FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

2-05
23 March 2005

FINAL ASSESSMENT REPORT

APPLICATION A522

DHA-RICH MICRO-ALGAL OIL FROM *ULKENIA* SP. AS A NOVEL FOOD

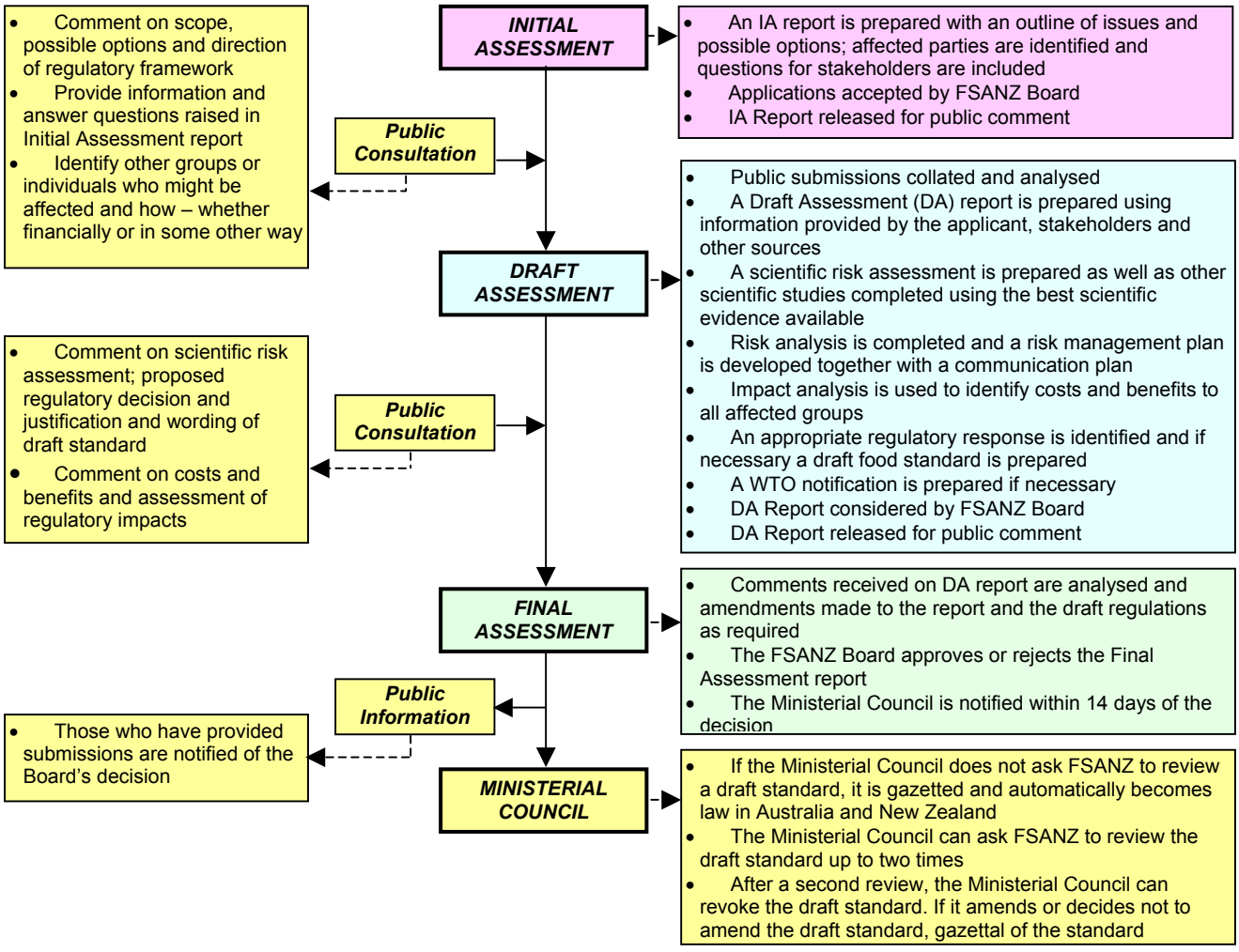
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Australian Government; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Australian Government, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Australian Government, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



Final Assessment Stage

FSANZ has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this Application. This Final Assessment Report and its recommendations have been approved by the FSANZ Board and notified to the Ministerial Council.

If the Ministerial Council does not request FSANZ to review the draft amendments to the Code, an amendment to the Code is published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

In New Zealand, the New Zealand Minister of Health gazettes the food standard under the New Zealand Food Act. Following gazettal, the standard takes effect 28 days later.

Further Information

Further information on this Application and the assessment process should be addressed to the FSANZ Standards Management Officer at one of the following addresses:

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Assessment reports are available for viewing and downloading from the FSANZ website www.foodstandards.gov.au or alternatively paper copies of reports can be requested from FSANZ's Information Officer at info@foodstandards.gov.au including other general enquiries and requests for information.

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Executive Summary and Statement of Reasons

FSANZ received an Application from Nutrinova Australasia Pty Ltd on 5 December 2003 to amend Standard 1.5.1 – Novel Foods, of the *Australia New Zealand Food Standards Code* (the Code) to approve the use of Docosahexaenoic acid (DHA)-rich oil derived from marine micro-algae (*Ulkenia* sp.), referred to as DHA-rich oil (*Ulkenia* sp.).

DHA is an omega-3 long chain polyunsaturated fatty acid derived from alpha-linolenic acid. Omega-3 long chain fatty acids, particularly DHA, have been identified as important dietary components. DHA is a normal constituent of the (non-vegan) human diet and the main source is cold-water fish.

Under the current food standards, novel foods are required to undergo a pre-market safety assessment, as per Standard 1.5.1 - Novel Foods. Although DHA is naturally present in certain foods such as fish and game meat, DHA-rich oil (*Ulkenia* sp.) is considered to be a non-traditional food because there is no history of significant human consumption of DHA from this marine micro-algae source in Australia or New Zealand. The safety of DHA-rich oil from this micro-algae source has not yet been determined. For these reasons, DHA-rich oil (*Ulkenia* sp.) is considered to be a novel food and is accordingly considered under Standard 1.5.1.

The objective of this assessment is to determine whether it is appropriate to amend the Code to permit the use of DHA-rich oil (*Ulkenia* sp.) as a novel food. Such an amendment would need to be consistent with the section 10 objectives of the FSANZ Act.

The safety assessment, dietary exposure assessment and nutrition assessment of DHA-rich oil (*Ulkenia* sp.) indicate that there are no public health and safety concerns at the anticipated levels of dietary exposure.

The only regulatory options identified were to approve or not approve the use of DHA-rich oil (*Ulkenia* sp.) as a novel food. The impact analysis indicates, that on balance, there is likely to be a benefit to consumers and public health professionals (by offering additional choice) and industry (potential to market new products) from the approval of this Application. There is unlikely to be a significant impact on government enforcement agencies as a result of approval for the use of DHA-rich oil (*Ulkenia* sp.) as a novel food.

Statement of Reasons

It is agreed to approve the use of DHA-rich oil (*Ulkenia* sp.) as a novel food, with no specified conditions of use for the following reasons:

- The Safety Assessment Report concluded that although the source organism does not have a history of safe use in food, information from scientific literature, toxicity studies, and a pathogenicity study, indicates that *Ulkenia* sp. is non-pathogenic. Toxicity studies on the DHA-rich oil (*Ulkenia* sp.) including acute toxicity, sub-chronic toxicity, a reproductive study and mutagenicity studies support the safety of the oil. There is no evidence of adverse effects in humans from the consumption of DHA from other sources (such as fish oil or other micro-algae) at low to moderate dose levels.

- The Dietary Exposure Assessment indicates that, when natural sources of DHA and all proposed food sources of added DHA (including DHA derived from either *Ulkenia* sp. or *Schizochytrium* sp.) were considered, there are no anticipated public health and safety concerns, even for high consumers.

- There is no anticipated nutritional risk attributable to the proposed addition of DHA-rich oil (*Ulkenia* sp.) to a range of foods. The overall nutritional impact of the addition of DHA-rich oil (*Ulkenia* sp.) to a range of foods is no different to the use of other non-novel oils in the food supply.

- The Food Technology Report indicates that DHA-rich oil (*Ulkenia* sp.) when used as a food/food ingredient, can be stabilised by food additives permitted in edible oils in the final food product to provide an alternative source of omega-3 fatty acids.

- The proposed changes to the Code are consistent with the section 10 objectives of the FSANZ Act.

- The Regulatory Impact Statement indicates that for the preferred option, namely, to approve the use of DHA-rich oil (*Ulkenia* sp.) as a novel food, the benefits of the proposed amendment outweigh the costs.

Specifications for DHA-rich oil derived from marine micro-algae (*Ulkenia* sp.) will be included in Standard 1.3.4 – Identity and Purity. DHA-rich oil (*Ulkenia* sp.) will be required to meet these specifications. The conditions of use for other novel DHA sources listed in Standard 1.5.1 (DHA-rich oil derived from marine micro-algae (*Schizochytrium* sp.) and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) will be removed as compliance with any specifications included in Standard 1.3.4 is a requirement of the Code. This variation is considered to be reasonably consequential within the scope of this Application. The requirements of Standard 1.3.4 will be referred to in an Editorial Note in Standard 1.5.1. As a food ingredient, the labelling requirements of Standard 1.2.4 – Labelling of Ingredients will apply. The proposed drafting for amendment to Standard 1.5.1 and Standard 1.3.4 is at Attachment 1 of the Draft Assessment Report.

