment
Content:	Outline of issues and scope of suggested amendments; requests for information and data / evidence pertinent to assessment and Regulatory Impact Statement.	Scientific risk assessment; examination of issues and conclusions as to regulatory response; proposed drafting for changes to Food Standards Code; Regulatory Impact Statement; WTO notification; request for comments and additional data / evidence relevant to review.	Evaluation of comments received and determination of final risk management and regulatory requirements.
Issues for consideration:	Comment on scope and direction of regulatory framework.	Review scientific risk assessment; confirm robustness of scientific assessment; review regulatory direction and justification; confirm draft standard; ensure all relevant issues addressed.	Review additional comments and evidence received to ensure all are addressed adequately and that no new evidence demands adjustment of final regulatory response.

3.2 Proposed uses

The dried marine algae is proposed to be used in the following foods at levels ranging between 200-300 mg per serving which corresponds to 40-60 mg DHA per serving:

- bread and other baked goods such as crispbreads;
- breakfast foods;
- table spreads;
- dressings/mayonnaise;
- modified milk products; and
- special purpose foods such as Formulated Meal Replacements/Supplementary Foods but excluding infant foods**.**

The DHA-rich oil derived from *Schizochytrium* sp. is proposed for use in the following foods (up to 150 mg per serving):

- liquid foods;
- beverages;
- fruit drinks;
- sport drinks;
- table spreads and dressings;
- dairy/non-dairy foods such as yoghurt and cheese products and ice creams;
- manufactured meat products and analogues; and
- infant foods and infant formulae.

3.3 Approval in other countries

In the United States, DHA-rich oil extracted from *Schizochytrium* sp. is sold as a dietary supplement under notification from the *Dietary Supplement Health and Education Act* (DSHEA), and as a nutritional ingredient in food following GRAS (generally recognised as safe) notification.

In the European Union, there is currently an application for the DHA-rich oil (but not the micro-algae) as a novel food.

In Japan, DHA-rich oil is considered a food and a regulatory filing is not required.

In Canada, there is no approval for these foods - Omega Tech Inc. intend to lodge an application for the use of DHA-rich oil in the near future.

4. OPTIONS

Parties affected by the options outlined include:

1. Food industry wishing to promote food products with DHA-rich oil and DHA-rich algae.
2. Consumers who may benefit from the use of DHA-containing products.
3. Government agencies enforcing the food regulations.

The various regulatory options are as follows:

Option 1. Not permit the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel food ingredients.

There are no perceived benefits to the stakeholders, government, consumers and industry, by maintaining the *status quo* and not giving specific permission in the *Food Standards Code* for the use of DHA- rich oil and DHA- rich dried marine micro-algae (*Schizochytrium* sp.) as novel food ingredients.

Although there is no perceived cost for the government at present, if, in the future, other countries approve the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel food ingredients, lack of approval in Australia or New Zealand may be construed as a non-tariff barrier to trade. Industry may be denied the use of an alternative source of omega-3 fatty acids in the form of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) in their food products. Consumers who do not eat fish on a regular basis may be denied the use of an alternative source of omega-3 fatty acids in the form of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) in their diet.

The parties who are disadvantaged by the current state of regulation, which would not permit the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.), are the applicant and the producers who may use them in their final food products. This option would essentially deny Australian and New Zealand industry and consumers who do not eat fish on a regular basis an alternative source of omega-3 fatty acids.

Option 2. Permit the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel food ingredients.

For the Government, the approval of the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel foods may in the future promote international trade and reduction of technical barriers to trade, while continuing to protect public health and safety.

For the industry this option will allow manufacturers to use an alternative source of omega-3 fatty acids in the form of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) in their food products. For the consumers who do not eat fish on a regular basis this option will give access to an alternative source of omega-3 fatty acids in the form of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) in their diet.

While the Government will incur the cost of amending the *Food Standards Code*, there are no perceived costs for the industry or the consumers.

5. IMPACT ANALYSIS

ANZFA is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, the food industry and governments in both Australia and New Zealand. The benefits and costs associated with the proposed amendment to the *Food Standards Code* have been analysed in a Regulatory Impact Assessment. Option 2 is the preferred option, because the assessment indicates that this Application raises no new issues, which would preclude the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.), being permitted under the *Food Standards Code*. The amendment to the *Food Standards Code* to permit the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) is cost effective and of benefit to both producers and consumers. For the preferred option, namely, approval of the use of DHA-rich *Schizochytrium* sp. dried marine micro-algae, and oil derived from DHA-rich *Schizochytrium* sp. marine micro-algae, the benefits of the proposed amendment outweigh the costs.

6. CONSULTATION

6.1 Public consultation

ANZFA conducted Initial and Draft Assessments (previously known as the Preliminary Assessment and Full Assessment respectively) of A428 - DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as a novel food ingredient. Public comments were called for between 8 May 2001 and 20 June 2001 (first round) and between 12 December 2001 and 6 February 2002 (round two). Six submissions were received during the first round and nine more during the second round. These are summarised in **Attachment 5**.

6.2 World Trade Organisation (WTO) Notification

Australia and New Zealand are members of the World Trade Organization (WTO) and are signatories to the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and on Technical Barriers to Trade (TBT Agreement). In some circumstances, Australia and New Zealand have an obligation to notify the WTO of changes to food standards to enable other member countries of the WTO to make comments.

This Application has been notified to the WTO because permission to use the DHA-rich micro-algae or the DHA-rich oil could lead to a liberalising effect on trade. There are no international standards in relation to DHA-rich micro-algae or the DHA-rich oil. No other submissions were received from other countries.

7. ISSUES ADDRESSED DURING ASSESSMENT

7.1 Role of DHA in human nutrition

Omega-3 long chain fatty acids, specifically, DHA have been identified as important dietary nutrients with specific roles in the developing foetus and pre-term infants.

There are also recent reports that indicate that DHA, as one of the omega-3 fatty acids, may have an important role in cardiovascular health and beneficial effects on the immune system in the general population. DHA is also considered to be important for the development and function of the brain and eyes, although the dietary essentiality of DHA in term infants is not unequivocally established.

7.2 Current sources of exposure to DHA in the diet

The principal dietary sources of DHA are oily fish species such as salmon, tuna, sardines, and herrings that feed on the micro-algae. Game meat is also a source of DHA.

However, the consumption of fish/game meats in Australia and New Zealand is relatively low and therefore the normal exposure to DHA is low.

7.3 Safety of DHA-rich *Schizochytrium* sp. micro-algae

A detailed report on the safety of DHA-rich *Schizochytrium* sp. micro-algae and oil derived from this micro-algae is provided at **Attachment 2**.

Schizochytrium micro-algal species are widely distributed in marine habitats although there are no reports of their being used for human consumption. There are no reports of toxins being found in this class of micro-algae. Detailed specifications for both the micro-algae and for the oil derived from the micro-algae have been provided.

The Applicant has submitted detailed toxicological studies to support the safety of DHA-rich *Schizochytrium* sp. micro-algae. These studies indicate that the micro-algae has low toxicity, is not genotoxic or teratogenic and has no effect on reproductive parameters. The no-observable-effect level (NOEL) from a 13-week feeding study in rats was 8% in the diet (equivalent to 4000 mg/kg bw/day, which equates to approximately 430 mg/kg/day of DHA). This was the highest dose level tested in this study. An earlier study in a different strain of rat at higher doses was not considered suitable to assess the safety of dried micro-algae.

There are no human toleration studies available on the micro-algae or on the oil derived from the micro-algae. However, the compositional analysis of DHA-rich micro-algae or DHA-rich oil derived from the micro-algae do not raise any particular concerns in relation to the safety of the components of these products. There are also numerous published studies available on the safety of DHA and other DHA-rich oils (see **Attachment 2**). None of the available studies indicate any toxicity associated with DHA, at the anticipated levels of exposure. Human breast milk contains low but significant levels of DHA and this source provides a daily intake of approximately 1.5 g of DHA for breast-milk fed infants. This level has been used as the basis for GRAS status for DHA-rich micro-algae use in the USA.

The data from the available animal studies, taken together with the composition data and the data on known levels of exposure to DHA, does not indicate any potential for toxicity associated with dried micro-algae or the oil derived from this micro-algae.

7.4 Composition of DHA-rich oil extracted from *Schizochytrium* sp. micro-algae

The compositional equivalence of this oil to other traditionally used DHA-rich oils available on the market has been examined (see **Attachment 3** – Draft Food Technology report).

The results indicate that the fatty acid profile of DHA-rich oil is similar to those of other marine based oils. About 60% (w/w) of the micro-algae is made up of fatty acids of which DHA is the major component (35%) followed by palmitic acid (24%), docosapentaenoic acid (DPA) (13.6%) and myristic acid (10.1%).

Sterols make up about 3% of the total mass of the DHA-rich oil. The following sterols have been identified: cholesterol (25%), brassicasterol (15%), ergosta-7,22-dien-3-ol (5-7%), ergosta-7,24-dien-3-ol (5-6%), stigmasta-5,22-dien-3-ol (19%) and stigma-5,23-dien-3-ol (8%). All sterols identified in the unsaponifiable fraction of DHA-rich oil are present in the human food supply. The exposure to these sterols when using proposed level of these DHA-rich products would not exceed the current consumption of sterols in the general population from other food sources. Their absorption, distribution and excretion profiles in mammalian species are well understood.

Comparison of fatty acid profiles of *Schizochytrium* sp. derived DHA-rich oil with other traditional oils shows similarities as well as differences in the fatty acid make up. For example, compared to fish oils (menhaden, salmon, cod-liver) the ratio of DHA/EPA is much greater in the micro-algal products. Further, the presence of DPA is much greater in *Schizochytrium* oil than in other fish oils. Vegetable oils and fats do not contain C20 or C22 n-6 or n-3 fatty acids in appreciable amounts, but these fatty acids can be found in foods of animal origin, such as egg yolk and meat. The longer-chain n-3 fatty acids, 20:5n-3 and 22:6n-3 are found in highest amounts in high-fat fish and marine species. In contrast, marine macro-algae such as seaweeds (*Undaria*, *Porphyra* and *Laminaria*), which are traditionally consumed in Japan, have only trace amounts of C20 or C22 n-6 or n-3 fatty acids. Fatty acid profiles of macro-algae from Australia and French Brittany Coast also show only trace amounts of longer chain (C20 and C22) polyunsaturated fatty acids.

In general, the iodine content in marine seaweeds consumed in Japan and Korea are high. The dried lavers from nori (*Porphyra* sp.) for example contains 4-6 mg of dietary iodine per 100g dry weight[1]. However, the iodine content in the *Schizochytrium* sp. micro-algal powder is 0.006 mg per 100 g. Thus there is no public health concern from excessive intake of this micro-algae leading to harmful levels of dietary iodine.

7.5 Potential dietary exposure to DHA-rich *Schizochytrium* sp. micro-algae

A detailed report on the potential dietary exposure to DHA-rich micro-algae or DHA-rich oil derived from the micro-algae is provided at **Attachment 4**.

[1] Watanabe et al., (1999) **J. Agric Food Chem**, *47(6)*, 2341-2343.

The principal dietary sources of DHA are oily fish species such as salmon, tuna, sardines, and herrings, which feed on the micro algae. Game meat is also a source of DHA. However, the consumption of fish/game meats in Australia and New Zealand is relatively low and therefore the normal exposure to DHA is low. The all *cis*-form of DHA in DHA-rich micro-algae or DHA-rich oil derived from the micro-algae is the same as that reported in many fish oils[2].

Various international organisations, such as British Nutrition Foundation, Health Canada, France-AFSSA, Centre National de la Reserche Scientifique (CNRS), International Society for the Study of Fatty Acids and Lipids and WHO, have recommended intakes of DHA from 200-2000 mg/day.

The Australian National Heart Foundation in 1999 recommended at least two fish meals/week, should be consumed, although they did not specify a recommended daily intake of DHA *per se*.

Using Australian nutrient composition data, ANZFA has estimated that mean exposure to DHA, from existing food sources, to be 100 mg per day for all respondents aged 2-100 years, with high consumers (95th percentile) exposed to 480 mg per day, as outlined in Table 3 in **Attachment 4**. Estimates of diet-derived DHA exposure were not able to be determined for New Zealanders, as information on the levels of DHA in New Zealand foods was not available.

ANZFA has also conducted dietary modelling on the proposed uses of this micro-algae and its oil in various foods to determine the dietary intakes for the mean consumer and the 95th percentile consumer. The mean total dietary exposure based on exposure to DHA solely from the use of micro-algae was determined to be 260 mg per day in Australia and 280 mg per day in New Zealand. The 95th percentile total dietary exposure based on the same exposure data was determined to be 600 mg per day in Australia and 690 mg per day in New Zealand.

Estimated 95th percentile total dietary exposure to DHA from all sources (micro-algae and other dietary sources) indicate that even in the population with greatest potential exposure (Australian adults 19-100 years, 950 mg/day), which is a conservative estimate of intake, the dietary exposure would be well below the DHA intake (1.5g/day) for infants from human breast milk.

7.6 Use of DHA-rich *Schizochytrium* sp. micro-algae or DHA-rich oil derived from *Schizochytrium* sp. in food products

A detailed Food Technology report is provided at **Attachment 3**.

The dried micro-algae are produced by a controlled fermentation process, and have a minimum crude fat content of about 37% corresponding to a minimum DHA content of 15%. DHA itself is a highly unsaturated fatty acid and susceptible to oxidative degradation, however, encapsulation of the DHA by the dried micro-algae provides stability and it can then be effectively used in various food products for DHA enrichment.

The Applicant has provided detailed product specifications for the dried micro-algae and its DHA-rich oil.

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[2] Omega-3-acid triglycerides (1999):1352, corrected 2000 in European Pharmacopoeia, *suppl.* 2000.

The Applicant has provided ANZFA with a product stability assay report, which demonstrates that the dried micro-algae product is very stable. Assessment of this data is included in the Safety Assessment report.

7.7 Use of DHA-rich oil derived from *Schizochytrium* sp. in infant formula

The Applicant has indicated that the DHA-rich oil may be used in infant formula, which is regarded as a Special Purpose Food, as well as in general purpose foods. The stated purpose of the Novel Food Standard is to ensure the safety of non-traditional foods in the general food supply. Ingredients of Special Purpose Foods, however, may have additional requirements in terms of safety and efficacy before use is permitted in these foods.

These additional requirements cannot be assessed under Novel Foods Standard. Therefore while the Novel Foods Standard does not prohibit the use of these products in infant formula, the DHA-rich oil derived from *Schizochytrium* sp. still needs to comply with any additional requirement of the Infant Formula Standards 2.9.1 normally that it be nutritionally adequate for infant feeding, before it can be used in infant formula preparations.

7.8 Other issues arising from public submissions

(i) Safety of DHA-rich *Schizochytrium* sp. micro-algae in infant formula

Issue: *Use of DHA alone in infant formula, without the presence of ARA, may lead to lower fatty acid levels of ARA compared with breast milk-fed infants and infants fed formula supplemented with both DHA and ARA.*

Response
In the draft Infant Formula Standard 2.9.1, ANZFA requires that supplemented infant food formula should contain ARA and DHA at levels of 2:1. Therefore it is the responsibility of the infant food formula manufacturers to meet the required specifications.

Issue: *The presence of eicosapentaenoic acid (EPA) in the marine oils, when used in infant formula may reduce ARA levels, which may lead to reduced growth and mental development.*

Response
Earlier studies linked poor growth to the use of marine oil containing EPA/DHA ratio of 2:1 to depressed arachidonic acid (AA)[1]. This concern has been addressed by using low EPA marine oil with an EPA/DHA ratio of 1:10[2,3]. This did not compromise weight gain and in fact resulted in higher Bayley mental scores at 12 months. The oil extracted from *Schizochytrium* sp. contains an EPA/DHA ratio of 1:13.6 and therefore it is unlikely to affect the synthesis of AA.

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[1] Carlson *et al*., (1992) **J Pediatr**., *120 (4 Pt 2),* S159-167.
[2] Carlson *et al*., (1993) **Proc. Natl. Acad. Sci. USA**, *90*, 1073-1077.
[3] Carlson *et al*., (1996) **J Nutr.,** *126 (4 Suppl)*, 1092S-1098S.

(ii) Other safety issues

Issue: *There is a lack of data with respect to long term exposure to higher intakes of DHA.*

Response
Humans have been exposed to DHA through traditional sources such as fish oils which have constituted part of human diet for centuries. It is known that consumption of high amounts of long chain n-3 polyunsaturated fatty acids (PUFA) leads to longer bleeding times[4]. Clinical trials using low to moderate doses of fish oil (0.5g to 2.0g per day of n-3 PUFA) did not increase bleeding times significantly[5]. The USFDA have stated that consumption of up to 3g/day of EPA plus DHA has been considered to have no effect on the bleeding times.

Issue: *The safety of the extraction of DHA-rich oil from the algal biomass which may concentrate the levels of unknown sterols*

Response
The extraction procedure uses hexane in which fats and oils are fully miscible. Hence the possibility of enriching sterols over fatty acids is unlikely and is confirmed by specification data. All the eight sterols found in the DHA-rich oil have been identified and well characterised.

Issue: *The safety of the DHA-rich oil with other food ingredients in matrices of products for infants and young children need to be demonstrated*

Response
Only known fatty acids or sterol components will be introduced into the diet by the use of these products. The components of the DHA-rich oil are known and have been shown to be safe by experimental studies and by the historical use of fish oils of similar composition.

Issue: *Analysis for the presence of common algal toxins in the dried micro-algal powder needs to be carried out especially since the powder is stored under different environmental conditions.*

Response
There are two known toxins, domoic acid and prymnesin, which are produced by the micro-algae in the Chromista, to which *Schizochytrium* sp. belongs. HPLC analysis carried out by the applicant for the presence of domoic acid did not find any trace of this compound in the micro-algal powder. A bioassay for the detection of prymnesin was also performed on dried micro-algae, which failed to detect the presence of this toxin.

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[4] NHMRC Working Party (1992) The role of polyunsaturated fats in the Australian diet. Australian Government Publishing Service, Canberra.
[5] Connor WE (1994) Omega-3 fatty acids and heart disease. *Kritchevsky D, Carroll KK eds. Nutrition and Disease Update: Heart Disease. Champaign, IL: AOCS Press. pp1-42.*

Issue**:** *What is the evidence that other phytoplanktons are not present in the fermented material? Respiratory problems experienced by one worker may have been due to phytoplankton exposure.*

Response
The strain of *Schizochytrium* sp. used for commercial production is genetically stable pure culture strain derived from a wild strain. This strain is used because it produces greater yields of DHA. The culture used for the commercial fermentations are grown up from pure starter cultures. Thus contamination with other phytoplankton sources will not occur.

The reported respiratory difficulties experienced by one worker following excessive exposure to an aerosol mist of the fermentation broth during product recovery of *Schizochytrium* sp. was determined to be pulmonary hypersensitivity. It is generally caused by overexposure to aerosols, which may contain microorganisms from a variety of sources including industrial bioprocesses. Removing sources of aerosol/dusts from the working environment has since eliminated health risk to workers.

Issue: *Some micro-algae support the growth of human pathogens notably Vibrio cholerae and Vibrio parahaemolyticus which are infective but non-culturable states and hence difficult to demonstrate pathogen-free status of harvested micro-algae.*

Response
As the culture used for the commercial fermentations is grown up from pure starter cultures under controlled environmental conditions, contamination with these microbial pathogens will be avoided.

(iii) Stability

Issue: *What is the stability of the DHA during improper storage that may lead to oxidation and production of radicals and peroxide?*

Response
The refined oil is encapsulated using a process similar to the standard industrial soft gel encapsulation process. The stability report submitted by the applicant shows that the encapsulated DHA-rich oil retains DHA levels to within 15% of Day 0 values and without the formation of any new product. The peroxide values also remained below 10 meq/kg limit for the duration of 24 month study suggesting that the oxidation and subsequent radical formation are minimal. In comparison, fish oils have much lower stability due to oxidation during production, and storage.

(iv) Labelling

Issue: *Submitter suggested that foods containing this ingredient should contain a label, which encourages a varied diet and acknowledges that fish and seafood are the best sources of DHA*

Response

General labelling

ANZFA considers labelling issues in regard to both public health and safety concerns and consumer choice. There are no public health and safety concerns regarding the use of this micro-algal powder or the oil derived from it. The submitter's labelling request pertains to consumer choice. There is no information to suggest that DHA derived from micro-algae is inferior to DHA from more traditional sources such as seafood and fish as suggested by the submitter. Consequently, the proposed labelling statement is not considered necessary.

Ingredient Labelling

With regard to ingredient labelling, ANZFA has considered whether the source of the DHA should be identified in the ingredient list on product labels. It was considered that such labelling would not necessarily provide meaningful information that would assist consumers in making informed choices. In addition it may result in extensive and highly detailed ingredient labelling on consumer products - this could potentially cause confusion for consumers who utilise ingredient list labelling and may result in increased labelling cost for industry. The labelling for these products will still need to comply with general labelling provisions contained in the *Food Standards Code* (for instance, Standard 1.2.4 – Labelling of Ingredients, which requires the declaration of ingredients using the common name of the ingredient, a name describing the true nature of the ingredient, or specified generic name). Consequently additional labelling provisions specifically requiring the source from which the DHA product is derived to be specified will not be required.

(v) Efficacy

Issue: *Efficacy of these products should be established before approval.*

Response

The efficacy of these products in relation to any potential nutritional/health claim is not being considered as part of this Application. Health claims are currently prohibited unless specific permission is given under the *Food Standards Code*. ANZFA has before it a Proposal (P153) to review the current regulatory measures in relation to health claims.

(vi) Novel food status for DHA-rich *Schizochytrium* sp. products

Issue: *i) These products are not novel based on the presence of components in other traditional food. ii) Equivalence in composition to other recognised sources is sufficient to allow the safe use of food components from non-traditional sources.*

Response
DHA-rich marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. are non-traditional foods because they do not have a history of significant human consumption by the broad community in Australia and New Zealand. They are considered to be novel foods for the purposes of the Standard because there is insufficient knowledge in the broad community to enable safe use of these foods in the form or context in which they are proposed to be presented.

Although the components of the micro-algal products, DHA and other fatty acids and sterols, are also present in traditional DHA sources such as seaweed and fish, the novel food requirement is based on the marine micro-algae and the oil derived from it which may contain other unknown components whose safety can only be determined after the evaluation of safety data by ANZFA.

(vii) Inconvenient timing of public consultation during the Holiday period

Issue**:** *National Council of Women of Australia considers that the submission period in January is inappropriate*

Response
ANZFA appreciates the difficulty this poses, however ANZFA is under statutory obligation to follow the 12-month timeframe for completion of the assessment of applications. The submissions period for this Application was extended by two more weeks for a total of eight weeks to give maximum opportunity for all interested parties to respond.

(viii) Effect on the marine ecology

Issue**:** *Does the collection of this micro-algal species affect the ecological balance of marine animals that feed on them?*

Response
The micro-algae are produced from a production fermentation process using pure starter culture. It does not involve harvesting of micro-algae from the natural sources and hence has no effect on the marine ecology.

8. RISK ANALYSIS

The safety of DHA-rich micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. is based on: (i) consideration of the safety of the source organism; (ii) the composition of the dried micro-algae and the oil derived from the micro-algae; (iii) toxicology studies conducted on the micro-algae; (iv) safety studies on DHA and DHA-rich oils; and (v) a history of human exposure to DHA in foods. Considered together, this data demonstrates that this micro-algae and the oil derived from it are a safe source of DHA. There is no evidence of toxicity associated with exposure to the micro-algae or to the DHA-rich oil derived from it at the anticipated levels of exposure.

The compositional analysis of the micro-algae or the oil derived from the micro-algae indicates that the oil is comparable to other traditional sources of DHA and does not raise any safety concerns regarding other minor ingredients.

The dietary exposure assessment indicates the potential exposure to DHA from the micro-algae or the oil derived from the micro-algae is well within the levels shown to be safe from the animal studies conducted on the micro-algae, and from the animal and human studies conducted on DHA derived from other sources.

On the basis of the available data, it is not proposed that there be any restriction on the level of use of DHA-rich micro-algae or DHA-rich oil derived from micro-algae as novel foods. It is proposed, however, that both products should be required to comply with certain specifications (see **Attachment 1** for details).

The use and extent of supplementation of the food supply with DHA should be monitored to ensure the safety of long-term exposure at high DHA levels.

9. CONCLUSIONS

1. The available data on DHA-rich marine micro-algae (*Schizochytrium* sp.) and on DHA-rich oil derived from *Schizochytrium* sp. does not raise any safety concerns at the anticipated levels of exposure.

2. The composition of the *Schizochytrium* sp. micro-algae and the oil derived from *Schizochytrium* sp. are comparable to other traditional sources of DHA.

3. *Schizochytrium* sp. micro-algae and oil derived from the micro-algae will provide an alternative source of omega-3 fatty acids in foods.

4. The proposed changes to Volume 1 and Volume 2 of the *Food Standards Code* are consistent with the section 10 objectives of the *Australia New Zealand Food Authority Act 1991* and the regulatory impact assessment.

10. FOOD STANDARDS SETTING IN AUSTRALIA AND NEW ZEALAND

The Governments of Australia and New Zealand entered an Agreement in December 1995 establishing a system for the development of joint food standards. On 24 November 2000, Health Ministers in the Australia New Zealand Food Standards Council (ANZFSC) agreed to adopt the new *Australian New Zealand Food Standards Code*. The new Code was gazetted on 20 December 2000 in both Australia and New Zealand as an alternate to existing food regulations until December 2002 when it will become the sole food code for both countries. It aims to reduce the prescription of existing food regulations in both countries and lead to greater industry innovation, competition and trade.

Until the joint *Australia New Zealand Food Standards Code* is finalised the following arrangements for the two countries apply:

- **Food imported into New Zealand other than from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code*, as gazetted in New Zealand, or the New Zealand *Food Regulations 1984,* but not a combination thereof. However, in all cases maximum residue limits for agricultural and veterinary chemicals must comply solely with those limits specified in the New Zealand *(Maximum Residue Limits of Agricultural Compounds) Mandatory Food Standard 1999.*

- **Food imported into Australia other than from New Zealand** must comply solely with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as the joint *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code*, but not a combination of the two.

- **Food imported into New Zealand from Australia** must comply with either Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* as gazetted in New Zealand, but not a combination thereof. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

- **Food imported into Australia from New Zealand** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code*, but not a combination of the two. However, under the provisions of the Trans-Tasman Mutual Recognition Arrangement, food may **also** be imported into Australia from New Zealand provided it complies with the New Zealand *Food Regulations 1984.*

- **Food manufactured in Australia and sold in Australia** must comply with Volume 1 (known as Australian *Food Standards Code)* or Volume 2 (known as *Australia New Zealand Food Standards Code)* of the Australian *Food Standards Code* but not a combination of the two. Certain foods listed in Standard T1 in Volume 1 may be manufactured in Australia to equivalent provisions in the New Zealand *Food Regulations 1984.*

In addition to the above, all food sold in New Zealand must comply with the New Zealand *Fair Trading Act 1986* and all food sold in Australia must comply with the Australian *Trade Practices Act 1974,* and the respective Australian State and Territory *Fair Trading Acts.*

Any person or organisation may apply to ANZFA to have the *Food Standards Code* amended. In addition, ANZFA may develop proposals to amend the Australian *Food Standards Code* or to develop joint Australia New Zealand food standards. ANZFA can provide advice on the requirements for applications to amend the *Food Standards Code*.

11. FURTHER INFORMATION

Submissions
No submissions on this matter are sought as the Authority has completed its assessment and the matter is now with the Australia New Zealand Food Standards Council for consideration.

Further Information
Further information on this and other matters should be addressed to the Standards Liaison Officer at the Australia New Zealand Food Authority at one of the following addresses:

Australia New Zealand Food Authority
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2258
email: slo@anzfa.gov.au

Australia New Zealand Food Authority
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
email: anzfa.nz@anzfa.gov.au

Assessment reports are available for viewing and downloading from the ANZFA website www.anzfa.gov.au or alternatively paper copies of reports can be requested from the Authorities Information Officer at info@anzfa.gov.au.

ATTACHMENTS

1 Draft Variation to Volume 1 and Volume 2 of the *Food Standards Code*.
2 Safety Assessment Report
3 Food Technology Report
4 Dietary Exposure Assessment Report
5 Summary of public submissions

ATTACHMENT 1

DRAFT VARIATIONS TO VOLUME 1 AND VOLUME 2 OF THE *FOOD STANDARDS CODE*

To commence: on gazettal

[1] ***Standard A11*** *of Volume 1 of the Food Standards Code is varied by –*

[1.1] *inserting in the* Schedule *into* Column 1 *and* Column 2 *respectively, immediately after the entry for* Divinylbenzene copolymer –

Docosahexaenoic acid (DHA) – rich dried marine micro-algae (*Schizochytrium* sp.)	Addendum 14
Docosahexaenoic acid (DHA) – rich oil derived from marine micro-algae (*Schizochytrium* sp.)	Addendum 15

[1.2] *inserting, immediately after* Addendum 13 –

ADDENDUM 14

SPECIFICATION FOR DOCOSAHEXAENOIC ACID (DHA) – RICH DRIED MARINE MICRO-ALGAE (*SCHIZOCHYTRIUM* SP.)

Full chemical name for DHA	4,7,10,13,16,19-docosahexaenoic acid (22:6n-3 DHA)
Appearance	Free flowing coarse powder
Colour	Golden (yellow to light orange)
Odour	Slight marine
Solids (%)	min. 95.0
Crude oil (%)	min. 37.0
DHA (%)	min. 15.0
Peroxide value (meq/kg)	max. 10.0
Ash (%)	max. 12
Sodium (%)	max. 3
Heavy metals (ppm) (as Pb)	max. 20
Lead (ppm)	max. 2
Arsenic (ppm)	max. 1
<u>Microbiological</u>	
Total count (cfu/g)	max. 10,000
Yeast (cfu/g)	max. 300
Mould (cfu/g)	max. 300
E. coli	Negative to test
Salmonella	Negative to test

ADDENDUM 15

SPECIFICATION FOR DOCOSAHEXAENOIC ACID (DHA) – RICH OIL DERIVED FROM MARINE MICRO-ALGAE (*SCHIZOCHYTRIUM* SP.)

Full chemical name for DHA	4,7,10,13,16,19-docosahexaenoic acid (22:6n-3 DHA)
Appearance	Free flowing oil
Colour	Pale light yellow to orange
Odour	Characteristic bland to fish-like
DHA (%)	min. 32 max. 45
Tetradecanoic acid 14:0 (%)	min. 5 max. 11
Hexadecanoic acid 16:0 (%)	min. 18 max. 25
Eicosapentaenoic acid 20:5n-3 (%)	min. 0.5 max. 4
Docosapentaenoic acid 22:5n-6 (%)	min. 10 max. 20
Peroxide value (meq/kg)	max. 10
Moisture and volatiles (%)	max. 0.10
Non-saponifiables (%)	max. 4.5
Trans fatty acids (%)	max. 2.0
Free fatty acid	max. 0.25
Lead (ppm)	max. 0.2
Arsenic (ppm)	max. 0.2
Copper (ppm)	max. 0.05
Iron (ppm)	max. 0.25
Mercury (ppm)	max. 0.2
Hexane (ppm)	max. 20

[2] Standard A19 *of Volume 1 of the Food Standards Code is varied by inserting in the Table to clause 2, into Column 1 and Column 2 respectively –*

Docosahexaenoic acid (DHA) – rich dried marine micro-algae (*Schizochytrium* sp.)	May only be added to food according to Standard A11.
Docosahexaenoic acid (DHA) – rich oil derived from marine micro-algae (*Schizochytrium* sp.)	May only be added to food according to Standard A11.