1. Introduction

FSANZ received an Application from Nutrinova Australasia Pty Ltd on 5 December 2003 to amend Standard 1.5.1 – Novel Foods, of the Code to approve the use of Docosahexaenoic acid (DHA)-rich oil derived from marine micro-algae (*Ulkenia* sp.), hereafter referred to as DHA-rich oil (*Ulkenia* sp.). The Application is made on behalf of Nutrinova Nutrition Specialities and Food Ingredients GmbH, Frankfurt, Germany.

DHA is an omega-3 long chain polyunsaturated fatty acid derived from alpha-linolenic acid. Omega-3 long chain fatty acids, particularly DHA, have been identified as important dietary components. DHA-rich dried marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from marine micro-algae (*Schizochytrium* sp.) were previously assessed by the then Australia New Zealand Food Authority (ANZFA) and are approved novel foods in Australia and New Zealand.

In preparing this Final Assessment Report FSANZ has assessed:

- the safety of DHA-rich oil (*Ulkenia sp*.) as a food ingredient;
- the estimated dietary exposure to DHA-rich oil (*Ulkenia sp*.) based on the proposed food uses and the proposed levels of use;
- the food technology considerations;
- the nutritional issues related to its use as a food ingredient; and
- other issues raised in submissions to the Initial and Draft Assessment Reports.

2. Regulatory Problem

Under the current food standards, novel foods are required to undergo a pre-market safety assessment, as per Standard 1.5.1 – Novel Foods. The purpose of Standard 1.5.1 is to ensure that non-traditional foods that have features or characteristics that may raise safety concerns will undergo a risk-based safety assessment before they are offered for retail sale in Australia or New Zealand.

Novel Food is defined in clause 1 of Standard 1.5.1 as:

> *a non-traditional food 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*
> *(e) patterns and levels of consumption of the product.*

Non-traditional food *means a food which does not have a history of significant human consumption by the broad community in Australia or New Zealand*.

Although DHA is a normal constituent of the (non-vegan) human diet with the main source being cold-water fish, DHA-rich oil (*Ulkenia* sp.) is considered **non-traditional** because it does not have a history of significant human consumption in the broad community in Australia and New Zealand.

Because this DHA-rich oil is derived from a micro-algal source, the potential exists for undesirable substances to be present in the product. The safety of DHA-rich oil (*Ulkenia* sp.) with respect to levels of undesirable substances in the product and the potential for adverse effects in humans had not been assessed prior to receipt of this Application. In addition, there have been some adverse effects noted in clinical studies using high levels of DHA, such as increased bleeding times, necessitating a dietary exposure assessment to determine the predicted intake of DHA based on current consumption and the proposed foods uses. As such, DHA-rich oil (*Ulkenia* sp.) is considered a **novel food** in accordance with the definition provided in Standard 1.5.1 because it is a non-traditional food 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 levels of undesirable substances in the product, the potential for adverse effects in humans and the patterns and levels of consumption of the product.

3. Objective

The objective of this assessment is to determine whether or not it is appropriate to amend the Code to permit the use of DHA-rich oil derived from marine micro-algae (*Ulkenia* sp.) as a novel food. Such an amendment would need to be consistent with the section 10 objectives of the FSANZ Act.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 10 of the FSANZ Act. These 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.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Background

4.1 Nature of the Novel Food

The DHA-rich oil is a refined oil containing typically 45% DHA derived from marine micro-algae (*Ulkenia* sp.) produced under controlled fermentation conditions. The Applicant's proposed marketing name for DHA-rich oil (*Ulkenia* sp.) is Nutrinova DHA. Other common names, as stated by the Applicant, include DHA45-TG, DHA containing lipid, micro-algal oil and Ulkenia oil. DHA45-oil does not have a chemical name, as it is primarily a complex mixture of triglycerides, containing mainly the omega-3 fatty acid DHA. The chemical name of the major fatty acid DHA is:

All-cis-4,7,10,13,16,19-docosahexaenoic acid (22:6) ;

and the molecular formula is: $C_{22}H_{32}O_2$

The Applicant stated their intention to add DHA-rich oil (*Ulkenia* sp.) as an ingredient in food products to provide an additional source of omega-3 fatty acids. Those food products containing DHA-rich oil (*Ulkenia* sp.) would be aimed at people interested in increasing their intake of omega-3 fatty acids, specifically DHA.

The Applicant states that while DHA-rich oil (*Ulkenia* sp.) is derived from a novel source and is high in DHA, it is similar to conventional sources of polyunsaturated fatty acids with the main difference being the different fatty acid composition. Omega-3 fatty acids found in fish oils are produced by marine micro-algae and proceed through the marine food chain into fish[1].

The Applicant has provided information on the taxonomical classification of *Ulkenia* sp. as follows:

- Domain *Eukaryota*
- Kingdom *Chromista*
- Subkingdom *Heterokonta*
- Phylum *Labyrinthulomycota*
- Class *Labyrinthulea (Labyrinthulomycetes)*
- Subclass *Thraustochytriade*
- Order *Thraustrochytriales*
- Family *Thraustochytiaceae*
- Genus *Ulkenia*
- Species *Ulkenia* sp.

4.2 Proposed uses of DHA-rich oil (*Ulkenia* sp.)

[1] Yazawa, K., Watanabe, K., Ishikawa, C. Kondo, K., and Kimura, S. (1992) Production of eicosapentanoic acid from marine bacteria. In: Industrial Applications of Single Cell Oils. Kyle, D.J. and Ratledge, C. (Eds). American Oil Chemists Society, Champaign, USA.

The Applicant has stated their intention to use DHA-rich oil (*Ulkenia* sp.) as a food ingredient in such foods as breads and rolls, cakes and biscuits, breakfast cereals, cream cheese, modified milk and milk products, beverages, fruit drinks, sports drinks, functional drinks, dairy/non-dairy products, grain-based energy bars, infant foods, infant cereals and infant drinks and margarines and spreads. More detail on the proposed food uses is provided in the Dietary Exposure Assessment at Attachment 3 of this Report.

4.3 Nutritional role of omega-3 fatty acids and DHA

There are two families of polyunsaturated fatty acids, the omega-6 (or n-6) family, and the omega-3 (or n-3) family. The omega-6 family is derived from linoleic acid (C18:2) which has two double bonds, and the omega-3 family is derived from alpha-linolenic acid (C18:3) which has three double bonds. These two fatty acids are referred to as essential fatty acids, as they cannot be made in the human body and must be obtained from foods.

DHA is a normal constituent of the (non-vegan) human diet and the main source is cold-water fish in a range of 20 to 2020 mg/100 g. DHA is present as triacylglycerol and follows the normal fat absorption pathway.

DHA is an important structural element of cell membranes and is essential for the formation of new tissues. It plays a role in foetal neural development and has been implicated in decreasing the risk factors for coronary heart disease.

Further background information on the nutritional role of DHA is provided in the Nutrition Risk Assessment at Attachment 4.

4.3.1 Omega-3 fatty acid claims

Clause 13 of Standard 1.2.8 – Nutrition Information Requirements, provides criteria that must be met in order for claims in relation to the omega fatty acid content of foods to be made. A claim must not be made in relation to the omega-3 fatty acid content of a food, other than fish or fish products that have no added saturated fatty acids, unless the:

(a) total of saturated fatty acids and trans fatty acids is less than 28 per cent of the total fatty acid content of the food; or
(b) food contains no more than 5 g of saturated fatty acids and trans fatty acids per 100 g of the food.

A nutrition claim must not be made in relation to the omega-3 fatty acid content of a food, unless the food satisfies the requirements of subclause (2) as above, and contains no less than –

(a) 200 mg alpha-linolenic acid per serving; or
(b) 30 mg total eicosapentaenoic acid and docosahexaenoic acid per serving.

A nutrition claim must not be made that a food is a 'good source' of omega-3 fatty acid or words of similar import, unless the food satisfies the requirements of subclause (2) and contains no less than 60 mg total eicosapentaenoic acid and docosahexaenoic acid per serving.

4.4 Previous Application for DHA-rich dried marine micro-algae and oil derived from *Schizochytrium* sp. as novel foods

DHA-rich dried marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. were considered as novel foods in Application A428 by the then ANZFA. DHA-rich dried micro-algae (*Schizochytrium* sp.) and DHA-rich oil (*Schizochytrium* sp.) were approved as novel foods for the following reasons:

- The available data on DHA-rich micro-algae (*Schizochytrium* sp.) and on DHA-rich oil derived from *Schizochytrium* sp. did not raise any safety concerns at the predicted levels of exposure.
- The fatty acid composition of the dried *Schizochytrium* sp. micro-algae and the oil derived from *Schizochytrium* sp. were comparable to other traditional sources of DHA.
- Dried *Schizochytrium* sp. micro-algae and the oil derived from *Schizochytrium* sp. would provide an alternative source of omega-3 fatty acids in foods.