[3] ***Standard 1.3.4*** *of Volume 2 of the Food Standards Code is varied by inserting in the* Schedule*, following the* Specification for diethyl aminoethyl cellulose ion exchange resin –

Specification for docosahexaenoic acid (DHA) – rich dried marine micro-algae (*Schizochytrium* sp.)

Full chemical name for DHA	4,7,10,13,16,19-docosahexaenoic acid (22:6n-3 DHA)
Appearance	Free flowing coarse powder
Colour	Golden (yellow to light orange)
Odour	Slight marine
Solids (%)	min. 95.0
Crude oil (%)	min. 37.0
DHA (%)	min. 15.0
Peroxide value (meq/kg)	max. 10.0
Ash (%)	max. 12

Sodium (%)	max. 3
Heavy metals (ppm) (as Pb)	max. 20
Lead (ppm)	max. 2
Arsenic (ppm)	max. 1
Microbiological	
Total count (cfu/g)	max. 10,000
Yeast (cfu/g)	max. 300
Mould (cfu/g)	max. 300
E. coli	Negative to test
Salmonella	Negative to test

Specification for docosahexaenoic acid (DHA) – rich oil derived from marine micro-algae (*Schizochytrium* sp.)

Full chemical name for DHA	4,7,10,13,16,19-docosahexaenoic acid (22:6n-3 DHA)
Appearance	Free flowing oil
Colour	Pale light yellow to orange
Odour	Characteristic bland to fish-like
DHA (%)	min. 32 max. 45
Tetradecanoic acid 14:0 (%)	min. 5 max. 11
Hexadecanoic acid 16:0 (%)	min. 18 max. 25
Eicosapentaenoic acid 20:5n-3 (%)	min. 0.5 max. 4
Docosapentaenoic acid 22:5n-6 (%)	min. 10 max. 20
Peroxide value (meq/kg)	max. 10
Moisture and volatiles (%)	max. 0.10
Non-saponifiables (%)	max. 4.5
Trans fatty acids (%)	max. 2.0
Free fatty acid	max. 0.25
Lead (ppm)	max. 0.2
Arsenic (ppm)	max. 0.2
Copper (ppm)	max. 0.05
Iron (ppm)	max. 0.25
Mercury (ppm)	max. 0.2
Hexane (ppm)	max. 20

[4] ***Standard 1.5.1*** *of Volume 2 of the Food Standards Code is varied by inserting in the* Table to clause 2*, into* Column 1 *and* Column 2 *respectively –*

Docosahexaenoic acid (DHA) – rich dried marine micro-algae (*Schizochytrium* sp.)	May only be added to food according to Standard 1.3.4.
Docosahexaenoic acid (DHA) – rich oil derived from marine micro-algae (*Schizochytrium* sp.)	May only be added to food according to Standard 1.3.4.

ATTACHMENT 2

FINAL SAFETY ASSESSMENT REPORT

DHA-RICH DRIED MARINE MICRO-ALGAE (*SCHIZOCHYTRIUM* SP.) AND DHA-RICH OIL DERIVED FROM *SCHIZOCHYTRIUM* SP.

SUMMARY

The safety of DHA-rich dried marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. is based on: (i) consideration of the safety of the source organism; (ii) the composition of the dried micro-algae and the oil derived from the micro-algae; (iii) toxicology studies conducted on the micro-algae; (iv) safety studies on DHA and DHA-rich oils; and (v) a history of human exposure to DHA in foods.

Safety of the source organism

Schizochytrium sp. is a member of the kingdom Chromista (also called stramenopiles) which includes golden algae, diatoms, yellow-green algae and thraustochytrids but not the toxic blue-green or dinoflagellate micro-algae. There are no reports of human consumption of *Schizochytrium* sp., however, the filter feeders (clams and mussels) that feed on this organism are part of the normal diet. The improved strain of *Schizochytrium* was developed from a patented wild-type parent strain and selected for its improved DHA productivity.

Composition of the dried micro-algae and oil derived from the micro-algae

Schizochytrium sp. powder has a high oil content (minimum 37 %) and the oil has a high DHA content (greater than 40%) encapsulated within the micro-algal cells. The dried micro-algal powder has a minimum DHA content of 15%. The product is stabilised by an approved food grade antioxidant.

Toxicology studies on the dried micro-algae (*Schizochytrium* sp.)

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

Published studies on DHA and DHA-rich oils

Studies are available in both animals and in humans exposed to DHA. DHA oil from algal sources is well absorbed by healthy adults with plasma and red blood cell levels of DHA increasing in proportion to the DHA dosage. Exposure to DHA derived from micro-algae also elevates DHA in the breast milk lipids of lactating women. None of the available studies in animals or humans demonstrate adverse effects associated the DHA exposure. The human studies available were conducted primarily for efficacy purposes but there were no reports of adverse effects at a dose level of 2.5 g/day for four weeks.

There are reported studies which indicate that consumption of high amounts of long chain n-3 polyunsaturated fatty acids (PUFA) leads to longer bleeding times. Clinical trials using low to moderate doses of fish oil (0.5g to 2.0g per day of n-3 PUFA) did not increase bleeding times significantly. The USFDA have stated that consumption of up to 3g/day of EPA plus DHA has been considered to have no effect on the bleeding times.

History of exposure to DHA in foods

The principal dietary sources of DHA are oily fish species such as salmon, tuna, sardines, and herrings that feed on the micro algae. Game meat is also a source of DHA. However, the consumption of fish/game meats in Australia and New Zealand is relatively low and therefore the normal exposure to DHA is low.

Conclusion

The safety of *Schizochytrium* sp. micro-algae and the DHA-rich oil derived from this species is well supported by the current knowledge of the safety of its components published in the literature and from the safety studies provided by the applicant. Species of *Schizochytrium*, while not directly used by humans as food, are consumed by marine animals that form part of human food supply.

The available toxicology studies conducted in animals do not raise any safety concerns. While there are no human studies available specifically on DHA-rich micro-algae or on the oil derived from the micro-algae, the compositional analysis of these products do not raise any particular concerns in relation to the safety of their components. There are also numerous published studies available on the safety of DHA and other DHA-rich oils at the anticipated levels of exposure. The effects of n-3 fatty acids on bleeding times has been observed at only extreme levels of exposure. The use of DHA-rich micro-algae and oil derived from this micro-algae in foods is not reported to lead to any adverse health effects.

DHA-RICH DRIED MARINE MICRO-ALGAE (*SCHIZOCHYTRIUM* SP.) AND DHA-RICH OIL DERIVED FROM *SCHIZOCHYTRIUM* SP.

BACKGROUND

ANZFA received an Application to amend the *Food Standards Code* to include dried micro-algae (*Schizochytrium* sp.) and the oil derived from it as permitted novel foods. Approximately 60% of the micro-algae is made up of fatty acids, and approximately 35% is the omega-3 fatty acid, docosahexaenoic acid (DHA).

History of Source Organism

Schizochytrium sp. is a member of the kingdom Chromista (also called stramenopiles) which includes golden algae, diatoms, yellow-green algae and thraustochytrids but not the toxic blue-green or dinoflagellate micro-algae. *Schizochytrium* sp. is a thraustochytrid and is found throughout the world in estuarine and marine habitats. Current molecular biological techniques have demonstrated that thraustochytrids are not fungi and they are related to the heterokont algae.

There are no reports of human consumption of *Schizochytrium* sp., however, the filter feeders (clams and mussels) that feed on this organism are part of the normal diet.

The improved strain of *Schizochytrium* was developed from a patented wild-type parent strain for its improved DHA productivity.

Chemistry of DHA

Docosahexaenoic acid (DHA) is a long chain, polyunsaturated fatty acid with the formula $C_{22}H_{32}O_2$. A shorthand nomenclature is 22:6n-3 which indicates 22 carbon atoms in the molecule, 6 double bonds and 3 carbon atoms from the methyl terminus to the first double bond. n-3 and n-6 fatty acids are essential to normal human growth and must be obtained from the diet, nominally from vegetable oils such as linolenic acid (18:3n-3) or linoleic acid (18:2n-6).

The longer chain n-3 fatty acids (e.g. eicosapentaenoic acid, 20:5n-3 and DHA, 22:6n-3) are found in high amounts in high fat-containing fish and marine animals. From the data supplied by the applicant there appears to be some variability in percent of DHA containing-fatty acids in fish species (e.g. 5% in Plaice to 26% in Red mullet).

In addition to dietary sources, longer chain unsaturated fatty acids (n-3, n-6, n-7 and n-9) are synthesized *in vivo* by enzymatic desaturation, and chain-elongation reactions and for the very long-chain fatty acids, by retro-conversion by specific enzymes (Sinclair, 1984).

Therefore, DHA is absorbed, distributed, metabolised and excreted via the normal biochemical pathways for other triglycerides and fatty acids in the human body. Previous studies have demonstrated that algal sources of DHA oil are well absorbed by healthy adults with plasma and red blood cell levels of DHA increasing in proportion to the algal DHA dosage (Innis and Hansen; Becker and Kyle, 1998).

DHA is found in both triglyceride and phospholipids in human breast milk. However, breast milk is primarily triglyceride (ca. 98%), with only about 1% phospholipid, and 1% unsaponifiable fats such as cholesterol and phytosterols (Jensen, 1996). While the DHA level in the phospholipid fraction of breast milk is relatively higher than in the triglyceride fraction (Jensen, 1996), the absolute amount of DHA in breast milk is much higher in the triglyceride fraction. Therefore, the majority of DHA in breast milk is found in the triglyceride fraction. DHA in DHA–rich oil derived from *Schizochytrium* sp. is found predominantly in the triglyceride fraction. This is also true for DHA present in tuna oils, other fish oils, and other micro-algal oils (e.g. *Chrypthecodinium cohnii* oil).

DHA in DHA–rich oil derived from *Schizochytrium* sp is esterified to both sn-1,3 and sn-2 positions on the triglyceride molecule. This is also true for DHA present in tuna oil triglyceride (Amate et al., 1999) and in *Chrypthecodinium cohnii* oil (Myher et al., 1996).

Makrides *et al* (1996) demonstrated bioavailability of DHA from oil derived from a micro-algae source in lactating women by the elevation of DHA in their breast milk lipids in a linear, dose-dependent fashion.

Approximately 80% of DHA is absorbed when provided in an infant formula which is similar to absorption rates from triglycerides in human milk (Carnielli *et al* 1998). Radiolabelled studies on ^{13}C-derived DHA have also demonstrated uptake of DHA from the gut, transportation to the vasculature and appearance in breast milk at similar rates to other fatty acids (Croset *et al* 1996). DHA is found in high concentrations in specific tissues such as brain, eye, testes and heart.

Fatty acids found in foods

The Applicant has supplied extensive data detailing typical fatty acid compositions of fatty acid components in DHA-rich oil and other foods (fats and oils). Lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0) and palmitoleic (16:1) acids are present in one or more of commercial fats and oils. Vaccenic acid (18:1n-7), arachidonic (20:4n-6), eicosapentaenoic and DHA are commonly found in meats and seafoods (Sinclair, 1984; Padley *et al*, 1994).

Three minor fatty acids, tetradecatrienoic acid (14:3n-3), eicosatetraenoic (20:4n-3) and docosatetraenoic acid (22:4 n-9) were identified in the DHA-rich oil in trace to small amounts. These acids are degradation or synthesis products of fatty acids present in the diet (linolenic, vaccenic and oleic acid).

SINGLE DOSE STUDIES

1. Acute oral limit study of DHA-rich oil derived from DHA-rich micro-algae in mice.
Study number EHL 97137 by Bechtel CL and Thake DV. Environmental Health Laboratory, USA. *October 24, 1997.*

Test material:	DHALIP-NS-yellowish DHA-rich oil derived from *Schizochytrium* sp.
Test Species:	Crl:CD-1 (ICR)BR (VAF/Plus) mice 5 males and 5 females per test dose, administration via gavage.
Dose:	Single acute doses at 2000 mg/kg bw.

Study conduct

Mice were administered test article (referred to as DHALIP-NS) via gavage as single doses at 2000 mg/kg bw/day. They were observed for clinical signs at 1, 2.5, four hours post dosing; and twice daily for any mortality. Mice were provided with rodent diet *ad libitum* except for a four or five hour fast period prior to dosing. Body weights were recorded before and after fasting on day 0 and on day seven post-dosing. Animals were necropsied on day seven post-dosing.

Results

There were no deaths, clinical signs, effects on bodyweights or gross necropsy findings related to treatment.

REPEAT DOSE STUDIES

A 13-week dietary toxicity study of DHA-rich micro-algae in the albino rat.
Project Number 86511 by Kangas L et al. ClinTrial Bioresearch. *Work completion date, 22 November 1994; final report date, 13 November 1997.*

Test material:	*Schizochytrium* sp. micro-algal biomass
Control material	Untreated basal diet
Test Species:	Sprague-Dawley BR strain rats 26 males and 26 females per test dose, administration in diet
Dose:	0, 0.6, 6, 18 or 30% w/w in diet for 13 weeks.
GLP:	USA GLP Regulations, 1994
Guidelines:	USFDA 1982-Toxicological Guidelines

Test article and control material

An analysis of the test article revealed that the micro-algae was an orange solid freeze dried powder which contained high levels of fat (approximately 41% w/w) of which long chain highly unsaturated fatty acids were a major component (22% DHA).

Study conduct

Four groups of rats (26/sex/group) were treated with micro-algae in the diet at 0, 0.6, 6, 18 or 30% (equivalent to 0, 380, 3810, 13,400 or 17,140 mg/kg bw/day for males; and 0, 440, 4270, 13,700 or 19,050 mg/kg bw/day for females). The control group received untreated basal diet.

Clinical observations were recorded daily and bodyweight and food consumption were measured weekly. Haematology, clinical chemistry and urinalysis were performed at week four, six and 13 and ophthalmology of all animals was performed before the study and near termination. At the end of the study, all animals were sacrificed and a complete necropsy performed (gross examination, organ weights and tissue sampling). Histopathology was performed on target organs (kidney, liver, adrenals and heart) and on any lesions observed macroscopically. Appendix 1 lists the histopathological parameters measured.

Results

One male from the high-dose group was found dead during the terminal sacrifice period, although the death was not attributed to treatment. No other animals died during the study. No specific treatment related clinical signs were observed other than incidental signs consisting of staining and/or scabbing and areas of thin fur not confined to specific doses.

No treatment related changes were observed in bodyweights or bodyweight gains of male rats up to the highest dose tested. In females, significantly increased bodyweights and bodyweight gains were observed at doses of 6% and 30%; particularly from weeks eight-13; although, significance was not reached in females receiving 18% in the diet (a trend of increased weight gain was observed). Food consumption in males was significantly reduced at doses of 30% throughout the study, and in females for the first two weeks of treatment. No dose-response relationship was observed for any of these changes. The ophthalmologist reported that no treatment related ocular changes were observed in week 13.

Isolated statistically significant changes in a range of haematological parameters were noted; however, these generally lacked a dose-response relationship and were not repeated throughout entire study. Increased prothrombin times (14%; $p<0.01$) and activated partial thromboplastin (APTT) (22%; $p<0.01$) times were observed in males at high dose. Increases in APTT also occurred in females at high dose (15%; $p<0.01$).

At week four, six and 13 there was a trend of an overall reduction in cholesterol, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and triglycerides at all treatment doses in both males and females.

For males at week four significant increases were noted in alanine amino-transferase (25% increase; $p<0.01$) at doses of 18 and 30%, and in alkaline phosphatase (25%, $p<0.05$) at 30%; however, there was no dose-response relationship in AP increases. Serum chloride was marginally increased at doses of 6, 18 and 30% in both males and females. In males at week six changes in liver enzymes (AST and ALT), calcium, and increases in sodium and chloride levels were observed at doses of 6% or above. However, as for week four, these changes did not demonstrate a definite dose-response relationship. By week 13 there were no treatment related changes in clinical chemistry parameters in either males or females.

At week four, urinalysis results showed significant increases in total protein (65%; $p<0.05$), specific gravity (2%; $p<0.05$), sodium (77%; $p<0.01$), phosphorous (62%' $p<0.01$) and a decrease in urine volume (42%; $p<0.01$) at the highest dose in males. Decreases in urine volume, increases in sodium and phosphorous were also observed at doses of 18%. In females at week four, increases were noted in sodium at 18 and 30% doses. At week six, significant increases in total protein, specific gravity, sodium, potassium and phosphorous levels were observed in males at doses of 18 and 30% (reduced volume only at 30%). In females, significant increases were observed in sodium, potassium, chloride and phosphorous values at 6% and in sodium and phosphorous levels at 18 and 30%. At week 13, the above effects were less notable, although sodium and phosphorous levels remained elevated in males and females at 30% and sodium at 6%. The sporadic nature of these changes indicated a treatment-related effect only at the two highest dose levels.

No changes were observed in absolute or relative organ weights for animals sacrificed at week five. At terminal sacrifice, liver and kidney (absolute and relative to brain weight) was slightly increased at doses of 6% or higher.

At doses of 30%, females had increased liver weights (absolute and relative to body and brain weight) and increased adrenal and kidney weights (absolute and relative to brain weight).

Histopathology revealed cortical vacuolation in the adrenals of male rats at six and 30% (not observed at 18%), and periportal hepatocellular vacuolation with incidence of 1/26, 0/26,13/26, 0/26, 17/26 in females at 0, 0.6, 6, 18 and 30%.

Incidence of cardiomyopathy was increased in females at high-dose. The incidence was 7/26,10/26,0/26,0/26 and 13/26 at 0, 0.6, 6, 18 and 30%. Although males had a higher incidence of cardiomyopathy than females, there was generally no differences in incidence across male groups – incidence was 12/26, 15/26, 19/26, 0/26 and 18/26 at 0, 0.6, 6, 18 and 30%. Lesions in the kidneys were observed in both sexes, although in males at 0.6 and 6% no lesions could be attributed to treatment. Generally, lesions were characterised by hyalinisation of the papilla, tubular basophilia and mineralisation of the cortico-medullary junction at doses of 6% or higher in females and at 18% or higher in males, although there was no dose-response relationship and at 18% in males and females no lesions were observed.

Conclusion

There was evidence of toxicity following treatment with DHA-containing micro-algae (sourced from *Schizochytrium* sp.) only at the highest dose level (30%). Although some changes in clinical parameters were observed at lower dose levels, these changes did not always demonstrate a dose-response relationship.

The study sponsors considered that the high levels of fat (approximately 48%w/w) and high levels of ash contributed to the above observed changes as well as to the hepatocellular vacuolation observed in male rats at the high dose levels. Cardiomyopathy was observed in the high dose females and in all male groups, including controls, and is considered characteristic of this particular strain of rat.

A Pathology Working Group (PWG) was formed in the United States to review the heart slides to assess the accuracy and consistency of the initial histopathological examinations of the hearts of male and female rats. The expert panel (PWG) 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 supplement for humans. A similar conclusion was drawn by the United Kingdom Food Standards Advisory Committee for Novel Foods and Processes, namely, that the presence of heart lesions in the rat was of no significance in the safety evaluation of DHA for use in humans.

Because of the conflicting results obtained in this study, a second study in rats was undertaken at more realistic dose levels.

Thirteen week feeding study in rats. Naylor MW and Ruecker FA. Environmental Health Laboratory and Experimental Pathology Laboratories. (see also Hammond *et al* 2001a) *Study Number EHL 95085. October 14, 1997.*

Test material:	*Schizochytrium* sp. biomass
Control Material	Fish oil
Test Species:	Crl:CD (SD) BR) rats 20 males and 20 females per test dose, administration in diet
Dose:	0, 400, 1500 or 4000 mg/kg bw/day in diet for 13 weeks.
GLP:	USA GLP Regulations, 1994
Guidelines:	USFDA 1982-Toxicological Guidelines

Test article and control material

An analysis of the test article and fish oil revealed that the micro-algae was an orange solid freeze dried powder which contained high levels of fat (approximately 41% w/w) of which long chain highly unsaturated fatty acids were a major component. DHA was analysed as 7.41% of total biomass. The fish oil (control material) was a yellow viscous liquid.

Study conduct

Four groups of rats (20/sex/group) were treated with 0, 0.7, 3.0 or 8.1% of micro-algae in the diet equivalent to 0, 400, 1500 or 4000 mg/kg bw/day. Another control group received fish oil in the diet at a dose of 1628 mg/kg bw/day. These doses represented micro-algae in the diets of rats.

Vitamin E was added to the test article at manufacture to compensate for the highly unsaturated fat content, and was also added to the fish oil to provide comparable levels of vitamin E.

Clinical observations, bodyweight and food consumption were recorded weekly; haematology, clinical chemistry and urinalysis mid study and at termination; and ophthalmology of all animals was performed before the study and near termination.

At the end of the study, all animals were sacrificed and a complete necropsy performed (gross examination, organ weights and tissue sampling). Heart, lungs and pituitary for males and liver for females were examined microscopically and in addition any gross lesions with possible histological correlations. Appendix 1 lists the histopathological parameters measured.

Results

No deaths were associated with treatment. There were no treatment related clinical signs, adverse effects on food consumption and body weights or bodyweight gains. The reporting ophthalmologist concluded that there were no ocular abnormalities associated with the test material.

In males significant increases in neutrophils ($p<0.05$) were observed at high dose (156% of control values) and mean platelet volume (113% of control values; $p<0.01$) in the terminal sampling period (day 90-93). In females increases in haematocrit were observed at low (104% of control values; $p<0.05$) and high dose only (104% of control values; $p<0.01$), increases in platelets at mid-dose (116% of control values; $p<0.05$) at day 40-44 sampling period, and increased mean platelet volume at mid-dose in the terminal sampling period. These increases were incidental and lacked dose-response relationship.

Analysis of the blood chemistry parameters revealed a significant increase (122% of untreated control values; $p<0.05$) in serum alanine aminotransferase (ALT) of males at high-dose in the terminal sampling period. It was stated that these values were within historical control ranges of this strain of rat at the Environmental Health Laboratory.

Significant decreases in HDL (high-dose group), cholesterol and LDL (in fish oil control groups) were observed. Other incidental decreases were noted in creatinine and creatine phosphokinase (CPK) and BUN in high-dose females, total protein (TP) and albumin (ALB) in low-dose females. Urinalysis was unremarkable in control and treated groups.

Organ weights, organ morphology and microscopic features were generally unaffected by treatment up to the highest dose. Exceptions were myocardial degeneration/fibrosis observed at 0, 1628 (fish-oil), 400, 1500 and 4000 mg/kg bw/day (incidence 5,6,9,3,13); and increased incidences of pelvic dilatation of the kidneys (0,2,0,3,5) at high-dose in males (both significant at $p<0.05$) when compared to untreated controls. A pathology Working Group was formed to review the heart slides. They concluded that myocardial fibrosis has been observed historically in male Sprague-Dawley rats fed high dietary levels of vegetable or fish oils and is not observed in other non-rodent species including primates fed similar dose levels in the diet. They considered that these myocardial effects observed above were specific to the strain of rat.

Females had significantly increased periportal hepatocellular vacuolation in all groups when compared to untreated controls (incidences; 8, 18, 16, 18, 19) at 0, 1628 (fish-oil), 400, 1500 and 4000 mg/kg bw/day. However, there were no differences between control and treated groups in the degree of severity for liver vacuolation. This finding may be attributable to the high fat content of the diets.

Conclusion

In conclusion, no evidence of toxicity was noted following treatment with DHA-containing micro-algae (sourced from *Schizochytrium* sp.) at levels up to 3% in the diet.

At the highest dose tested (8%) there was a slight increase in incidence of cardiac myopathy in male rats, although there were no differences in severity between groups. However, an expert panel concluded that these effects were specific to Sprague-Dawley rats and thus were of no significance to humans. The NOEL was the highest dose tested, namely, 4000 mg/kg bw/day for 90 days.

REPRODUCTION STUDIES

A dietary 1-generation (1 litter) range-finding reproduction study of DHA-rich micro-algae in the rat. Robinson K. ClinTrial Bioresearch. *Project No. 95708* (see also Hammond *et al,* 2001c). *3 November 1997.*

Test material:	*Schizochytrium* sp. micro-algal biomass
Test Species:	Crl:CD (SD) BR) rats rat (Charles River, Kingston, New York) 30 females and 30 males per F_0 dose group; administration in diet
Dose:	0, 0.6, 6.0 or 30% (w/w) in diet.
GLP:	OECD/EC
Guidelines:	USFDA 1982-Toxicological Guidelines

Test article and control material

An analysis of the test article and fish oil revealed that the micro-algae was a yellow course powder/flake which contained high levels of fat (approximately 41% w/w) of which long chain highly unsaturated fatty acids were a major component (21% DHA). The fish oil (control material) was a yellow viscous liquid.

Study conduct

Groups of 30 male and 30 female Sprague-Dawley rats were treated with micro-algae in the diet at 0, 0.6, 6.0 or 30% (w/w) (equivalent to 0, 400, 3900 or 17,800 mg/kg bw/day for males and 0, 480, 4600 or 20,500 mg/kg bw/day for females). F_0 males were treated 10 weeks before mating, throughout mating and for 3 weeks after. F_0 females were treated 2 weeks before mating and throughout gestation and lactation to day 21 after littering. F_0 females were then sacrificed. At four weeks of age selected F1 males and females (30/sex/group) were dosed as above for 12 weeks prior to terminal sacrifice.

A clinical examination was performed daily, food consumption weekly (except during pairing) and bodyweights measured weekly (pre-mating, gestation and lactation for females). Reproductive parameters measured included:

- oestrus cycle length, mating performance, fertility, gestation length, parturition and gestation index for F_0 parents;

- litter size, offspring weights, offspring viability indices and physical development were assessed for F1 generation.

Detailed necropsy was performed on adult F0 and F1 animals and for F0 males the testes were examined histologically including an assessment of spermatogenic cycle and histological analysis was performed on livers of F0 females.

Results

F_0 generation - There were no deaths or abnormal or dose related clinical observations during the study that was attributed to treatment. Increases in mean bodyweight were observed only in females at the 30% dose level during pre-mating, gestation and lactation periods. Food consumption was significantly lower in females at 30% during gestation only; whereas, for males, food consumption was decreased for males throughout most of the treatment period.

F_0 and F_1 generation - Fertility and reproductive performance parameters were not significantly altered by treatment in either generation. There were also no dose related changes in litter data. There were no consistent or dose related effects on organ weights or histopathology in either generation attributed to treatment other than periportal hepatocellular vacuolation observed in F_0 females at doses of 6% or higher. This finding is expected given the high fat content of the diets.

Also, there was a slight increase in the number of days to mating at the highest dose (5.8 days compared to 3.79) and length of gestation at 6% in the F_0 generation; however, the significance of this is probably minor given that there were no effects on other parameters and there was no dose-response relationship.

The NOEL for reproductive effects was the highest dose level tested, namely, 30% in the diet (equivalent to 17,800 mg/kg bw/day in males and 20,500 mg/kg bw/day in females).

DEVELOPMENTAL STUDIES

1. A dose range-finding study of embryo/foetal developmental of DHA-rich micro-algae in rabbits. Holsen JF. Wil Research Laboratories. *Project Number WIL-50242. 2 October 1997.*

Test material:	*Schizochytrium* sp. micro-algal biomass in aqueous methylcellulose and polysorbate
Test Species:	Rabbits (NZ White) 5 artificially inseminated females per dose group; administration by gavage
Dose:	500, 1000, 1500, 2000 or 2500 mg/kg bw/day from days 6 to 18 of gestation (plus 2 control groups).
GLP:	USFDA and Japanese GLP Standards
Guidelines:	FDA Redbook 1-Guideline for teratology testing in the rabbit, 1982.

Study conduct

Groups of five artificially inseminated female rabbits were treated with micro-algae (41% crude fat w/w) by gavage at 0, 500, 1000, 1500, 2000 or 2500 mg/kg bw/day. The objective of the study was to determine dose levels for a definitive developmental toxicity study in rabbits.

One vehicle control group and a fish oil control group (fish oil/vitamin E) suspended in vehicle control article were also run concurrently. Administration was via gavage from days six to 18 gestation. Rabbits were examined twice daily and bodyweights were recorded on days 0, 6-24 (daily) and 29. Food consumption was measured daily from gestation day 0-29. Rabbits were sacrificed on day 29 and examined for gross abnormalities. The uteri and ovaries were examined for the number of corporal lutea, number of implantation sites, early and late resorptions, live and dead foetuses, sex of foetuses, and any malformed foetuses.

Ovarian and uterine weights were determined. Gross necropsy was performed on any females which died during the study or aborted.

Results

One female in the fish oil control group died. Two animals each from fish oil control and 2500/mg/kg/day groups and one from 2000 mg/kg/day group aborted between gestation days 20 and 25. These animals had food consumption less than 15g/day for 4 to 9 days. Clear association between the two events could not be made because of 3 other animals with low food consumption for four days or more showed normal reproductive outcome. No treatment-related internal findings were observed in any of the animals. Based on the results of this study, dose levels of 180, 600 and 1800 mg/kg/day were selected for an embryo/foetal developmental toxicity study in rabbits (below).

2. A study of DHA-rich micro-algae on embryo/foetal development in rabbits. Holson JF. Wil Research Laboratories. *Project Number WIL-50243* (see also Hammond *et al,* 2001b). *2 October 1997.*

Test material:	*Schizochytrium* sp. micro-algal biomass in aqueous methylcellulose and polysorbate
Test Species:	Rabbits (NZ White) 22 artificially inseminated females per dose group; administration by gavage
Dose:	180, 600 or 1800 mg/kg bw/day from days 6 to 18 of gestation (plus 2 control groups).
GLP:	USFDA
Guidelines:	FDA Redbook 1-Guideline for teratology testing in the rabbit, 1982.

Test article and control material

An analysis of the test article and fish oil revealed that the micro-algae was an yellowish, brown flaky powder which contained high levels of fat (approximately 41% w/w) of which long chain highly unsaturated fatty acids were a major component (22% DHA). Vehicle control mixtures were a white powder (methyl cellulose) and a clear yellow liquid (polysorbate). The fish oil was a clear yellow liquid.

Study conduct

Groups of 22-artificially inseminated female rabbits were treated with micro-algae (41% crude fat w/w) by gavage at 0, 180, 600 or 1800 mg/kg bw/day. One vehicle control group and a fish oil control group (fish oil/vitamin E) suspended in vehicle control article were also run concurrently. Administration was via gavage from days six to 18 gestation. Rabbits were examined twice daily and bodyweights were recorded on days 0, 6-24 (daily) and 29. Food consumption was measured daily from gestation day 0-29. Rabbits were sacrificed on day 29 and examined for gross abnormalities. The uteri and ovaries were examined for the number of corporal lutea, number of implantation sites, early and late resorptions, live and dead foetuses, sex of foetuses, and any malformed foetuses. Ovarian and uterine weights were determined. Gross necropsy was performed on any females that died during the study or aborted.

Results

One female in the fish oil control group aborted on gestation day 23, and two females in the high-dose group aborted on gestation days 25 and 26. Abortions occur spontaneously more frequently in the rabbit than in other experimental animals. One animal in the 600 mg/kg bw/day dried micro-algae group died during gestation day 10 unrelated to the treatment. No treatment-related clinical signs were observed in the test groups. The fish oil control and 1800 mg/kg/day groups showed reductions in body weight gain and food consumption during gestation days 12-19. These parameters remained reduced in these animals during the first half of the post-treatment period.