4.5 Regulation in other countries

The Applicant has indicated that DHA-rich oil (*Ulkenia* sp.) is permitted in the USA, Europe and Japan. The Applicant states that:

- In the USA, a panel of independent experts conducted a safety review of DHA-rich oil (*Ulkenia* sp.) and generally recognised as safe (GRAS) status was granted.

- DHA-rich oil derived from *Schizochytrium* sp. was notified as a novel food under article 5 of the EC Regulation 258/97 and can now be marketed across the EU.

- The German Competent Authority (Federal Authority for Consumer Protection and Food Safety, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)) determined that DHA-rich oil (*Ulkenia* sp.) was substantially equivalent to DHA-rich micro-algal oil from *Schizochytrium* sp. and was notified as a novel food under article 5 of the EC Regulation 258/97 and can now be marketed across the EU.

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

The Applicant states that there is no approval for DHA-rich oil derived from *Ulkenia* sp. in either Brazil or Canada and indicated that Nutrinova intend to lodge applications for the approval of DHA-rich oil in those countries in the near future.

The United Kingdom's Advisory Committee on Novel Foods and Processes (ACNFP) met on 4 February 2004 to consider the positive opinion on equivalence from the German Competent Authority on DHA-rich oil derived from *Ulkenia* sp. with the comparable product obtained from *Schizochytrium* sp. The ACNFP considered that the information summarised in the opinion did not appear to be sufficient to support the substantial equivalence of the two products. The Secretariat of the ACNFP agreed to seek clarification from the German Competent Authority on a number of points.

5. Relevant Issues

5.1 Safety assessment

The safety of DHA-rich oil (*Ulkenia* sp.) was assessed based on the available studies for this micro-algal source rather than on the basis of studies made available for DHA-rich oil derived from *Schizochytrium* sp. or other sources.

Two studies were submitted to establish the safety of the source organism, *Ulkenia* sp.: an acute oral toxicity study in mice; and a bacterial mutation assay. Three animal toxicological studies and three *in-vitro* genotoxicity studies were submitted in support of the safety of the DHA-rich oil (*Ulkenia* sp.). These consisted of an acute toxicity study, a sub-chronic oral toxicity study, a reproduction study, two bacterial reverse mutation assays and a chromosomal aberration study.

The safety assessment of DHA-rich oil (*Ulkenia* sp.) concluded that:

- although the source organism does not have a history of safe use in food, information from the scientific literature, toxicity studies, and the pathogenicity study indicates that *Ulkenia* sp. is non-pathogenic;
- acute toxicity studies in rats indicate that the LD_{50} for DHA-rich oil is more than 2000 mg/kg bw in rats;
- there was no evidence of toxicity in the sub-chronic toxicity study in rats fed DHA-rich oil for 90 days at the highest dose tested (2000 mg/kg bw/day). This is the equivalent of 900 mg/kg bw/day of DHA;
- a reproductive study showed no DHA-rich oil related adverse effects on reproductive parameters;
- the DHA-rich oil preparation produced no evidence of genotoxic potential in *in vitro* assays;
- there is no evidence to show adverse effects in infants based on the use of DHA from other sources (such as fish oil or other micro-algae) in infant formula; and
- there is no evidence of adverse effects in humans from the consumption of DHA from other sources (such as fish oil or other micro-algae) at low to moderate dose levels.

The full Safety Assessment is at Attachment 2.

5.2 Dietary exposure assessment

A dietary exposure assessment has been undertaken by FSANZ to determine the impact of allowing DHA-rich oil (*Ulkenia* sp.) to be added to a variety of foods in Australia and New Zealand. The assessment took into account naturally occurring levels of DHA in food (the 'naturally occurring' scenario – Scenario 1). The requested uses of DHA-rich oil (*Ulkenia* sp.) from the current application were also considered in a separate scenario (the 'A522' scenario - Scenario 2). Under Standard 1.5.1 there is an existing permission to add DHA derived from *Schizochytrium* sp. to various foods. Consequently, naturally occurring DHA concentrations were considered in conjunction with the current and proposed permission to add DHA addition from either micro-algae sp (the 'naturally occurring plus micro algal DHA' scenario – Scenario 3).

Dietary intakes of DHA were calculated for the Australian and New Zealand populations, and for the population sub-groups of infants aged 9 months, children aged 2-6 years and females aged 16-44 years. As no data were available for infants aged 9 months, a diet was constructed to estimate dietary intake of DHA. This was thought necessary due to the proposed addition of DHA to infant formula and follow on formula, infant cereal products, infant foods and infant drinks.

When naturally occurring and all proposed food sources of added DHA were considered (Scenario 3), the estimated mean dietary intakes of DHA were lowest for Australian infants aged 9 months (310 mg/day) and highest for the whole New Zealand population aged 15 years and above (498 mg/day). The estimated 95th percentile dietary intakes were lowest for Australian children aged 2-6 years (785 mg/day) and highest for the whole New Zealand population aged 15 years and above (1150 mg/day).

The major contributors to intake of DHA were breads and related products, oil emulsions and liquid milk for all population groups assessed, except infants where infant formula and follow on formula and bread and related products were the major contributors.

The full Dietary Exposure Assessment Report is at Attachment 3.

5.3 Nutrition assessment

Approval of DHA-rich oil (*Ulkenia* sp.) for use as a novel food ingredient will give manufacturers increased opportunities to add DHA to foods that have not traditionally contained significant levels of this fatty acid. The potential nutritional impact posed by the permission to add DHA-rich oil (*Ulkenia* sp.) to foods is no greater than that posed by the use of other non-novel oils.

*5.3.1 Interaction Between DHA-rich oil (*Ulkenia *sp.) and Other Nutrients*

There is evidence linking an increased intake of polyunsaturated fatty acids (such as DHA) to increasing population vitamin E requirements, however:

- DHA is only one of several polyunsaturated fatty acids that can influence vitamin E requirements; and
- the use of other polyunsaturated-rich oils is not limited by their impact on the vitamin E requirements.

  Vitamin E is often added to oils for technological (i.e. antioxidant) purposes or for nutrient restoration. Therefore, the potential consequences for vitamin E requirements are very minor, and do not constitute a significant public health problem.

- There is no evidence to suggest that DHA-rich oil (*Ulkenia* sp.) will impact on the bioavailability of other nutrients, or will be digested any differently to other sources of fat.

5.3.2 The ratio of Eicosapentaenoic acid to DHA

Traditionally rich sources of DHA (e.g. marine oils) are also the primary source of eicosapentaenoic acid (20:5) (EPA) in the diet. DHA-rich oil (*Ulkenia* sp.) has an EPA to DHA ratio of 1:16, which is comparable with some fish such as coral trout.

5.3.3 Nutrition Claims

The potential for the approval of DHA-rich oil (*Ulkenia* sp.) to increase the number of foods carrying omega-3 fatty acid claims within Australian and New Zealand markets is already managed by mandatory eligibility criteria. The eligibility criteria for omega-3 fatty acid claims prevent these claims from appearing on the labels of nutritionally inappropriate foods, by addressing inappropriate nutritional characteristics (e.g. saturated fatty acid content).

Omega-3 fatty acid claims are currently under review as part of Proposal P293 – Nutrition, Health and Related Claims. It is proposed under P293 that eligibility criteria will continue to apply to omega-3 fatty acid content claims. The relationship between omega-3 fatty acids and cardiovascular disease is also under review as a possible high-level claim (i.e. a claim that will require permission in the Code under the proposed new nutrition, health and related claims standard); this diet disease relationship may promote use of DHA-rich oil (*Ulkenia* sp.) if approved, although such an outcome will depend on any criteria that are set in relation to use of the claim.

The full Nutrition Assessment Report is at Attachment 4.

5.4 Risk assessment

The public health and safety risk to Australian and New Zealand populations have been assessed on the basis of the findings of the safety assessment (section 5.1) and the dietary exposure assessment (5.2). The data from the available animals studies, taken together with the composition data and the dietary exposure assessment for DHA-rich oil (*Ulkenia* sp.) do not indicate any potential for toxicity.

There is no evidence of adverse effects in humans from the consumption of DHA from other sources (such as fish oil or other micro-algae) at low to moderate dose levels. The human studies available on DHA from other sources were conducted primarily for efficacy purposes but there were no reports of adverse effects at dose levels up to 6 g/day for 90 days in adults. The USFDA have stated that consumption of up to 3 g/day of EPA plus DHA has been considered to have no effect on bleeding times (high amounts of long chain n-3 polyunsaturated fatty acids has been reported to be associated with longer bleeding times in some studies).

The highest estimated 95th percentile dietary intake was 1150 mg/day when natural sources of DHA and all proposed food sources of added DHA (including DHA derived from either *Ulkenia* sp. or *Schizochytrium* sp.) were considered. The estimated mean intakes were much lower (the highest mean intake was 498 mg/day). The highest estimated intakes are still lower than the dose level of 6 g/day in adults where no adverse effects were reported and also lower than the 3 g/day of n-3 polyunsaturated fatty acids that is stated by the USFDA to have no effect on bleeding times.