Intrauterine growth and survival were unaffected by treatment at all dose levels. There were no significant differences between the treated or fish oil control group and the normal control in mean number of corpa lutea, implantation sites, litter size, post implantation loss, and foetal body weight. Based on the results of these studies, dried micro-algae is not teratogenic in the rabbit.

3. A dietary teratology study of DHA-rich micro-algae the rat. Pinsonneault L et al. ClinTrials Bioresearch. *Project Number 95706* (see also Hammond *et al,* 2001b). *3 November 1997.*

Test material:	*Schizochytrium* sp. micro-algal biomass
Test Species:	Rats Crl:CD (SD) BR) (Sprague-Dawley)-25 mated females per dose group; administration in diet
Dose:	0, 0.6, 6.0 or 30% w/w from days 6 to 15 of gestation.
GLP:	USFDA

Test article and control material

An analysis of the test article and fish oil revealed that the micro-algae was a yellow coarse powder which contained high levels of fat (approximately 41% w/w) of which long chain highly unsaturated fatty acids were a major component (22% DHA).

Study conduct

Groups of 25 mated female rats were treated with micro-algae (41% crude fat w/w) at 0, 0.6, 6.0 or 30% w/w in the diet (equivalent to 0, 500, 4800 or 22,000 mg/kg bw/day) from days 6 to 15 gestation. Females were mated with males of the same strain and source. When sperm was identified in the vaginal lavage of females this was considered Day 0 of gestation.

Rats were examined twice daily and bodyweights were recorded on gestation days 0, 6, 9,12,16,18 and 20. Food consumption was measured on gestation days 0 to 6, 6 to 9, 9 to 12, 16 to 18 and 18 to 20. All females were sacrificed on day 20 and examined for gross abnormalities. The uteri and ovaries were examined and the foetuses were weighed and given an external and internal examination. All foetuses were examined for skeletal development.

The following was examined in detail:

- number and position of live foetuses, dead foetuses and any resorptions recorded;
- major, minor and common skeletal findings in the foetuses; and
- pregnancy rates, pre and post-implantation loss.

Results

No rats died during the course of the study. There were no treatment-related clinical signs. Animals in the 30% dried micro-algae group exhibited a reduction in weight gain from gestation days 16 to 18. Food consumption was also reduced in the latter group during gestation days six to nine and between gestation days 16 and 18. Examination of the uteri confirmed that 88%, 88%, 92% and 80% of the mated animals in the control through high-dose DRM groups were pregnant and produced foetuses by gestation day 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 was noted in low- and mid-dose dried micro-algae groups (mainly due to a low percentage (39.1%) of male litter/litter in control group).

The incidences of litter with ossification centres in the first lumbar vertebrae (2%) was significantly lower in high-dose- dried micro-algae group but was within 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 dried micro-algae groups. This resulted from a single litter with a number of affected pups (mid-dose, eight foetuses, high-dose, 5 foetuses). Treatment with dried micro-algae did not result in other skeletal and visceral anomalies in rats.

GENOTOXICITY STUDIES

1. Mouse Bone Marrow Micronucleus Assay. Stegeman SD et al. Environmental Health Laboratory. *Study number ML-96-133. 6 October 1997.*

Test material:	*Schizochytrium* sp. micro-algal biomass
Test Species:	Mouse bone marrow cells
Dose:	500, 1000 and 2000 mg/kg bw
GLP:	USFDA
Guidelines:	OECD (1995), US FDA (1993) and ICH (1994)

Study conduct

This study was performed to evaluate the ability of the *Schizochytrium* sp. micro-algae biomass, administered via oral gavage, to induce chromosomal effects as measured in mouse bone marrow cells. The animals used in the study were eight to ten weeks old male and female CD-1 mice. Animals were treated by a single oral gavage dose of water, *Schizochytrium sp.* micro-algae in water (500, 1000 and 2000 mg/kg bw) or cyclophosphamide in water as positive control (40 mg/kg bw). All animals were observed for visible toxic effects and mortality on the day of dosing, and daily thereafter for up to 48 hours after dosing. Animals were weighed at the time of treatment and at the time of sacrifice for bone marrow extraction.

Results

Preliminary range finding experiments showed that the substance was not toxic to male or female animals up to 2000 mg/kg bw. The main micronucleus experiment consisted of male mice dosed with 500, 1000 and 2000 mg/kg bw and their bone marrow extracted for micronucleus assay.

There were no deaths or signs of toxicity were observed in the test, control or positive control groups. Also, no statistically significant decreases in the mean body weight change or in mean PCE/total erythrocyte ratio were observed in any of the animals.

Conclusions

Analysis of the data indicated no treatment related increases in micronucleated PCE (MN PCE) frequency in the test group while the positive control (cyclophosphamide) group yielded the expected positive response. It is concluded that *Schizochytrium* sp. did not induce increases in micronucleated PCE frequencies in mouse bone marrow cells under the experimental conditions.

2. An Evaluation Of The Mutagenic Potential Of the Oil Derived from DHA-rich micro-algae in the Ames Salmonella/ Microsome Assay. Balwierz PS and Bunch RT, G.D. Searle & Co., *Study No. PCR1216, 17 November 1997.*

Test material:	DHALIP-NS DHA-rich oil from *Schizochytrium* sp.
Test Species:	TA1535, TA100, TA 102, TA98 and TA97a
Dose:	10, 50, 100, 500, 1000, and 5000 µg/plate
Guidelines:	OECD (1995), US FDA (1993) and ICH (1994)

Study conduct

The mutagenicity of oil derived from *Schizochytrium* sp. was examined using Ames/Salmonella test strains TA1535, TA100, TA 102, TA98 and TA97a in the presence or absence of an Aroclor 1254-induced rat metabolic activation system (S-9 mix) at a concentration of 10, 50, 100, 500, 1000, and 5000 µg/plate in experiments.

Conclusions

Precipitation occurred from 500 µg/plate onwards. There was no toxicity observed at all test article concentrations. 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. The results indicate that the test substance is not mutagenic towards any of the *S. typhimurium* strains used in the in the presence or absence of an Aroclor 1254-induced rat metabolic activation system (S-9 mix).

3. Ames/*Salmonella* Mutagenicity Assay. Stegeman SD et al. Environmental Health Laboratory. *Study number ML-96-134. 6 October 1997.*

Test material:	DHALIP-NS DHA-rich oil from *Schizochytrium* sp.
Test Species:	TA98, TA100, TA102, TA1535 and TA1537
Dose:	0.005, 0.015, 0.05, 0.15 and 0.5 mg /plate
Guidelines:	OECD (1995), US FDA (1993) and ICH (1994)

Study conduct

The mutagenicity of *Schizochytrium* sp. micro-algae biomass was examined using Ames/Salmonella test strains TA98, TA100, TA102, TA1535 and TA1537 in the presence or absence of an Aroclor 1254-induced rat metabolic activation system (S-9 mix).

Results

In the mutagenicity test, three replicate plates were prepared for each strain/S-9/dose level along with positive and negative controls. Plates were examined after at least 48 hours at 37^{o} C. Statistical analysis were performed on plate incorporation assay results after transforming revertant/plate values as log 10 (revertants/plate). Analysis included Bartlett's test for homogeneity of variance and Grub's test for significance of outlying observations. The test sample precipitated out at concentrations above 0.5 mg/plate treatment level and at higher levels precipitation interfered with counting of revertant colonies. No toxicity was observed at levels of up to 5 mg/plate with and without S-9 mix. For mutagenicity testing, 0.5 mg/plate was chosen as the highest level because of the precipitation problem. Lower treatment levels used were 0.005, 0.015, 0.05 and 0.15 mg/plate.

Conclusions

The results indicate that the test substance is not mutagenic towards any of the *S. typhimurium* strains used in the in the presence or absence of an Aroclor 1254-induced rat metabolic activation system (S-9 mix).

4. *In Vitro* Mammalian Cytogenetic Test Using Human Peripheral Lymphocytes. Gudi et al. Microbiological Associates, Inc. *Study Number G94BR06.346. 13 October 1997.*

Test material:	*Schizochytrium* sp. micro-algae biomass
Test Species:	Human peripheral blood lymphocytes (HPBL)
Dose:	0 to 5000 μg/ml
Guidelines:	OECD (1995), US FDA (1993) and ICH (1994)

Study conduct

The mutagenicity assay was performed in two phases, both in the absence and in the presence of metabolic activation. The first phase, the initial chromosome aberration assay, was conducted to establish the dose range for testing and to evaluate the clastogenic potential. In the non-activated portion of the initial test, HPBL cells were exposed to the test article for 20 hours; in the activated portion of the assay, HPBL cells were exposed for four hours. Metaphase cells were collected for microscopic examination at 20 hours after the initial treatment.

Results

In the initial chromosome aberration assay, the maximum dose tested was 5000 μg/ml. Visible precipitate was observed in treatment medium at all observed concentrations tested. Due to excessive precipitation of the test article on the slides, the highest scorable dose level (50 μg/ml) was the dose which had sufficient number of unobstructed metaphase cells. Mitotic inhibition was around 0 and 8% at the 500 μg/ml dose level both in the absence and in the presence of metabolic activation. No statistically significant increases in chromosome aberrations were observed in non-activated or S9-activated test systems relative to the solvent group up to 500 μg/ml.

The second phase, the independent repeat chromosome aberration assay, was performed to confirm the test system response to the test article seen in the initial assay. In the non-activated portion of the repeat test, HPBL cells were exposed to the test article for 20 and 44 hours; in the activated portion of the assay, HPBL cells were exposed to the test article for four hours.

Metaphase cells were collected for microscopic examination at 20 and 44 hours after the initial treatment (activation and non-activation) and 20 hours after the initiation of pulse treatment (non-activated).

Based on the initial assay, a confirmatory chromosome aberration assay was conducted in the absence and in the presence of an Aroclor-induced S9 metabolic activation system at dose levels up to 750 μg/ml. Visible precipitation was observed in treatment medium at all tested concentrations. Toxicity measured by mitotic inhibition was approximately 17% (20 hour harvest) and 53% (44 hour harvest) at the highest dose tested in the absence of metabolic activation.

In the four hour pulse treatment group, toxicity was 37.5% at the highest dose tested. No statistically significant increases in structural or numerical chromosome aberrations were observed, regardless of dose level or harvest time, either in the absence of or presence of metabolic activation.

Conclusion

The micro-algae tested was not clastogenic and did not induce structural or numerical chromosome aberrations in human peripheral blood lymphocytes at doses up to 750 μg/ml, either in the presence or in the absence of metabolic activation.

5. ***Salmonella/ Escherichia coli*** **Mutagenicity Assay.** San HC. Microbiological Associates, Inc. *Study Number G94BR06.503. 10 October 1997.*

Test material:	*Schizochytrium* sp. micro-algae biomass
Test Species:	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strains WP2 *uvr*A (pKM101) and WP2 (pKM101)
Dose:	0 to 5000 μg/ml
Guidelines:	OECD (1995), US FDA (1993) and ICH (1994)

Study conduct

In this study the mutagenic potential of the micro-algae *Schizochytrium* sp. was investigated by measuring its ability to induce back mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* tester strains WP2 *uvr*A (pKM101) and WP2 (pKM101) in the presence of or in the absence of S9 activation.

The test article was tested in the bacterial reverse mutation assay using *S. typhimurium* tester Strains TA98, TA100, TA1535 and TA1537 and absence of Aroclor-induced rat liver S9. The assay was performed in two phases. The first phase, the dose range-finding study, was used to establish the dose range for the mutagenicity assay. The second phase, the mutagenicity assay (initial and confirmatory experiments), was used to evaluate the mutagenic potential of the test article.

The plate incorporation method was used for the dose-range finding study and the initial mutagenic assay. The pre-incubation method was used for the confirmatory assay.

Results

In the preliminary toxicity assay, the maximum dose tested was 5000 μg/plate; precipitate was observed at ≥ 6.7 μg per plate but no appreciable toxicity was observed.

Based on the findings of toxicity assay, the maximum dose selected for the mutagenicity assay was 5000 μg per plate.

In the mutagenicity assay, no positive response was observed. In the initial mutagenicity assay (using the plate incorporation method), the precipitate was generally observed at ≥ 1000 μg per plate with all tester strain/activation combinations and toxicity was generally observed at ≥ 3333 μg per plate only with several of the tester strain/ activation combinations.

In the confirmatory mutagenicity assay (using the pre-incubation method), precipitate was generally observed at ≥ 100 μg per plate but no appreciable toxicity was observed. The range of doses tested was from 10 to 5000 μg/plate.

Conclusion

The test substance was not mutagenic in the *Salmonella/ Escherichia coli* Mutagenicity Assay when tested at doses ranging from 10 to 5000 μg per plate either with or without metabolic activation.

6. AS52/XPERT Gene Mutation Assay. Stegeman SD et al. Environmental Health Laboratory. *Study Number ML-96-132. 6 October 1997.*

Test material:	*Schizochytrium* sp. micro-algae biomass
Test Species:	cultured Chinese hamster ovary/xanthine-guanine phosphoribosyl transferase (AS52/XPRT) gene locus
Dose:	200 to 5000 μg/ml
Guidelines:	OECD (1995), US FDA (1993) and ICH (1994)

Study conduct

The mutagenic potential of the *Schizochytrium* sp. algae biomass was tested in cultured Chinese hamster ovary/xanthine-guanine phosphoribosyl transferase (AS52/XPRT) gene locus assay. The AS52 cell line was developed through genetic engineering of Chinese hamster ovary (CHO) cells and contains one copy of the *E. coli gpt* gene per cell. Mutagenicity testing was performed initially using a range of Aroclor-1254-induced rat liver homogenate (S9) concentrations (0, 1, 5 and 10%) followed by a confirmatory experiment with 0 and 5% S-9 Mix. The range of test concentrations, up to clearly cytotoxic levels, varied depending on the concentrations of S-9 Mix utilized. Overall, greater cytotoxicity was observed with increasing concentrations of S-9. No significant cytotoxicity (≤ 50% relative survival) was observed at any of the concentrations tested without S-9 Mix.

Results

The initial mutagenicity experiment was conducted at 200, 500, 2000 and 5000 μg/ml without S-9 Mix; at 200, 1250, 1300 and 1350 μg/ml with 1% S-9 Mix; at 200, 700, 850 and 1000 μg/ml with 5% S-9 Mix; and at 200, 950, 1050 and 115 μg/ml with 10% S-9 Mix. No statistically significant increases in mean mutant frequency or dose responses were observed in any of the treated cultures without S-9 Mix and with 5% S-9 Mix. Statistically significant increases in mean mutant frequency and a statistically significant linear dose response was observed with 1% S-9 Mix. However the increases observed were very small, less than 1.7 fold over control values.

In a subsequent repeat experiment with 1% S-9 Mix, no statistically significant increases in mean mutant frequency or statistically significant dose responses were observed. The increases observed in the initial experiment were not reproducible and may have been due to the small standard deviation of the concurrent medium controls and not related to test article administration.

In the confirmation experiment, the test substance was tested at 200, 500, 1000, 2000 and 5000 μg/ml without S-9 Mix and at 200, 700, 850, 900 and 1000 μg/ml with 5% S-9 Mix. A statistically significant linear dose response was observed without S-9 Mix. The result is not consistent with the results of the initial mutagenicity experiment and the subsequent repeat experiment when the treatment levels and cytotoxic effects are compared. The observed dose response is clearly not consistent with a treatment related effect.

Conclusion

The *Schizochytrium* sp. algae biomass was not mutagenic in the AS5/XPRT assay in the absence of S-9 Mix or in the presence of 1%, 5% or 10% S-9 Mix. Although increases in mean mutant frequency were observed at two treatment levels in the presence of 1% S-9 Mix, the data do not indicate a treatment related mutagenic effect under these conditions.

Summary of the genotoxicity studies

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse point mutation (*In vitro*)	Micro-algae from *Schizochytrium* sp.	Up to 5 mg/plate (+/- S9)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 and *E. Coli* WP2uvrA and pKM101	-ve
Forward point mutation (*In vitro*)	Micro-algae from *Schizochytrium* sp.	200 to 1000μg/mL (+/-S9)	AS52/XPRT Chinese Hamster cells	-ve
Chromosome aberrations (*In vitro*)	Micro-algae from *Schizochytrium* sp.	125 to 750 μg/mL (+/-S9)	Human peripheral lymphocytes	-ve

In vivo micronucleus test (oral gavage)	Micro-algae from *Schizochytrium* sp.	500 to 2000 mg/kg bw	Mouse bone marrow *in vivo* (CD-1 mice)	-ve

STABILITY STUDIES

Storage Stability Study of Encapsulated DHA-Rich Oil Prepared from the HD-1 Process, Diltz, S.I., Monsanto Life Science Company. *Study No. SD-FS-AN-9702, February 04, 2000.*

Test material:	DHA-rich oil from *Schizochytrium* sp.
Dose:	0.005, 0.015, 0.05, 0.15 and 0.5 mg /plate
GLP:	US FDA (1993)

This study was conducted in accordance with the Food and Drug Administration (FDA) and Good Laboratory Practice Standards (21 CFR 58).

Study conduct

The test articles consisted of two lots of DHALIPNS. The refined oil was encapsulated using a process similar to the standard industrial softgel encapsulation process. The DHA content of the test article at different time points for up to 24 months storage at uncontrolled room temperature showed that all samples were within 15% of the DHA content on Day 0. In addition, no new peaks in the chromatograms were observed over the course of the study. The peroxide value of the test articles did not rise above the 10 meq/kg limit of the duration of the 24 month study.

Conclusion

DHA encapsulated oil is stable for 24 months when stored at uncontrolled room temperature. The peroxide level remained below 10 meq/kg and the DHA content was within 15% of its value on day 0.

Toxicological summary of key published papers on related oils and fatty acid components

Study	Species	Summary of study and results
Acute toxicological studies		
Shibutani *et al* (1989)	Rats and mice, 10g/kg	***Mice and rats were administered a series of polyunsaturated fatty acids (stearidonic, arachidonic, EPA and DHA).*** ***LD_{50}>10g/kg***

Study	Species	Summary of study and results
Boswell *et al* (1996)	SD Rats, 20g/kg bw	DHA from a single cell micro-algae (*Crypthecodinium cohnii*) DHASCO (38.4% DHA) and an oil with high levels of arachidonic acid (from a fungus, *Mortierella alpina*) –referred to as ARASCO) were administered separately as a single dose by *gavage* *No deaths were observed. Soft stools during first four hours and dark stained urogenital areas for two days. No clinical signs observed from day 3-14. No visible lesions observed on histopathology*.
Short-term repeat dose studies		
Boswell *et al* (1996)	SD Rats, 25 to 1250 mg/kg bw/day (DHA)	High arachidonic acid and DHA oils (38.4%) *administered for four weeks* by *gavage* at doses of 50, 1000, 2500 mg/kg bw/day (ARA) and 25, 500 and 1250 mg/kg bw/day (DHA). *Source of oils as for acute studies.* All animals survived to the scheduled sacrifice. No significant changes were noted in bodyweights or bodyweight gains, clinical chemistry/haematology, organ weights or histopathology.
Wibert *et al* (1997)	SD Rats, 1800 to 12000 mg/kg bw/day	A blend of high-DHA oil (40%) from the algae *Crypthecodinium cohnii* and high-ARA oil (41%) from *Mortierella alpina* administered for four weeks in diet at doses of 18000, 60,000 or 120,000 ppm. One male rat was found dead in the high-dose-group; however, this was not considered related to treatment. No significant changes were noted in bodyweights or bodyweight gains, clinical chemistry/haematology, organ weights or histopathology other than isolated changes in some blood chemistry parameters at high dose (reversible by week four), and increased liver weight/body weight (mid and high dose) and brain weight/body weight (low and mid dose)
Reproduction studies		
Burns *et al* (1999)	SD Rats, 1800 to 12 000 mg/kg bw/day	A blend of DHA from a single cell micro-algae (*Crypthecodinium cohnii*) (16% DHA) and an oil with high levels (27%) of arachidonic acid (from a fungus, *Mortierella alpina*) were administered in the diet at doses of 0, 18000, 60,000 or 120,000 ppm. Rats received treatment over a pre-mating interval, mating, gestation and lactation. F1 pups consumed the diets from weaning for a further period of 90 days.

Study	Species	Summary of study and results
		No deaths were observed other than three control animals. No significant adverse effects were seen in reproductive parameters or fertility. Mid and high dose F1 animals had increased white cell counts, neutrophil counts, blood urea nitrogen, liver and spleen weights and increased pro-thrombin times. Decreased haemoglobin (males only), partial thromboplastin times and haematocrit was observed in mid and high dose animals. However, all these values were within historical control ranges and lacked dose-response. The NOEL was 12, 000 mg/kg bw/day.
Genotoxicity assays		
Arterburn *et al* (2000)	CD BR Rats, 500 and 1250 mg/kg bw/day	DHASCO oil containing 52% DHA from (source not stated) was administered in the diet at doses of 0, 500 and 1250 mg/kg bw/day. . No treatment related effects were noted. The NOEL was 1250 mg/kg bw/day.
Genotoxicity tests		
Arterburn *et al* (2000)	*Bacterial species TA98, TA 100, TA1535, TA1537, TA 1538* 100-5000µg/plate	DHASCO oil containing 38% DHA from *Crypthecodinium cohnii* was tested in the Ames mutagenicity assay. Negative
Arterburn *et al* (2000)	*Mouse lymphoma cells,* 1000-5000µg/mL	DHASCO oil containing 38% DHA from *Crypthecodinium cohnii* was tested the mouse lymphoma forward mutation assay. Negative
Arterburn *et al* (2000)	*Chinese hamster ovary cells,* 1260-5000µg/mL	DHASCO oil containing 38% DHA from *Crypthecodinium cohnii* was tested for chromosome aberrations. Negative
Humans		
Agren *et al* (1996)	1.68g DHA/day	Healthy males received DHA from micro-algae source for a period of 15 weeks. Decreases were noted in level of fasting triglyceride levels. No adverse effects were reported; however, the study was done for efficacy purposes.

Conquer and Holub (1996)	1.62g/day	Males and females consumed DHA for a period of six weeks. Significant decreases were noted in total cholesterol/HDL ratio (16%), LDL/HDL ratio (22%) and serum tri-glycerides. No other effects were reported; however, the study was done for efficacy purposes.
Davidson *et al* (1997)	1.25 or 2.5g/day	Subjects received DHA from a micro-algal source* (DHASCO; 42% DHA) at doses of 1.25 or 2.5g/day for a period of four weeks. A dose-dependent increase in DHA content of plasma phospholipids was noted and decreases in triglycerides, and increases in HDL cholesterol were observed. *An increase in LDL cholesterol (14%; $p<0.001$) was observed at doses of 2.5g/day.* No other effects were reported; however, the study was done for efficacy purposes.
Innis and Hansen (1996)	0 to 2.9g/day	Supplemented diets of healthy men with mixture of high DHA micro-algal oil* and a high arachidonic acid fungal oil* at doses of 0, 0.6g, 1.7g or 2.9g/day for 14 days. No clinically dose-related effects were noted on physical examination or routine laboratory tests; *although a significant ($p<0.05$) increase in cholesterol at low (14%), mid (18%) and high doses (17%) was observed. However, the significance of this effect is unknown.*
O'Dea, and Sinclair 1985	14 volunteers; 2 weeks on a diet of tropical seafood rich in arachidonic acid (AA) and PUFA followed by 3 weeks on diet rich in linoleic acid and AA	The concentration of AA in plasma lipids doubled while linoleic acid halved; Bleeding times increased in all subjects from 4.1 min to 5.9 min. Association between bleeding times and modification of plasma lipids suggests a more complex mechanism of homeostasis modulation.
Connor WE, 1994	0.5 to 2.0 g per day of n-3 PUFA	No significant changes to bleeding times.

***Source not stated in the methods**

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APPENDIX 1

LIST OF CLINICAL CHEMISTRY, HAEMATOLOGY AND URINALYSIS PARAMETERS TESTED

Clinical chemistry	Haematology	Urinalysis
Albumin and albumin/globulin ratio	Platelet count	Appearance
Alkaline phosphatase (AP)	Mean platelet volume (MPV)	Specific gravity
Alanine aminotransferase (ALT)/glutamic pyruvic transaminase (SGPT)	Mean Corpuscular Volume (MCV)	Glucose
Aspartate aminotransferase (AST)/glutamic oxaloacetic transaminase (SGOT)	Mean Corpuscular Haemoglobin Concentration (MCHC)	Ketones
Bilirubin (total)	Mean Corpuscular Haemoglobin (MCH)	Sediment (microscopic)
Calcium	Leucocyte total count	Occult blood
Chloride	Leucocyte differential count	pH
Cholesterol (total)	Haemoglobin	Protein
Creatinine and creatine phosphokinase (CPK)	Haematocrit (packed cell volume)	Bilirubin
Gamma-glutamyl transpeptidase (GGTP)	Total Erythrocyte count	Calcium
Globulin	Red Blood Cell distribution list	Chloride
Glucose	Activated Partial Thromboplastin Time (APTT)	Potassium
Low density lipoproteins (LDL) and high density lipoproteins (HDL)	Reticulocyte count	Sodium
Phosphorus (inorg)		Nitrite
Potassium		Urobilinitrogen
Protein (total)		Osmolality
Sodium		Volume
Triglycerides		
Urea nitrogen and uric acid		

ORGANS/TISSUES FOR ORGAN WEIGHT DETERMINATION AND HISTOPATHOLOGICAL EXAMINATION

Organs weighed

Organ weighed	**Tissues examined for histopathology**	
Adrenals	Heart	Various other tissues were retained but all were not examined
Brain	Kidneys	
Heart	Liver	
Lungs	Pituitary	
Ovaries	Adrenals	
Testes		
Pituitary		
Kidneys		
Liver		
Thymus		
Pituitary		
Thyroid/parathyroid		

ATTACHMENT 3

FOOD TECHNOLOGY REPORT

A428 – DHA-rich dried marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. as novel food ingredients

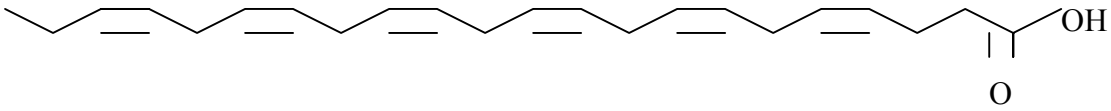
Introduction

ANZFA received an Application (A428) on 13 March 2001, from Omega Tech Inc., to amend Standards A19 and 1.5.1 (the Novel Foods Standards) of the *Food Standards Code* to permit the use of dried marine micro-algae (*Schizochytrium* sp.) which is rich in the omega-3 long chain polyunsaturated fatty acid DHA (docosahexaenoic acid) as a novel food ingredient in a limited range of foods. The Applicant subsequently amended their application on 28 August 2001 to include DHA- rich oil derived from the same species for use as a novel food ingredient in a limited range of foods.

Chemical Structure of DHA

DHA (docosahexaenoic acid) is an omega-3 long chain polyunsaturated fatty acid. It contains 22 carbon atoms and 6 C=C double bonds. Its molecular formula is $\mathbf{C_{22}H_{32}O_2}$. The CAS number for fatty acids containing 14-22 carbon atoms (C14-C22), and 16-22 carbon atoms (C16-C22) esterified to glycerol is **[68424-59-9]** (described in the CAS registry as "glycerides", C14-C22 and C16-C22). The correct name of the acid is 4,7,10,13,16,19-docosahexaenoic acid. The short hand nomenclature often used is 22:6n-3, where 22 refers to the number of carbon atoms, 6 refers to the number of double bonds and 3 refers to the number of carbon atoms from the final methyl group to the first double bond. All the double bonds in DHA are in the *cis* orientation.

The following diagram represents the structural formula of DHA:



Description of the product

There are two possible products containing DHA covered by this application.

Micro-algal Powder

Micro-algal powder is a free flowing yellow to light orange coarse powder (flakes) with a malt-like and/or slight marine odour prepared from the ground, dried micro-algae *Schizochytrium* sp. This powder has a high oil content (minimum 37 %) and the oil has a high DHA content (greater than 40%) encapsulated within the micro-algal cells. The dried micro-algal powder has a minimum DHA content of 15% and exists in the all-*cis*-docosa-4, 7,10,13,16,19-hexaenoic acid form. This is based on fatty acid methyl ester analysis by gas-liquid chromatography and comparison to all-cis-docosa-4, 7,10,13,16,19-hexaenoic acid analytical reference standard. No evidence of trans DHA isomeric form(s) is evident in the fatty acid profile of DHA-rich oil. All peaks greater than 4 mg/g oil were identified and quantified.

The product is stabilised by an approved food grade antioxidant.

Extracted DHA Rich Oil

The other product covered by this Application is the extracted oil from the micro-algae. This oil contains a number of various long chain fatty acids (C12 –C22) with DHA being the major fatty acid. The extracted oil is a free flowing pale light-yellow to orange colour with a characteristic "bland to fish-like" odour. The oil also contains a small percentage of trans-fatty acids (less than 2%) and unsaponifiables (essentially identified and unidentified sterols, less than 4.5%).

The extracted oil is less dense than water, with a freezing point just above 0°C and a flash point between 165-215°C.

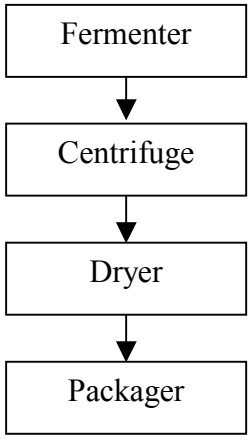
Production Process

The Applicant requested that the production processes, for both the micro-algal powder and extracted DHA oil, be treated as commercial in-confidence. The processes will not be detailed here but a brief overview is provided. The technology used is similar to comparable processes such as fed-batch fermentations and food oil extraction processes.

Micro-algal Powder

The micro-algae are produced from a production fermentation process using a carbohydrate substrate. The strain of *Schizochytrium* sp. used for commercial production is a genetically stable pure culture strain ND23OD, derived from a wild strain ATCC 20888 using classical mutagenesis and as such is not categorised as genetically modified. This strain was used because it produced greater yields of DHA. The production process can be classified as being a typical commercial, food grade fed-batch fermentation process using common techniques and equipment expected for such processes and performed under GMP with food grade materials. Once fermentation has completed the micro-algae cells are separated and dried.