The safety assessment indicated that there is no evidence to show adverse effects in infants based on the use of DHA from other sources (such as fish oil or other micro-algae) in infant formula. However, a diet was constructed to estimate the dietary intake of DHA for infants of 9 months of age. The estimated mean dietary intake of DHA for Australian infants aged 9 months was 310 mg/day. This intake is significantly lower than the dose level of DHA (6 g/day for 90 days in adults) at which adverse effects were reported. Even considering the lower body weight of infants, there are no public health and safety concerns associated with the use of DHA-rich oil (*Ulkenia* sp.) in infant formula or infant foods. The overall nutritional impact of the addition of DHA-rich oil (*Ulkenia* sp.) to a range of foods is no different to the use of other non-novel oils in the food supply.

In conclusion there are no public health and safety concerns associated with the use of DHA-rich oil (*Ulkenia* sp.) in the range of foods and at the maximum levels proposed by the Applicant.

5.5 Food technology considerations

Food technology issues have been considered in preparing this Draft Assessment Report and the Food Technology Report is at Attachment 5. The following points are derived from the Food Technology Report:

- DHA-rich oil (*Ulkenia* sp.) is prepared using commonly employed techniques of fed-batch fermentation for micro-organisms performed in accordance with GMP and food grade oil extraction and purification processes. DHA-rich oil (*Ulkenia* sp.) then undergoes the normal processing and preparation requirements for the particular food to which it is added.

- Unsaturated fatty acids are readily oxidised by contact with oxygen. DHA-rich oil (*Ulkenia* sp.) is stabilised by: the addition of antioxidants permitted in Standard 1.3.1 – Food Additives – of the Code, primarily tocopherols; packaging in containers with limited oxygen content; preventing contact with light; and storage at low temperatures.

- DHA-rich oil (*Ulkenia* sp.) contains a number of long chain fatty acids (C12-C22) with DHA being the major fatty acid (typically being 45% of total fatty acids). The oil also contains a small percentage of trans-fatty acids (less than 2%) and non-saponifiables (mainly sterols, less than 2%). The oil is colourless to pale yellow, fluid to waxy oil, with a characteristic "bland to fish-like odour".

The proposed use of DHA-rich oil (*Ulkenia* sp.) is as a food/food ingredient, providing a source of omega-3 fatty acids. The use of the extracted DHA-rich oil obtained from the micro-algae *Ulkenia* sp. as a food ingredient is consistent with the use of DHA derived from the micro-algae *Schizochytrium sp*. that is already approved as a novel food in Standard 1.5.1 of the Code. The Food Technology Report indicates that DHA-rich oil (*Ulkenia* sp.) when used as a food ingredient can be stabilised in the final food product, enabling manufactures to make omega-3 content claims in accordance with Standard 1.2.8 – Nutrition Information Requirements.

5.6 Risk management

Standard 1.5.1 of the Code, in the Table to clause 2, makes provision for conditions of use for a particular novel food to be specified in column 2 of that table, associated with permission for that novel food. Conditions of use may be specified where a particular public health and safety risk is identified for either the general population or an identified population sub-group. Such conditions of use may be referred to as risk management strategies and include limiting the maximum level of use of the novel food or novel food ingredient, limiting the categories of foods to which the novel food ingredient may be added, or requiring statements to be provided on novel foods that advise against consumption by particular sub-groups of the population or provide the consumer with information about the appropriate use of the novel food.

The risk assessment indicates that there is no identified public health and safety concern for the use of DHA-rich oil (*Ulkenia* sp.) as a novel food in the proposed range of foods, including infant formula and infant foods, at the maximum levels of use as provided by the Applicant. Therefore, the use of risk management strategies in conjunction with a permission for DHA-rich oil (*Ulkenia* sp.) as a novel food is not deemed necessary.

The specifications for DHA-rich oil (*Ulkenia* sp.), as provided by the Applicant, will be included in Standard 1.3.4 – Identity and Purity – of the Code and when used as a food ingredient, DHA-rich oil (*Ulkenia* sp.) will be required to meet these specifications.

In the course of preparing the Draft Assessment, FSANZ reviewed the need for compliance with Standard 1.3.4 being stated as a condition in the table to Clause 2 of Standard 1.5.1. The specifications for DHA-rich oil (*Ulkenia* sp.) will be listed in the Schedule to Standard 1.3.4, and the Authority regards compliance with that Standard as a requirement of the Code, with which there must be compliance in any event. Therefore FSANZ has not, for this food, imposed compliance with Standard 1.3.4 as a condition of use for this food, and has removed that condition for other novel DHA sources listed in Standard 1.5.1 (DHA-rich oil derived from marine micro-algae (*Schizochytrium* sp.) and DHA-rich dried marine micro-algae (*Schizochytrium* sp.)). The requirements of Standard 1.3.4 will be referred to in an Editorial note in Standard 1.5.1 and this is included in the proposed drafting at Attachment 1. FSANZ considers this variation to be reasonably consequential within the scope of this Application

FSANZ has, in Application A433 and Application A508, also acted to remove that condition for phytosterol esters and tall oil phytosterols.

As a food ingredient, the labelling requirements of Standard 1.2.4 – Labelling of Ingredients – of the Code will apply. This requires ingredients to be declared in the statement of ingredients using: the common name of the ingredient; or a name that describes the true nature of the ingredient; or where applicable, a generic name set out in the table to clause 4 of that Standard.

5.7 Issues raised in submissions

5.7.1 Issues raised in response to the Initial Assessment Report

5.7.1.1 Safety assessment

The New Zealand Food Safety Authority (NZFSA) noted in its submission that the UK ACNFP did not agree with the positive opinion of the German Competent Authority that there is sufficient evidence to support the substantial equivalence of DHA-rich micro-algal oil from *Ulkenia* sp. and the product obtained from *Schizochytrium* sp. NZFSA suggested that the safety assessment for this Application should be undertaken based on the safety studies undertaken on DHA-rich oil (*Ulkenia* sp.) itself rather than on the basis of the safety data for *Schizochytrium* sp.

NZFSA also requested clarification, for the purposes of the safety assessment and dietary exposure assessment, on whether it was proposed to use DHA-rich oil (*Ulkenia* sp.) in infant formula and follow-on formula in addition to the other infant food products listed in the Application. If it is intended to use the oil in infant formula, the safety assessment should consider the safety of DHA-rich oil (*Ulkenia* sp.) for use by infants.

FSANZ consideration

FSANZ has considered the outcome of the UK ACNFP discussion of the German Competent Authority's determination of substantial equivalence. In preparing this Draft Assessment Report, the safety of DHA-rich oil (*Ulkenia* sp.) has been assessed based on the available studies for this micro-algal source and not on the basis of safety studies made available for DHA-rich oil derived from *Schizochytrium* sp.

The Applicant does propose to use DHA-rich oil (*Ulkenia* sp.) in infant formula and follow-on formula as well as in other infant foods. Therefore, the safety of DHA-rich oil (*Ulkenia* sp.) has been considered, to the extent possible, for infants and young children.

As no human studies have been conducted using DHA-rich oil from *Ulkenia* sp., the assessment of safety in humans (including infants) is based on the scientific literature in which the effects of DHA or DHA-containing foods are presented. DHA is present in human breast milk at varying levels depending on the mother's diet. No studies have consistently shown a negative effect on the growth or developmental indices of DHA-supplemented formula-fed term infants or weaning infants fed diets supplemented with n-3 fatty acids.

This is discussed further in Section 3.3.2 of the Safety Assessment Report at Attachment 2.

5.7.1.2 Dietary modelling considerations

NZFSA requested that dietary modelling be conducted for infants and young children in addition to the general population because of the intended use in infant formula (including follow-on formula) and other infant foods. The Dietitians Association of Australia (DAA) requested that FSANZ determine the potential total intake of DHA for high consumers if DHA-rich oil (*Ulkenia* sp.) were to be used in all the proposed products.

FSANZ consideration

FSANZ has examined all of the issues raised by submitters with respect to dietary modelling as described in section 5.2 of this Report and in the Dietary Exposure Assessment Report at Attachment 3.

Dietary exposure assessment was conducted for infants (aged 9 months) and young children (aged 2-6 years). When DHA from all sources was taken into consideration, the estimated mean dietary intake for children aged 2-6 years was 378 mg/day and the estimated 95th percentile dietary intake was 785 mg/day. A diet was constructed to estimate the dietary intake of DHA for infants of 9 months of age. The estimated dietary intake from all sources was 310 mg/day.

The potential total intake of DHA for high consumers if DHA-rich oil (*Ulkenia* sp.) were to be used in all the proposed products was also calculated. The estimated 95th percentile intake of DHA was highest for all New Zealanders aged 15 years and above at 1150 mg/day, considerably less than the level of DHA which has not been found to cause adverse effects of 3 gm/day. Further information on potential total intake of DHA for high consumers is presented in Attachment 3 (Dietary intake assessment report).