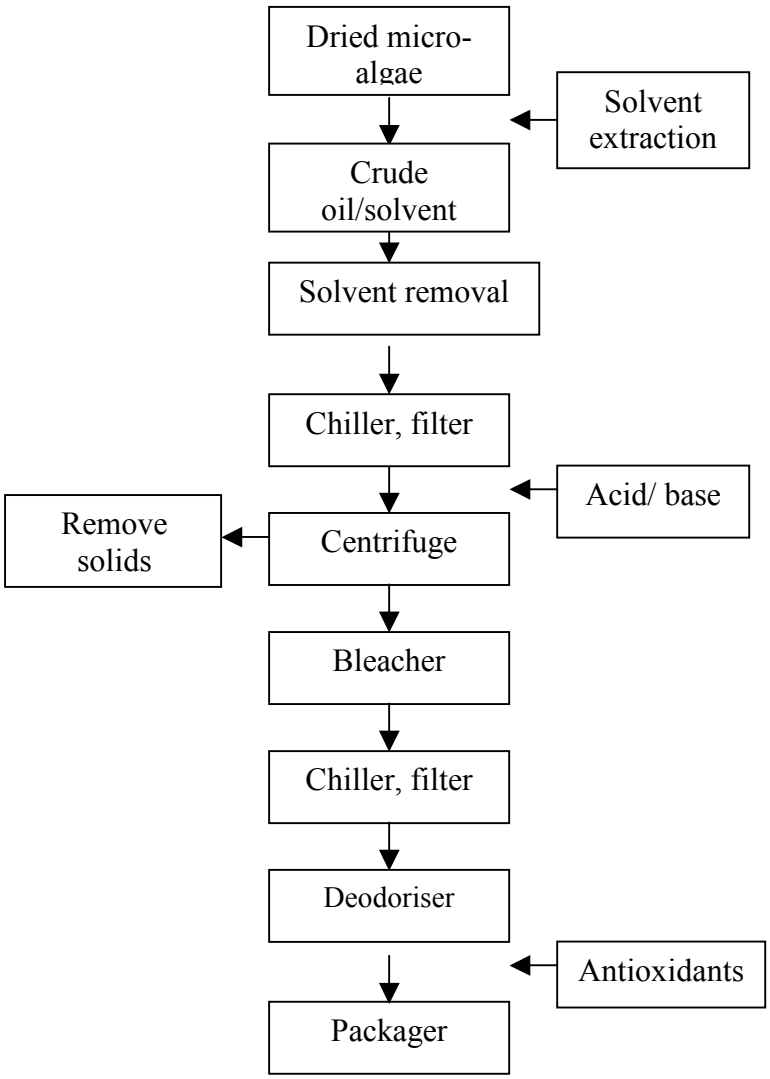
The culture used for the commercial fermentations are grown up from pure starter culture. The media contains a carbon source, a nitrogen source, various nutrients including trace minerals and vitamins. This is fed batch throughout the fermentation. Air (providing a source of dissolved oxygen) is pumped through the broth in a controlled manner during the fermentation. Agitation and temperature are also controlled. The fermentations are performed in cleaned and sterile fermenters using GMP. Once the fermentation has reached the required mass the broth is chilled and the micro-algae separated and dried and ultimately packaged. The dried micro-algae can be further processed to extract and purify the DHA oil as explained below.



DHA Oil

The dried micro-algae are crushed via wet milling and the oil extracted with an approved organic solvent (hexane). The crude oil/solvent mixture is chilled and filtered to remove solid impurities. The solvent is removed and the crude oil is purified by treatment with acid and base and the resultant solid impurities removed by filtration. The crude oil is further bleached with solid adsorbents to remove colour compounds and other impurities. It may be further cleaned by chilling and filtering out any solid impurities formed. The oil is further treated for a short time at high temperature (deodoriser) to remove low molecular weight contaminants as well as destroying peroxides (which can later irreversibly oxidize the oil and so limit its shelf life). Antioxidants are then added to the purified oil and it is packaged to limit oxidation.

The chemicals and filtration materials that have contact with the oil are common processing aids commonly used by the food industry for a range of applications.



Composition of the extracted oil

The typical fatty acid and sterol composition of the DHA rich extracted oil is found in Tables I and II. This oil is compared to other similar food oils that contain similar long chain fatty acid profiles, including another DHA rich oil extracted from a single cell organism, *Crypthecodinium cohnii*, and marine algae used in traditional Japanese food.

Most of the fatty acid components of DHA-rich oil are present in substantial amounts in other foods. Lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0) and palmitoleic (16:1) acids are present in high amounts in one or more commercial fats and oils, namely menhaden oil, salmon oil, palm oil, butter and lard, to name a few. Vaccenic acid (18:1n-7) is found in meats and seafood[3]. Arachidonic acid (20:4n-6), EPA, DPA(n-6), and DHA are commonly found in significant amounts in meats and seafood[4].

Tetradecatrienoic (14:3n-3) and hexadecatrienoic (16:3n-6) acids are beta-oxidation products of alpha-linolenic (18:3n-3) and gamma-linolenic (18:3n-6), respectively. Stearidonic acid (18:4n-3) and eicosatetraenoic acid (20:4n-3) are intermediates in the synthesis of EPA and DHA from alpha-linolenic acid. Dihomo-gamma-linolenic acid (20:3n-6) is an intermediate in arachidonic acid synthesis from gamma-linolenic acid. Eicosatetraenoic acid (20:4n-7) is an elongation, desaturation product of cis-vaccenic acid. Docosatetraenoic acid (22:4n-9) is an elongation, desaturation product of oleic acid. Eicosatetraenoic acid (20:4n-7) has been identified in animal phospholipids[5]. It is concluded that all of these minor fatty acids are likely to be present at low concentrations in a variety of foods, especially animal derived foods.

The three principal sterols in DHA-rich oil from *Schizochytrium* sp., cholesterol, stigmasterol and brassicasterol, are common in human foods including fish and shellfish[6,7,8]. Three other sterols, 23-dehydrositosterol, 7,24(28)-ergostadienol and 5,6-dihydroergosterol, are present in the oil in very small amounts. All have been identified in food except 23-dehydrositosterol which has been identified in Vanilla bean species and other non-grain parts of the corn plant.

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[3] Douglass JS, Server BE, Reich AG, Chew S. (1995). Mean daily intake and three-day average intake of 5,8,11,14,17-eicospentaenoic acid (EPA), 4,7,10,13,16,19-docosahexaenoic acid (DHA), 11-octadecenoic acid (VA), and 4,7,10,13,16-docosapentaenoic acid (DPA) by the U.S. population and population subgroups. TAS, Inc. Report.

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[5] Kunau WH, Bartnik F. (1974). Studies on the partial degradation of polyunsaturated fatty acids in rat-liver mitochondria. *Eur J Biochem*. 48(1):311-318.

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

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

[8] Sinclair, A.-J., Murphy, K.-J., Li, D. (2000). Marine Lipids: Overview "News Insights and Lipid Composition of Lyprinol™" *Allergie et Immunologie* – volume XXXII – nº7.

Table I Comparison of fatty acid profiles of DHA-rich oil from *Schizochytrium* sp. with oils derived from other micro-algae and a macro-algae (*Laminaria japonica)* found in the Sea of Japan used for food (% of total fatty acids)

CHEMICAL acid	**ABBREV**	**DHA OIL[1]**	**DHASCO OIL[2]**	***Thalassiosira pseudonana*[3]**	***Pavlova lutheri*[3]**	***Chroomonas salina*[3]**	***Laminaria japonica*[4,5]**	***Porphyridium cruentum*[6,7]**
Lauric	12:0	0.4	4.4	TR	0.3	TR	-	-
Myristic	14:0	10.1	12.7	14.3	11.5	8.4	5.4	-
Palmitic	16:0	23.7	9.7	11.2	21.3	14.0	20.8	24.2
Palmitoleic	16:1n-7	1.8	-	18.0	16.8	0.6	3.4	-
Stearic	18:0	0.5	1.1	0.7	1.3	0.8	-	-
Vaccenic	18:1n-7	0.7	27.0	0.1	1.4	3.4	-	-
Linoleic	18:2n-6	-	1.2	0.4	1.5	11.1	6.9	5.7
Linolenic	18:3n-3, n-6	-	-	0.3	2.2	15.9	5.6	-
Octadecatetraenoic	18:4n-3	0.6	-	5.3	6.0	20.6	10.5	-
Dihomo-gamma-linolenic & Eicosatetraenoic n-7	20:3n-6 20:4n-7	2.2	-	-	-	-		-
Arachidonic (ARA)	20:4n-6	1.8	-	0.3	TR	1.0	11.8	19.8
Eicosatetraenoic n-3	20:4n-3	-	-	0.3	-	1.0	-	-
Eicosapentaenoic n-3 (EPA)	20:5n-3	2.6	0	19.3	19.7	11.4	8.2	19.4
Docosatetraenoic	22:4n-9	0.6	-	-	-	-	-	-
Docosapentaenoic (DPA)	22:5n-6	13.6	-	-	2.0	0.1	-	-
Docosahexaenoic (DHA)	22:6n-3	35.0	40.0	3.9	9.4	5.5	-	-

NOTES:

1. Derived from *Schizochytrium* sp.; Monsanto derived 1997 analytical data from 5 bench lots.
2. Derived from *Crypthecodinium cohnii*; oil composition data from Martek Home Page, Martek Biosciences Corp., 1996.
3. J.K. Volkman, S.W. Jeffery, P.D. Nichols, G.I. Rogers and C.D. Garland, **J. Exp. Mar. Biol. Ecol.,** *128*, 219-240, 1989.
4. S.V. Khtimchenko and I.V. Kulikova, **Botanica Marina**, *43*, 87-91, 2000.
5. The reported results are from the middle parts of the blade of the brown algae.
6. M.M. Rebolloso Fuentes, G.G. Acien Fernandez, J.A. Sandez Perez and J.L. Guil Guerrero, **Food Chemistry**, *70*, 345-353, 2000.
7. Only data for the four fatty acids listed where reported. The data in the article were converted to % of total fatty acids by dividing by the total lipid content of 6.53 g/100g dry biomass.

Table II Typical sterol profiles (% of sterols) of different oil sources

Sterol	DHA-rich oil[1]	DHASCO oil[2]	Lyprinol oil[3]	*Laminaria japonica*[4]	Tuna oil[4]
cholesterol	25	2	31.8	tr	98.5
stigmasterol	19		0.8		0.1
brassicasterol	15		23.1		
23-dehydrositosterol	8				
7,24(28)-ergostadienol	<5-6				
5,6-dihydroergosterol	<5-7				
trans-22-dehydrocholesterol			10.9		0.2
24-methylene cholesterol			7.0	9-28	0.1
Fucosterol				72-88	
campesterol			1.7		0.1
beta-sitosterol			6.4		
dinosterol		40			
dehydrocholesterol		14			
4α-24-dimethyl cholestanol		minor			
dehydrodinosterol		major			
lathosterol		minor			
dinosterone		14			
cholesta-x,x-dienol		trace			
23 or 24-methyl cholesta-5,7-dienol		trace			

[1]Derived from *Schizochytrium* sp.
[2]Derived from *Crypthecodinium cohnii;* sterol profile from Withers *et al*., 1978
[3]Lyprinol (oil extracted from New Zealand Green Lipped Mussels) and tuna oil profile from Sinclair *et al*., 2000.
[4]Seasonal variation of sterol composition in Japanese macroalgae from Honya *et al*., **J Appl Phycology** *6*, 25-29, 1994.

ATTACHMENT 4

DIETARY EXPOSURE ASSESSMENT REPORT

A428 – DHA-rich dried marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. as novel food ingredients

ANZFA received an Application (A428) on 13 March 2001, from Omega Tech Inc., to amend Standards A19 and 1.5.1 (the Novel Foods Standards) of the *Food Standards Code* to permit the use of dried marine micro-algae (*Schizochytrium* sp.) which is rich in the omega-3 long chain polyunsaturated fatty acid DHA (docosahexaenoic acid) as a novel food ingredient in a limited range of foods. The Applicant subsequently amended their application on 28 August 2001 to include DHA-rich oil derived from the same species for use as a novel food ingredient in a limited range of foods

A dietary exposure assessment was conducted to estimate likely dietary exposure of Australians and New Zealanders to micro-algae-derived DHA, the main fatty acid present in this substance, if the substance were added to the foods nominated by the applicant at the maximum proposed level of use. The dietary exposure assessment also compares estimated total DHA exposure from use of this substance to existing dietary exposure from other food sources of DHA within Australia and New Zealand.

Predicted mean respondent exposure to DHA resulting from addition of micro-algae to the foods nominated by the applicant, at a level that would provide 60 mg DHA per serve of the food, ranges from 190-280 mg per day, across different age groups in Australia and New Zealand. Exposures for high consumers (95th percentile) of foods containing the micro-algae ranged from 400-750 mg per day, with exposure highest in New Zealanders aged 15-18 years. When background DHA exposure is taken into account, exposure of the highest 95th percentile consumer group (Australian adults) to all sources of DHA was 950 mg per day.

Background

DHA is a component of the oil present in the micro-algae *Schizochytrium* sp. The oil content of the dried micro-algae varies from batch to batch, and therefore the amount of DHA present varies also. The maximum reported DHA content in the micro-algae is 45% with a minimum content of 20%.

The Applicant has indicated that two levels of addition of the micro-algae (or its extracted oil) are proposed: addition of sufficient micro-algae (or its oil) to supply either 30 mg or 60 mg of DHA per serve of nominated food. These levels have been chosen to reflect requirements under Standard 1.2.8 (13) for nutrition claims for foods containing omega-3 fatty acids.

Table 1 identifies the foods to which the Applicant proposes to add the micro-algae or its oil. Typical levels of micro-algae addition are specified; these levels appear to assume a DHA concentration in the micro-algae of 20%.

The Applicant did not identify specific special purpose foods in which the micro-algae would be used, but noted this would be likely to include formulated meal replacements, infant foods and infant formula.

There is no Acceptable Daily Intake (ADI) established for the micro-algae or for DHA. The United States has set a GRAS level for DHA of 1.5 g per day based on the DHA level in breast milk.

Table 1: Foods proposed to contain the micro-algae *Schizochytrium* sp.

Food	Typical serve	mg algae/serve	mg algae/100 g (ml)
Bread	36 g	200 – 300	555 – 833
Crispbread biscuits	35 g	200 – 300	570 - 860
Breakfast foods	60 g	200 – 300	330 – 500
Table spreads	10 g	200 – 300	2000 – 3000
Dressings/mayonnaise	25 g	200 – 300	800 – 1200
Modified milk products	250 mL	200 – 300	50 – 75
Special purpose foods	Variable	200 – 300	-

Dietary Exposure Assessment provided by the Applicant

The Applicant provided estimates (see Table 2) of likely DHA intakes from the micro-algae, based on the 1995 Australian National Nutrition Survey, the proposed concentrations of DHA and the likely serve sizes of the foods containing DHA.

Table 2: Applicant's estimates of likely exposure to micro-algae-derived DHA

Food	Estimated daily intake (mg DHA)
Breads & crispbreads	115 - 175
Breakfast foods	15 – 25
Table spreads	30 – 47
Modified milk	33 – 49
TOTAL	198 - 303

The dietary exposure assessment provided by the Applicant was not considered to be sufficiently comprehensive to assist ANZFA in assessing the suitability of this novel food, as the assessment only provided estimates of mean or median intake across the entire Australian population (two years and above), did not include exposure estimates for special purpose foods, nor estimate exposure of high consumers or of particular age groups within the Australian population. The Applicant also failed to estimate exposure of New Zealanders to the novel food or to DHA derived from it. ANZFA therefore conducted its own dietary exposure assessment.

Food Consumption data

As a novel food, there appears to be no existing consumption of this micro-algae in Australia or New Zealand. A recent Australian study estimated daily intake of all omega-3 very long chain polyunsaturated fatty acids, including DHA, to be 180 mg, with fish and meats being the major sources of these fatty acids (Ollis, Meyer & Howe 1999).

However, using ANZFA's DIAMOND dietary modelling program and Australian nutrient composition data, diet-derived mean exposure to DHA, from existing food sources, was estimated to be 100 mg per day for all respondents aged two-100 years, with high consumers (95th percentile) exposed to 480 mg per day, as outlined in Table 3. Estimates of diet-derived DHA exposure were not able to be determined for New Zealanders, via the DIAMOND program, as information on the levels of DHA in NZ foods was not available. The Applicant did not provide any information on estimated New Zealand exposure to DHA from existing foods. The DIAMOND estimates do not take into account exposure to DHA from therapeutic goods, such as fish oil capsules.

Table 3: Australian exposure to DHA through existing dietary patterns and food sources

Population group	Mean mg/day	Median mg/day	95th percentile mg/day
2 -6 years	30	< 10	160
7 – 12 years	60	10	290
13 – 18 years	70	10	320
19 years and over	110	20	570
Women 16 – 44 yrs	80	10	410
All (2-100 years)	100	10	480

Dietary Modelling

The dietary exposure assessment was conducted using dietary modelling techniques that combine food consumption data with food chemical concentration data to estimate the exposure to the food chemical from the diet. The dietary exposure assessment was conducted using ANZFA's dietary modelling computer program, DIAMOND.

The exposure was estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data, with proposed levels of use of DHA derived from micro-algae in foods.

Dietary exposure = food chemical concentration x food consumption

Once this process has been completed for all of the foods specified to contain DHA, the total amount of DHA consumed from all foods is summed for each individual. Population statistics (mean, median and high percentile exposures) are then derived from the individuals' ranked exposures. This process was repeated using the assumption that all foods, other than those nominated by the Applicant, contain no DHA, in order to estimate exposure from the micro-algae alone.

Dietary Survey Data

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia that surveyed 13 858 people aged two years and above, and the 1997 New Zealand NNS that surveyed 4 636 people aged 15 years and above. Both of the NNSs used a 24-hour food recall methodology.

The dietary exposure assessment was conducted for both Australian and New Zealand populations. Modelling was conducted for the whole population, as well as for the age groups two - six years, seven -12 years, 13-18 years and 19 years and over. An exposure assessment was conducted on these age groups to determine whether or not particular age groups would have an exposure pattern markedly different to the population as a whole. For example, children generally have higher exposures, on a body weight basis, due to their smaller body weight and higher consumption of food per kilogram of body weight compared to adults. An exposure assessment was also conducted for women of childbearing age (assumed to be women aged 16-44 years) as it is possible that women in this age group may be likely to consume foods fortified with DHA due to its role in foetal and infant brain development.

DHA concentration levels

The levels of DHA in foods that were used in the models were derived from the Application. The Applicant had indicated that micro-algae would be added to achieve either a 30 mg or 60 mg intake of DHA per serve. For modelling purposes the upper proposed use level (60 mg) was selected in order to provide estimates of potential maximum exposure. The serve sizes proposed by the Applicant were used to estimate concentration of DHA per 100 g or 100 mL of the food. However as the Applicant provided only limited information on the particular special purpose foods in which they propose to use the substance, a number of assumptions were made about the types of special purpose foods and their serving sizes. Infant formula was not included as neither the Australian nor New Zealand dietary surveys studied infants under the age of two years. Some other infant foods were included in the modelling as there is some use of them in older age groups.

The foods and proposed levels of use are shown below in Tables 4 and 5. As different food codes and food names are used in the Australian and New Zealand databases used in DIAMOND, these are described in separate tables

Estimating Risk

As there is no established ADI for either DHA or the micro-algae *Schizochytrium*, estimated exposure levels were not compared to a particular safety standard in order to determine the number of consumers above this standard. However the estimated exposure to DHA based on the DHA levels in breast milk is 1.5 g per day for breast fed infants.

Table 4 : Proposed levels of use of micro-algae-derived DHA in foods - Australia

Food Code (NNS)	Food Name	Serve size	Concentration Level (mg/100 g or ml)
122	Regular breads and rolls	36 g	166
123	Breakfast cereals, plain, single source	60 g	100
127	Breakfast cereals, mixed source	60 g	100
132	Savoury biscuits	35 g	171
1421	Polyunsaturated margarine & spreads	10 g	600
1423	Monounsaturated margarine & spread	10 g	600

1424	Table margarines & spreads	10 g	600
1425	Reduced fat margarine spreads	10 g	600
1426	Unspecified margarine or marg.. spreads	10 g	600
145	Unspecified fats	10 g	600
1911	Milk, fluid, fat increased	250 ml	24
1913	Milk fluid reduced fat <2%	250 ml	24
1915	Milk fluid skim non-fat	250 ml	24
1919	Milk fluid unspecified	250 ml	24
224	Salad dressings	25 g	240
2911	Biscuit and bar meal replacement	150 g	40
2912	Milk based liquid meal replacements	250 ml	24
2913	Milk based powder meal replacements	50 g	120
2914	Oral supplement liquids	250 ml	24
2914	Oral supplement powders	50 g	120
312	Infant cereal products	25 g	240
313	Infant foods	75 g	80
314	Infant drinks	125 ml	48

Table 4: Proposed levels of use of micro-algae-derived DHA in foods – New Zealand

Food Code (NNS)	Food Name	Serve size	Concentration Level (mg/100 g or /100 ml)
021	Regular breads and rolls	36 g	166
031	Muesli	60 g	100
033	Processed bran cereals	60 g	100
035	Single cereal puffed, flakes or extruded	60 g	100
036	Wheat based biscuits & shredded wheat	60 g	100
042	Biscuits savoury	35 g	171
113	Polyunsaturated margarine	10 g	600
114	Monounsaturated margarine	10 g	600
0814	Milk, trim	250 ml	24
0815	Milk calcium enriched fluid	250 ml	24
295	Salad dressings	25 g	240
3221	Meal replacement bars	150 g	40
3222	Meal replacement drinks	250 ml	24
323	Protein supplement powder	50 g	120
324	Carbohydrate supplement	50 g	120
07110010	Infant baby rice with fruit (dry powder)	25 g	240

Assumptions in the dietary modelling

Assumptions made in the dietary modelling include:

- all the foods within the group contain DHA at the maximum proposed level of 60 mg;
- food consumption patterns reported in 1995 (Australia) and 1997 (New Zealand) are assumed to represent current patterns;
- all respondents are considered to be consumers of the substance given the broad range of staple foods proposed; and
- any possible influence of dietary long chain polyunsaturated fatty acids on synthesis of DHA within the human body is ignored.

These assumptions are likely to lead to an overestimate of dietary exposure to DHA derived from the novel food.

Limitations of the dietary modelling

A limitation of estimating dietary exposure over a period of time associated with the dietary modelling is that only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. Therefore, predicted high percentile exposures are likely to be higher than actual high percentile exposures over a lifetime.

Results

Estimated dietary exposures to DHA derived from micro-algae

The estimated dietary exposures for DHA derived from micro-algae are shown in Table 6. Estimated mean intakes for respondents/consumers are 260 mg per day in Australia and 280 mg/day in New Zealand. Estimated 95th percentile exposures are 600 and 690 mg/capita/day in Australia and New Zealand respectively. Exposure at the 95th percentile is highest in teenage respondents (13-18 years in Australia and 15-18 years in New Zealand), which is to be expected given the high food consumption of this group.

Similar exposure levels were seen in Australia and New Zealand, although exposure tended to be slightly higher in New Zealand.

Table 6: Estimated dietary exposures to micro-algae-derived DHA and to total DHA for all consumers

Country	Age group (both sexes unless specified)	Number of consumers	Mean exposure to DHA from micro-algae alone* mg/day	Mean exposure to DHA from micro-algae and from other foods** mg/day	Median exposure to DHA from micro-algae alone* mg/day	Median exposure to DHA from micro-algae and from other foods** mg/day	95th percentile exposure to DHA from micro-algae alone* mg/d	95th percentile exposure to DHA from micro-algae and from other foods** mg/d
Australia	Whole population (2 years+)	13 858	260	350	220	270	600	890
	2-6 years	989	190	220	170	190	400	480
	7-12 years	1090	250	310	230	270	550	740
	13-18 years	928	270	340	240	280	680	830
	19-100 years	10851	260	370	230	290	620	950
	16-44 years Women	3178	210	280	180	230	470	730
New Zealand	Whole population (15 years+)	4636	280	-	230	-	690	-
	15-18 years	246	270	-	220	-	750	-
	19-97 years	4390	280	-	240	-	680	-
	16-44 years Women	1509	230	-	200	-	580	-

* Assumes DHA addition at 60 mg per serve of selected foods (breads, spreads, breakfast cereals, crispbreads, dressings, modified milk, special purpose foods. **Estimates not available for New Zealand.

All consumers had estimated exposure levels to micro-algae-derived DHA well below the 1.5 g per day breast milk level, even at the 95th percentile level in the highest consumer group, children aged 13-18 years (15-18 years in New Zealand).

Results have not been reported on a bodyweight basis. However, as expected, the highest estimated dietary exposures per kilogram bodyweight were found among children aged two - six years (mean 10 mg/kg bw/day).

Estimated dietary exposures to DHA derived from micro-algae in addition to existing diet-derived exposures

Table 6 also contains results for estimated exposure of Australians to DHA from both the background diet (predominantly fish and fish products) and from the proposed use of the micro-algae. High consumers in the age group 19-100 years had the highest potential exposure to DHA (950 mg/head/day), which is still well below the United States GRAS level.

Total DHA exposure was not able to be estimated for New Zealanders due to the lack of data available to the DIAMOND.

Major contributing foods

Foods contributing to the total estimated exposures of DHA from micro-algae are displayed in Table 7. For all groups studied, the two major contributors were predicted to be breads and table spreads, with lesser contributions from breakfast cereals and modified milks.

Table 7: Major dietary exposure contributors to DHA derived from micro-algae, for Australia and New Zealand, and for different age groups

Country	Age group	*Major contributing foods and percent of total DHA exposures*
Australia	Whole population (2+ years)	Breads 58% Margarine & spreads 16% Breakfast cereals 9% Modified milk 8%
	2-6 years	Breads 60% Margarine & spreads 19% Breakfast cereals 11% Modified milk 4%
	7-12 years	Breads 56% Margarine & spreads 20% Breakfast cereals 12% Modified milk 7%
	13-18 years	Breads 57% Margarine & spreads 18% Breakfast cereals 12% Modified milk 8%
	19-100 years	Breads 58% Margarine & spreads 19% Breakfast cereals 9% Modified milk 8%

	16-44 years Women	Breads 58% Margarine & spreads 16% Breakfast cereals 9% Modified milk 8%
New Zealand	Whole population (15+ years)	Breads 62% Margarine & spreads 20% Breakfast cereals 6% Modified milk 6%
	15-18 years	Breads 63% Margarine & spreads 21% Breakfast cereals 7% Modified milk 5%
	19-97 years	Breads 62% Margarine & spreads 21% Breakfast cereals 6% Modified milk 6%
	16-44 years Women	Breads 59% Margarine & spreads 18% Breakfast cereals 9% Modified milk 9%

Conclusion

Dietary modelling of exposure to DHA derived solely from the use of the micro-algae *Schizochytrium*, indicates that mean and median exposures in Australia and New Zealand would be in the range 190-280 mg/day and 170-240 mg per day respectively. The highest exposure at the mean and median level would occur in Australian children aged 13 – 18 years and in New Zealand adults (19 years and above). High consumers (those at the 95th percentile) were exposed to micro-algae-derived DHA levels between 400 mg (Australians two - six years) and 750 mg per day (New Zealanders 15-18 years). When exposure to DHA from existing food use is taken into account, predicted total exposure to DHA in Australians increases to 280 mg/head/day (mean all ages) or 230 mg/head/day (median all ages). Estimated 95th percentile total dietary exposure to DHA derived from both the micro-algae and from background dietary exposure indicates that, even in the population group with the greatest potential exposure (Australian adults, 950 mg/day), this exposure is well below the breast milk level of 1.5 g per day. Major contributors to dietary exposure were breads and table spreads for all population groups studied.

It must be recognised that the estimates produced in this dietary exposure assessment are likely to exceed actual exposure if this novel food were to be approved, due to the assumptions on which they are based (e.g. that all foods in the selected groups contain DHA at the maximum proposed level). In addition, as estimates are based on one-day food recalls, they are likely to overestimate exposure at the 95th percentile.

REFERENCES

Ollis TE, Meyer BJ, Howe PR. (1999) Australian food sources and intakes of omega-6 and omega-3 polyunsaturated fatty acids. **Ann Nutr Metab**, *43(6)*: 346-55.

ATTACHMENT 5

SUMMARY OF PUBLIC SUBMISSIONS

Round 1

No.	Organisation	Position	Comments
1	**Weston Technology**	Support Option 2.	Approve the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel foods in specified range of foods.
2	**Food Technology Association, Victoria Inc**	Support Option 2.	Approve the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel foods but argues if the list is restricted initially as shown in the list, additional permits for other foods will require special application with inherent delays, costs and loss of market potential. Request that they be maintained on the circulation lists for further changes to this Application.
3	**Dietitians Association of Australia**	Supports Option 2 conditionally.	Approves the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel foods but is concerned about the lack of long term safety data. DAA recommends that consideration be given to a labelling requirement on foods containing this source of DHA, which encourages varied diet, and acknowledges that fish and seafood are the best sources of DHA.
4	**National Council of Women of Australia**	Supports Option 1.	Opposes the approval of the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel foods on the grounds of dietary considerations, safety issues and Regulatory Impact Assessment. Dietary considerations: concerned that the 'various international organisations who have recommend omega-3 fatty acids' use' are not named in the Preliminary Assessment report. Safety issues: Expresses concern that there are no safety data on the oil from this source. Regulatory Impact Assessment: Argues that the efficacy of these products should be established before approval.
5	**Wyeth Nutritionals**	Does not state a position.	Expresses concern about the safety of this source and its oil with respect to infant food. They were concerned that the use of this marine oil which contains eicosapentaenoic acid (EPA) may lower the arachidonic acid (AA) levels if used in infant food formula without supplementing with AA.
6	**Queensland Health**	Does not state a position.	Expresses concerns about the stability of DHA when not in encapsulated form. Points out that oxidation of fatty acids under inadequate storage conditions could lead to production of radicals and peroxide. Further, advises carrying out safety evaluation of possible production of toxins by the algae under certain environmental conditions.

Round 2

No.	Organisation	Position	Comments
1	**Australian Food and Grocery Council**	Support Option 2.	Approve the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) foods in specified range of foods. But questions whether these substances can be classified as novel foods based on the presence of key components in other traditional DHA sources.
2	**Fonterra Co-operative Group, Wellington, NZ**	Support Option 2.	Approve the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel foods. Advises against the use of the species name in the ingredient list as it would not add benefit to the consumer
3	**National Council of Women of Australia**	No comments	Unhappy about ANZFA's timing of public comments during the holiday period as the Council does not resume activities until February.
4	**Anji Christian, Westland, NZ**	Support Option 1	Concerned about the fate of marine animals dependent on micro-algal species if production of these products involved harvesting from the sea.
5	**Queensland Health**	Support Option 2	While supporting the approval for the use of the micro-algal products, the submitter is concerned about: • the potential contamination of the products by other phytoplanktons; • the availability of sufficient data on all age groups regarding the health implications arising from both DHA and sterols; and • Approval for a wide range of food stuff rather than selected permission of food items for those requiring additional DHA.
6	**Goodman Fielder, NSW**	Support Option 2	Contends that the approval of this Application has established that equivalence in composition to other recognised is sources is sufficient grounds to allow the safe use of food components from non-traditional sources
7	**Environmental Health Service, Department of Health, WA**	Support Option 1	Main points: • A thorough safety evaluation of the cellular components of the micro-algae needs to be done before permission given for its use; • Analysis for the presence of common algal toxins need to be carried out; and • Micro-algae may support the growth of human pathogens.
8	**Dietitians Association of Australia**	Support Option 2 conditionally	The submitter reiterates its concerns expressed in the first round regarding proposed uses, safety and labelling. In addition, • Does not recommend the addition of DHA – rich micro-algae in fruit juice, sports and energy drinks which normally contain no fat or oil; • Recommends additional long-term safety assessment and monitoring of its use in infant formulae and the effects of higher DHA intakes by pregnant and lactating women; and • Recommends monitoring of allergenic reactions to dried micro-algae.

9	**Food Technology Association of Victoria Inc.**	Support Option 2	Approve the use of DHA-rich oil and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) as novel foods in specified range of foods.