5.7.1.3 Nutrition claims

One submitter expressed concern that there may be conflicting health messages if DHA-rich oil (*Ulkenia* sp.) is added to some of the proposed foods as proposed by the Applicant, e.g. sweet biscuits and the product is marketed as an additional source of omega-3 fatty acids.

FSANZ consideration

As described in section 5.3 of this Report, in accordance with Standard 1.2.8 – Nutrition Information Requirements, a claim must not be made in relation to the omega-3 fatty acid content of a food, other than fish or fish products that have no added saturated fatty acids, unless the:

(a) total of saturated fatty acids and trans fatty acids is less than 28 per cent of the total fatty acid content of the food; or
(b) food contains no more than 5 g of saturated fatty acids and trans fatty acids per 100 g of the food.

It is possible that a product such as sweet biscuits containing added DHA would not meet these criteria and therefore, would be disqualified from making a claim in relation to omega-3 fatty acid content. This is currently the status for foods containing DHA-rich oil derived from *Schizochytrium* sp. for which permission is already given in Standard 1.5.1. There are no restrictions on the foods to which the DHA-rich oil derived from *Schizochytrium* sp. may be added and so long as the qualifying criteria (that the food contains a minimum prescribed amount of either alpha-linolenic acid or total of eicosapentanoic acid (EPA) and docosahexaenoic acid per serving) and disqualifying criteria as set out above are met, a claim in relation to the omega-3 fatty acid content of the food can be made.

Further detail on omega-3 fatty acid claims is provided at Attachment 4.

5.7.1.4 Ratio of DHA to EPA

The DAA requested that FSANZ examine the ratio of EPA to DHA in the DHA-rich oil (*Ulkenia* sp.) and compare this with the EPA to DHA ratio in some of the main natural sources of these fatty acids such as meat and fish.

FSANZ consideration

The level of EPA specified in DHA-rich oil (*Ulkenia* sp.) is not specifically stated, however, the amount of 'other fatty acids' (which may include EPA) is quantified at 2.8%. Based on a DHA content of 45% for the DHA-rich oil (*Ulkenia* sp.), the minimum EPA:DHA ratio is 1:16.1. FSANZ has some limited data for levels of the two omega-3 fatty acids, EPA and DHA in Australian foods. A comparison of the ratio of EPA to DHA in various foods is present in the Nutrition Report (Attachment 4) and was discussed in section 5.3 of this Report. This comparison indicates that the ratio of EPA:DHA is highly variable, ranging from approximately 1:0.1 in lamb to 1:16 in coral trout.

5.7.1.5 Food technology issues

The NZFSA requested that FSANZ examine the stability of DHA-rich oil (*Ulkenia* sp.) and name any antioxidants used to limit the oxidation. It was also requested that FSANZ assess whether any antioxidants used continue to function in the final food and as such, whether additive labelling may be required.

FSANZ consideration

FSANZ has assessed the stability of DHA-rich oil (*Ulkenia* sp.) and antioxidants used in the preparation in the Food Technology Report (Attachment 5). Unsaturated fatty acids (fatty acids with at least one double bond) are readily oxidised by contact with oxygen which limits the quality of the extracted oil. The DHA-rich oil is stabilised by: the addition of antioxidants permitted in category 2 – edible oils and oil emulsions in Standard 1.3.1 – Food Additives – of the Code, primarily tocopherols; packaging in containers with limited oxygen content; preventing contact with light; and storage at low temperatures.

Antioxidants used in DHA-rich oil (*Ulkenia* sp.) may need to be labelled depending on whether the antioxidant has a technological function in the final food. This is covered in clause 6 and the subsequential editorial note in Standard 1.2.4 – Labelling of Ingredients – of the Code.

5.7.1.6 Consideration of DHA-rich oil (*Ulkenia* sp.) against Standard 1.5.1 – Novel Foods

The Australian Food and Grocery Council (AFGC), in its submission, contended that FSANZ has not fulfilled the requirements of section 13(2)(a) of the FSANZ Act in determining, at the initial assessment, whether the Application warrants a variation to a food regulatory measure. The AFGC argues that DHA-rich oil (*Ulkenia* sp.) should not be considered as a novel food for the following reasons:

- DHA-rich oil (*Ulkenia* sp.) is a standardised food under Standard 2.4.1 – Edible Oils – of the Code.

- DHA has a long history of safe consumption and is not a 'non-traditional' food in accordance with the definition in Standard 1.5.1 and therefore, cannot be considered novel. The fact that a food is produced from a novel source does not make it a novel food.

- If FSANZ determines that DHA-rich oil (*Ulkenia* sp.) is safe and does not impose any restrictions on its use, it should be declared to be a food rather than a novel food. Without any conditions of use there must be sufficient knowledge in the community to allow safe use and if that is the case, the food cannot be novel, as it fails to fulfil the definition.

FSANZ consideration

DHA-rich oil (*Ulkenia* sp.) is a complex mixture of triglycerides containing mainly DHA and as such, could meet the definition of edible oils in Standard 2.4.1. However, the possibility of a food meeting a particular definition in the Code does not exclude that food from also being considered novel. For example, a definition is provided in Standard 1.2.8 – Nutrition Information Requirements, for 'biologically active substances' however, many substances that meet this definition would also be considered to be novel.

Although DHA is a normal constituent of the (non-vegan) human diet with the main source being cold-water fish, DHA-rich oil (*Ulkenia* sp.) is considered non-traditional because DHA derived from the source organism does not have a history of significant human consumption in the broad community in Australia and New Zealand.

Because this DHA-rich oil is derived from a micro-algal source, the potential exists for undesirable substances to be present in the product. In addition, there have been some adverse effects noted in clinical studies using high levels of DHA, such as increased bleeding times, necessitating a dietary exposure assessment to determine the predicted intake of DHA based on current consumption and the proposed foods uses.

As such, DHA-rich oil (*Ulkenia* sp.) is considered a novel food in accordance with the definition provided in Standard 1.5.1 because it is a non-traditional food 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 levels of undesirable substances in the product, the potential for adverse effects in humans and the patterns and levels of consumption of the product.

AFGC has regularly provided submissions in response to assessment reports for novel food applications indicating that, in their opinion, the novel food being assessed does not meet the definition of novel food and should not require pre-market assessment. The Novel Foods Standard is currently under review and consideration will be given to the definitions for 'non-traditional food' and 'novel food'.

5.7.2 Issues raised in response to the Draft Assessment Report

5.7.2.1 Draft variations to the Code with respect to conditions of use for DHA derived from *Schizochytrium* sp.

The Food Technology Association of Victoria (FTA Vic) expressed concern that the draft variations to the Code that were included in the Draft Assessment Report proposed removing the conditions of use for DHA derived from *Schizochytrium* sp. from the Table to clause 2 of Standard 1.5.1. The reasons given for this concern were that: public comment should be specifically sought from stakeholders; and this amendment is not the subject of this application or any other applications. It was suggested that the proposed amendment with respect to *Schizochytrium* sp. should be made through the next minor amendments omnibus proposal.

FSANZ consideration

It is appropriate to include the removal of the conditions of use for DHA derived from *Schizochytrium* sp. in the proposed drafting for this Application. While it is not the subject of the Application, there is scope to make consequential and related amendments as a result of review during the course of assessing any Application. As stated in section 5.6 of this Report, specifications for DHA-rich oil derived from marine micro-algae (*Schizochytrium* sp.) and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) are in Standard 1.3.4 – Identity and Purity, and FSANZ regards compliance with that Standard as a requirement of the Code, with which there must be compliance in any event. Because compliance with the specifications listed in Standard 1.3.4 is a requirement of the Code, the removal of this condition of use has no impact on any affected party.

An explanation of the reasoning for the removal of the conditions of use for DHA derived from *Schizochytrium* sp. was provided in section 5.6 of the Draft Assessment Report and the proposed amendment was included in the proposed drafting put forward that Report. Therefore, FSANZ has sought stakeholder comments on this matter.

5.7.2.2 Stability of DHA-rich oil (*Ulkenia* sp.)

Queensland Health has asked if the Applicant is proposing to provide storage and shelf-life instructions to food manufacturers and advice on the need to carry out stability tests on finished products containing the oil given that DHA-rich oil (*Ulkenia* sp.) is not stable at temperatures above 5^0C.

FSANZ consideration

Although polyunsaturated fatty acids are sensitive to air, heat and light, the Applicant has provided stability tests indicating that DHA-rich oil is stable for 12 months when stored at 5^0C or 35^0C under an inert atmosphere. The Applicant has provided a further response stating that even at temperatures higher than 5^0C, the oil is stable in originally closed packaging since 3 measures are implemented in the production of the oil: the exclusion of oxygen by packaging under nitrogen; exclusion of light by stainless steel containers; and the addition of tocopherols as antioxidants. DHA-rich oil should be used within 4 weeks after opening. It should be stored (both before and after opening) in tightly closed original packing in a cool (5-15^0C) and dry place under inert atmosphere.

Freezing will prolong the stability. The Applicant has indicated that they will provide food manufacturers with product information sheets including: the specifications; and comprehensive advice on the storage conditions and how to enhance the shelf-life of opened packages.

5.7.2.3 Risk management strategies

NZFSA requested that FSANZ address the following points in relation to risk management in the Final Assessment Report:

- The reason why the maximum intake of DHA recommended by the US FDA has not been recommended after assessing this application. Although toxicity tests for the DHA-rich oil (*Ulkenia* sp.) and the source material *Ulkenia* sp. itself did not indicate any safety issues, and the data from other food sources of DHA confirms the absence of such risks, there was limited data overall and FSANZ should consider restrictions.

- The need for an upper limit on use since the high consumption figure for children is about 50% higher than the US FDA figure and the predicted New Zealand intake figures are all higher than the Australian figures.

In addition, Queensland Health has asked whether the Applicant is proposing any self-regulation measures (e.g. maximum intake levels) around restricting the use in products targeted at particular population sub-groups, e.g. infant foods.

FSANZ consideration

While the USFDA have stated that consumption of up to 3 g/day of EPA plus DHA has been considered to have no effect on bleeding times, this is not a restriction on the use of DHA (or EPA) from any particular source and is not employed as a risk management strategy.

FSANZ has determined that it is not necessary to specify an upper limit on the use of DHA-rich oil (*Ulkenia* sp.) or to include any other conditions of use as a risk management strategy. This is because the risk assessment indicates that there is no identified public health and safety concern for the use of DHA-rich oil (*Ulkenia* sp.) as a novel food in the proposed range of foods, including infant formula and infant foods, at the maximum levels of use as provided by the Applicant. There were no reports of adverse effects at dose levels up to 6 g/day for 90 days in adults. The highest estimated 95th percentile dietary intake were much lower than this at 1150 mg/day when natural sources of DHA and all proposed food sources of added DHA (including DHA derived from either *Ulkenia* sp. or *Schizochytrium* sp.) were considered. The estimated mean intakes were much lower than the highest estimated 95th percentile dietary intake with the highest mean intake being 498 mg/day. FSANZ believes that there was adequate information for the thorough assessment of the safety of DHA-rich oil (*Ulkenia* sp.).

The safety assessment indicated that there is no evidence to show adverse effects in infants based on the use of DHA from other sources (such as fish oil or other micro-algae) in infant formula. However, a diet was constructed to estimate the dietary intake of DHA for infants of 9 months of age. The estimated mean dietary intake of DHA for Australian infants aged 9 months was 310 mg/day. This intake is significantly lower than the dose level of DHA (6 g/day for 90 days in adults) at which adverse effects were reported.

Even considering the lower body weight of infants, there are no public health and safety concerns associated with the use of DHA-rich oil (*Ulkenia* sp.) in infant formula or infant foods.

In relation to the question asked by Queensland Health about whether the Applicant is proposing to restrict levels of use of DHA-rich oil (*Ulkenia* sp.) in products marketed to specific population sub-groups, the Applicant has proposed levels of use for DHA in various products. This information is included in the Dietary Intake Assessment Report at Attachment 3. For example, the Applicant is proposing to add 60 mg/serve of DHA-rich oil (*Ulkenia* sp.) to infant formula and follow-on formula, infant cereal products, infant foods and infant drinks. The Applicant has indicated that they will consider any relevant dietary recommendations in determining the levels of DHA-rich oil added to products, particularly infant formula and infant foods. There are no restrictions on the use of DHA from other sources in infant formula or infant foods in accordance with Standard 2.9.1 – Infant Formula Products, or Standard 2.9.2 – Foods for Infants.

5.7.2.4 Labelling

Queensland Health questioned whether FSANZ has given any consideration to prescribing the name that should appear in the statement of ingredients for foods containing DHA-rich oil (*Ulkenia* sp.) or elsewhere on the product label.

FSANZ consideration

As a food ingredient, the labelling requirements of Standard 1.2.4 – Labelling of Ingredients, of the Code will apply. This requires ingredients to be declared in the statement of ingredients using: the common name of the ingredient; or a name that describes the true nature of the ingredient; or where applicable, a generic name set out in the table to clause 4 of that Standard. The names of ingredients should be sufficiently detailed and accurate to ensure they are not false, misleading or deceptive, or likely to mislead or deceive.

The Applicant has indicated that they propose to use the common name 'DHA-rich oil' for ingredient labelling purposes. They have proposed alternative common names such as 'microalgal oil'. FSANZ believes that the names proposed by the Applicant provide sufficient detail and are not likely to mislead the consumer. For this reason, FSANZ is not proposing to prescribe the name that is to be declared in the statement of ingredients for DHA-rich oil (*Ulkenia* sp.). The name to be declared in the statement of ingredients for some other novel foods has been prescribed as a condition of use in the table to clause 2 of Standard 1.5.1. A name to be used for an ingredient would generally be prescribed only if there was some ambiguity as to what the ingredient should be called and had the potential to mislead consumers.

5.7.2.5 Considerations of DHA-rich oil (*Ulkenia* sp.) against Standard 1.5.1 – Novel Foods

Consistent with its submission to the Initial Assessment Report, the AFGC contends that DHA-rich oil (*Ulkenia* sp.) is not a non-traditional food and therefore cannot be considered novel. The AFGC argues that:

- DHA-rich oil (*Ulkenia* sp.) is traditional because DHA itself is traditional as a normal constituent of the (non-vegan) human diet and the definition of 'non-traditional food' in Standard 1.5.1 does not refer to novel sources or processes. DHA is the same whether it is obtained directly from a micro-algal source or indirectly through the food chain from fish.
- Although DHA derived from marine micro-algae is regulated as a novel food in the EU, this is legitimate because the EU definition for novel food includes reference to novel sources/processes.
- The risk management conclusion that it is not necessary to employ risk management strategies in conjunction with a permission for DHA-rich oil (*Ulkenia* sp.) provides support that it is not a novel food.

FSANZ consideration

As stated in response to AFGC's comments to the IAR, FSANZ considers DHA-rich oil (*Ulkenia* sp.) to be a novel food in accordance with the definition provided in Standard 1.5.1 and the Novel Foods Guidelines because it is a non-traditional food 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 levels of undesirable substances in the product, the potential for adverse effects in humans and the patterns and levels of consumption of the product.

Responses are provided to the 3 points summarised from the AFGC's submission as follows:

- Although DHA is a normal constituent of the (non-vegan) human diet with the main source being cold-water fish, the Applicant has applied for the approval of the oil derived from a specific micro-algal species. DHA-rich oil from this micro-algal species is considered to be non-traditional because the source organism does not have a history of significant human consumption in the broad community in Australia and New Zealand.

- Although the definitions for 'non-traditional food' and 'novel food' in Standard 1.5.1 do not explicitly include food from novel sources and/or processes, DHA-rich oil (*Ulkenia* sp.) is still subject to the Novel Foods Standard. This is because it is deemed 'non-traditional' as described in the point above and because the potential exists for undesirable substances to be present in the product. In addition, there have been some adverse effects noted in clinical studies using high levels of DHA, such as increased bleeding times, necessitating a dietary exposure assessment to determine the predicted intake of DHA based on current consumption and the proposed foods uses.

- Standard 1.5.1 of the Code, in the Table to clause 2, makes provision for conditions of use for a particular novel food to be specified in column 2 of that table, associated with permission for that novel food. It is not necessary for conditions to be specified for the food to be considered novel.

AFGC has regularly provided submissions in response to assessment reports for novel food applications indicating that, in their opinion, the novel food being assessed does not meet the definition of novel food and should not require pre-market assessment. The Novel Foods Standard is currently under review and consideration will be given to the definitions for both 'non-traditional food' and 'novel food'.

6. Regulatory Options

FSANZ 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, governments in both Australia and New Zealand and often public health professionals. The benefits and costs associated with the proposed amendment to the Code have been analysed in a Regulatory Impact Assessment.

Novel foods or novel food ingredients used in Australia and New Zealand are required to be listed in Standard 1.5.1 – Novel Foods. As the use of DHA-rich oil (*Ulkenia* sp.) is being considered as a novel food, which requires pre-market approval under Standard 1.5.1, it is not appropriate to consider non-regulatory options to address this Application.

Two regulatory options have been identified for this Application:

Option 1 – Not permit the use of DHA-rich oil (*Ulkenia* sp.) as a novel food.

Option 2 – Permit the use of DHA-rich oil (*Ulkenia* sp.) as a novel food.

7. Impact Analysis

7.1 Affected Parties

Parties possibly affected by the regulatory options outlined in Section 6 include:

1. Consumers who may benefit as a result of new products containing DHA-rich oil (*Ulkenia* sp.).

2. Public health professions because of the role of DHA in human nutrition.

3. Those sectors of the food industry wishing to market foods containing DHA-rich oil (*Ulkenia* sp.) including potential importers, manufacturers of DHA-rich oil (*Ulkenia* sp.) and manufacturers of foods that may potentially contain DHA-rich oil (*Ulkenia* sp.).

4. Government agencies enforcing the food regulations.

7.2 Impact Analysis

Some information relevant to the impact analysis was included in submissions received in response to the Draft Assessment Report. This information has been incorporated into the impact analysis that follows.

7.2.1 Option 1 – Not permit the use of DHA-rich oil (Ulkenia sp.)

7.2.1.1 Consumers

There are no significant costs or benefits of not permitting the use of DHA-rich oil (*Ulkenia* sp.) identified for consumers. Consumers wishing to ensure they have an adequate dietary intake of omega-3 fatty acids, or DHA specifically, can obtain them from existing sources such as cold-water fish.

Foods containing DHA-rich oil (*Schizochytrium* sp.) and DHA-rich marine micro-algae (*Schizochytrium* sp.) may also be available to consumers as these are approved novel food ingredients.

7.2.1.2 Public health professionals

There is no clear cost or benefit to public health professionals by not permitting DHA-rich oil (*Ulkenia* sp.) as a novel food. There are existing food sources of DHA which health professionals can recommend to clients for the purposes of increasing or maintaining their intake of omega-3 fatty acids, or DHA specifically.

7.2.1.3 Industry

The current situation of no permission for the use of DHA-rich oil (*Ulkenia* sp.) represents an opportunity cost to those industry sectors wishing to manufacture or import DHA-rich oil (*Ulkenia* sp.) for incorporation into food products or those wishing to manufacture or import final food products containing DHA-rich oil (*Ulkenia* sp.). The current situation also limits competition between suppliers of DHA-rich oils. The Applicant has indicated that the cost of using DHA-rich oil (*Ulkenia* sp.) is comparable to other DHA-rich oils. However, DHA-rich oil (*Schizochytrium* sp.) is an existing alternative to those industry sectors wishing to manufacture final food products, which provides a source of DHA.

7.2.1.4 Government

There is no benefit identified to government by not permitting DHA-rich oil (*Ulkenia* sp.) as a novel food. A potential cost to government of not permitting DHA-rich oil (*Ulkenia* sp.) is that it would be necessary for enforcement agencies to ensure that any food to which DHA is added does not contain DHA-rich oil from *Ulkenia* sp.

7.2.2 Option 2 – Permit the use of DHA-rich oil (Ulkenia sp.)

7.2.2.1 Consumers

Consumers may benefit from additional choice, particularly if manufacturers promote DHA-rich oil (*Ulkenia* sp.) as an vegetarian source of DHA. As stated by the Applicant, the purpose of adding DHA-rich oil (*Ulkenia* sp.) to products is to provide the consumer with a value-added product consumed for its nutritional properties. There are existing sources of omega-3 fatty acids, including DHA. The Applicant has stated that the cost of using DHA-rich oil (*Ulkenia* sp.) is comparable to other DHA-rich oils and therefore there should be no negative or positive price implications for consumers. Permitting the use of DHA-rich oil (*Ulkenia* sp.) is unlikely to significantly benefit consumers as there are existing alternatives, however, it would provide additional choice of DHA sources for vegetarians.

7.2.2.2 Public health professionals

Public health professionals may benefit from a wider range of foods providing omega-3 fatty acids, specifically DHA, which could be recommended to clients, particularly vegetarians, for the purposes of increasing or maintaining their omega-3 fatty acid intake.

7.2.2.3 Industry

Food manufacturers and importers are likely to benefit from permitting DHA-rich oil (*Ulkenia* sp.) as a novel food as there will be potential to develop and market new processed foods, which are a source of DHA. Manufactures of DHA-rich oil (*Ulkenia* sp.) will benefit from sales to food manufacturers. Permission for DHA-rich oil (*Ulkenia* sp.) would also potentially bring competition between suppliers of DHA-rich oils.

7.2.2.4 Government

It is unlikely that there will be any significant costs or benefits to government agencies enforcing the food regulations. DHA-rich oil (*Schizochytrium* sp.) and DHA-rich marine micro-algae (*Schizochytrium* sp.) are already permitted as novel foods and there is no indication that this permission has had a significant impact on resources. Approval of DHA-rich oil (*Ulkenia* sp.) as a novel food would promote international trade in food products, potentially benefiting government.

7.2.3 Assessment of impacts

On the basis of this Final Assessment, there is likely to be a slight benefit to consumers and public health professionals in offering additional choice of dietary omega-3 fatty acid sources. There is likely to be a benefit to industry sectors involved in the marketing of DHA-rich oil (*Ulkenia* sp.) as food. There is unlikely to be a significant impact on government enforcement agencies as a result of approval for the use of DHA-rich oil (*Ulkenia* sp.) as a novel food.

8. Consultation

8.1 Public consultation

8.1.1 Initial assessment

FSANZ received five submissions in response to the Initial Assessment Report. Only two of these submissions nominated a preferred regulatory option, which was in both cases, Option 2 – Permit the use of DHA-rich oil (*Ulkenia sp*.) as a novel food. A summary of submissions is at Attachment 5. Issues raised in submissions have been addressed in section 5 of this Report.

A sixth submission was received well after the close of the comment period without a request for an extension and as such, the issues raised in the submission could not be specifically addressed. However, the Safety Assessment Report at Attachment 2 contains information on the issues raised in this submission.

8.1.2 Draft Assessment

FSANZ received seven submissions in response to the Draft Assessment Report. Five of these submissions nominated Option 2 – Permit the use of DHA-rich oil (*Ulkenia* sp.) as a novel food as their preferred regulatory option. A summary of these submissions is at Attachment 5.

Issues raised in submissions have been addressed in section 5 of this Report and those comments that relate to the impacts on various affected parties have been incorporated into the impact analysis in section 7 of this Report.

8.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Amending the Code to permit the use of DHA-rich oil (*Ulkenia* sp.) as a novel food will not be notified to the WTO under either the Technical Barrier to Trade (TBT) or Sanitary and Phytosanitary Measure (SPS) agreements as the permission is unlikely to significantly effect trade, particularly since FSANZ would be expanding permissions. The potential food applications for DHA-rich oil (*Ulkenia* sp.) are limited in terms of market size. While Application A428 – DHA-rich dried marine micro-algae (*Schizochytrium* sp.) and DHA-rich oil derived from *Schizochytrium* sp. as novel food ingredients was notified to the WTO because the permission could lead to a liberalising effect on trade, it is generally not considered necessary to notify the WTO in these circumstances. No comments were received in response to the notification.

9. Conclusion and Recommendation

It is agreed to approve the use of DHA-rich oil (*Ulkenia* sp.) as a novel food for the following reasons:

- The Safety Assessment Report concluded that although the source organism does not have a history of safe use in food, information from scientific literature, toxicity studies, and a pathogenicity study, indicates that *Ulkenia* sp. is non-pathogenic. Toxicity studies on the DHA-rich oil (*Ulkenia* sp.) including acute toxicity, sub-chronic toxicity, a reproductive study and mutagenicity studies support the safety of the oil. There is no evidence of adverse effects in humans from the consumption of DHA from other sources (such as fish oil or other micro-algae) at low to moderate dose levels.

- The Dietary Exposure Assessment indicates that, when natural sources of DHA and all proposed food sources of added DHA (including DHA derived from either *Ulkenia* sp. or *Schizochytrium* sp.) were considered, there are no anticipated public health and safety concerns, even for high consumers.

- There is no anticipated nutritional risk attributable to the proposed addition of DHA-rich oil (*Ulkenia* sp.) to a range of foods. The overall nutritional impact of the addition of DHA-rich oil (*Ulkenia* sp.) to a range of foods is no different to the use of other non-novel oils in the food supply.

- The Food Technology Report indicates that DHA-rich oil (*Ulkenia* sp.) when used as a food/food ingredient, can be stabilised by food additives permitted in edible oils in the final food product to provide an alternative source of omega-3 fatty acids.

- The proposed changes to the Code are consistent with the section 10 objectives of the FSANZ Act.

- The Regulatory Impact Statement indicates that for the preferred option, namely, to approve the use of DHA-rich oil (*Ulkenia* sp.) as a novel food, the benefits of the proposed amendment outweigh the costs.

Specifications for DHA-rich oil derived from marine micro-algae (*Ulkenia* sp.) will be included in Standard 1.3.4 – Identity and Purity. DHA-rich oil (*Ulkenia* sp.) will be required to meet these specifications. The conditions of use for other novel DHA sources listed in Standard 1.5.1 (DHA-rich oil derived from marine micro-algae (*Schizochytrium* sp.) and DHA-rich dried marine micro-algae (*Schizochytrium* sp.) will be removed as compliance with any specifications included in Standard 1.3.4 is a requirement of the Code. This variation is considered to be reasonably consequential within the scope of this Application. The requirements of Standard 1.3.4 will be referred to in an Editorial Note in Standard 1.5.1. As a food ingredient, the labelling requirements of Standard 1.2.4 – Labelling of Ingredients will apply.

10. Implementation and review

FSANZ has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this Application. This Final Assessment Report and its recommendations have been approved by the FSANZ Board and notified to the Ministerial Council.

If the Ministerial Council does not request FSANZ to review the proposed amendments to the Code, the amendments to the Code with respect to Standard 1.5.1 – Novel Foods, would come into effect upon gazettal.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Dietary Exposure Assessment Report
4. Nutrition Report
5. Food Technology Report
6. Summary of submissions to the Initial Assessment Report

ATTACHMENT 1

Draft Variations to the *Australia New Zealand Food Standards Code*

To commence: on gazettal

[1] ***Standard 1.3.4*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Schedule –

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

Full chemical name for DHA	All cis-4,7,10,13,16,19-docosahexaenoic acid (22:6n-3 DHA)
Appearance	Fluid to waxy oil
Colour	Colourless to pale yellow
Odour	Characteristic bland to fish-like
DHA (%)	min. 32
Docosapentaenoic acid 22:5n-6 (%)	min. 8
Saturated fat (%)	max. 45
Trans fatty acids (%)	max. 2
Peroxide value (meq/kg)	max. 10
Moisture and volatiles (%)	max. 0.1
Non-saponifiables (%)	max. 2
Acid value (mg KOH/g)	max. 0.5
Lead (ppm)	max. 0.2
Arsenic (ppm)	max. 0.2
Mercury (ppm)	max. 0.2
Hexane (ppm)	max. 10

[2] ***Standard 1.5.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[2.1] *inserting in column 1 of the* Table to clause 2 –

Docosahexaenoic acid (DHA) – rich oil derived from marine micro-algae (*Ulkenia* sp.)	

[2.2] *omitting the conditions of use in* column 2 *of the* Table to clause 2 *for the following entries –*

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

[2.3] *omitting the* Editorial note *after the* Table to clause 2, *substituting –*

Editorial note:

Novel Foods must meet the requirements of Standard 1.3.4 - Identity and Purity.

The Table to Clause 2 contains conditions relating to novel foods. Nothing contained in this Code permits the mixing of phytosterol esters and tall oil phytosterols.

ATTACHMENT 2

Safety Assessment Report

Summary

Introduction

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

Safety of the source organism

DHA-rich oil is derived from a fermentation process using micro-alga, *Ulkenia* sp., which has no history of use in food. *Ulkenia* sp. is a member of the non-pathogenic, non toxigenic family of Thraustochytriaceae.

There is no information in the scientific literature that would indicate that *Ulkenia* species are pathogenic to humans or exhibit any other health risks. No algal toxins were detected in DHA-rich oil or *Ulkenia* biomass. *Ulkenia* sp. was found to be non-toxic, non-pathogenic, and non-mutagenic in an acute oral toxicity study in mice and in a bacterial mutation assay.

There are no reports of human consumption of *Ulkenia* sp., however, filter feeders (e.g. clams and mussels) feed on *Ulkenia* and are part of the normal diet. Furthermore, the production organism is not present in DHA-rich oil.

Composition of DHA-rich oil

Ulkenia sp. derived DHA-rich oil has a high DHA content (≥ 45%). It consists mainly of triacylglycerols. Further components as diacylglycerols or free fatty acids are either not-detectable or exist only as traces.

Toxicology studies on DHA-rich oil

Three toxicity studies have been conducted with DHA-rich oil from *Ulkenia* sp.; an acute study, a sub-chronic study and a reproductive toxicity study. The acute study established that the LD_{50} for DHA-rich oil in rats is >2000 µg/kg body weight. No significant adverse effects were reported in the sub-chronic study, and the NOEL was determined to be >2000 µg/kg body weight/day (the highest dose studied). There was no evidence that DHA-rich oil had any effect on reproductive parameters or progeny development in a rat one-generation dietary reproduction study (doses up to 7.5% DHA-rich oil in the diet).

Three genotoxicity studies were conducted with DHA-rich oil. DHA-rich *Ulkenia* sp. oil was not mutagenic in *Salmonella* or *E. coli* test strains TA97, TA98, TA100, TA102, TA1535, TA1537 and WP2 *uvr* in the presence or absence of activation. DHA-rich oil did not induce chromosomal aberrations in cultured Chinese hamster cells.

Published studies on DHA and DHA-rich oils from other sources

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 with the DHA exposure. The human studies available were conducted primarily for efficacy purposes but there were no reports of adverse effects at dose levels up to 6 g/day for 90 days in adults.

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 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.

DHA-rich oil and biomass from the marine micro-algae, *Schizochytrium* sp. are permitted food ingredients. DHA-rich oil from the algae *Crypthecodinium cohnii* is a permitted food ingredient in infant formula.

Overall Conclusion

The safety of DHA-rich oil derived from *Ulkenia* sp. 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 *Ulkenia*, 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 oil from *Ulkenia* sp., 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 oil derived from this micro-algae in foods at the levels proposed by the Applicant is not expected to lead to any adverse health effects.

DHA-RICH OIL DERIVED FROM *ULKENIA* SP.

1. Introduction

The purpose of this assessment is to determine the safety of DHA-rich oil from a novel source. The DHA-rich oil is a refined oil containing typically 45% DHA derived from marine micro-algae (*Ulkenia* sp.) produced under controlled fermentation conditions.

Other common names, as stated by the Applicant, include DHA45-TG, DHA containing lipid, micro-algal oil and Ulkenia oil. DHA-rich oil does not have a chemical name, as it is primarily a complex mixture of triglycerides, containing mainly the omega-3 fatty acid DHA. The chemical name of the major fatty acid DHA is all-cis-4,7,10,13,16,19-docosahexaenoic acid (22:6), and the molecular formula is $C_{22}H_{32}O_2$.

For the purposes of this safety assessment the term 'DHA-rich oil' is used to refer to DHA-rich oil derived from *Ulkenia* sp. Where reference is made to DHA from any other source (e.g. from fish oil or *Schizochytrium* sp.) this is stated.

1.2 Specifications for DHA-rich oil

The following specifications for DHA-rich oil were provided by the Applicant.

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

Full chemical name of DHA	All-cis-4,7,10,13,16,19-docosahexaenoic acid (22:6n-3 DHA)
Appearance	Fluid to waxy oil
Colour	Colourless to pale yellow
Odour and taste	Characteristic bland to fish-like
DHA (%)	min. 32
All-cis-4,7,10,13,16-docosapentaenoic acid (22:5n-6) (DPA) (%)	min. 8
Saturated fat (%)	max. 45
Trans fatty acids (%)	max. 2
Peroxide value (meq/kg)	max. 10
Moisture and volatiles (%)	max. 0.1
Non-saponifiables (%)	max. 2
Acid value (mg KOH/g)	max. 0.5
Lead (ppm)	max. 0.2
Arsenic (ppm)	max. 0.2
Mercury (ppm)	max. 0.2
Hexane (ppm)	max. 10

DHA-rich oil produced on a commercial scale meets the specifications indicated in the table above.

DHA-rich oil consists mainly of triacylglycerols. Further components as diacylglycerols or free fatty acids are either undetectable or exist only as traces.

Component (% w/w)	*Typical composition of DHA-rich oil*
Triacylglycerols	>98.0
Diacylglycerols	<1.0
Monoacylglycerol and phospholipids	<2.0
Unsaponifiables	<2.0
Free fatty acids	<0.25

The unsaponifiable fraction of DHA-rich oil is generally below 2.0% and is made up primarily of sterols. The sterol content of DHA-rich oil is comparable to conventional vegetable oils e.g. sunflower oil contains 0.25-0.75% and corn oil 0.58-1.5% sterol (Kochlar, 1983).

1.3 Chemistry of DHA

There are several distinct families of polyunsaturated fatty acids, the most important are the n-6 (also known as omega-6) family and the n-3 (omega-3) family. These two families are not inter-convertible. The n-6 family is derived from linoleic acid (C18), which has two double bonds, and the n-3 family from alpha-linoleic acid (C18), which has three double bonds. These two essential fatty acids cannot be made by the human body and therefore have to be provided through the diet.

DHA (22:6 n-3) is formed from alpha-linoleic acid by elongase and desaturase enzymes (Sinclair, 1984). It 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.

1.4 Natural occurrence, absorption and bioavailability

As well as being formed from alpha-linoleic acid, DHA is a normal constituent of the non-vegan human diet as it is found in high amounts in cold-water fish and in lower quantities in game meats. In the human body, DHA is found in most cell membranes and tissues. The cerebral cortex of the brain, the retina, testes and sperm are particularly rich in DHA (cited in BNF 1999).

DHA is present in food in triacylglycerides and is absorbed, distributed, metabolised and excreted via the normal biochemical pathways for other triglycerides and fatty acids in the human body. Triacylglycerides are broken down in the intestine (by lipolytic enzymes produced mainly in the pancreas) into fatty acids and 2-monoacylglycerols. These form micelles, which facilitates their movement to the intestinal brush border where the micelles are broken down and the lipolytic products can be translocated across the intestinal epithelium (cited in Kaliviankis, 1998).

DHA from foods has been shown to bioavailable. Although no studies have been done with DHA-rich oil from *Ulkenia* sp, the bioavailability of DHA from other sources has been the subject of a number of studies. 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, 1996; 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 *Ulkenia* 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. *Schizochytrium* and *Chrypthecodinium cohnii* oil).

Makrides *et al* (1996) demonstrated bioavailability of DHA from oil derived from *Schizochytrium* sp. 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).

1.5 Dietary intake of DHA

The main source of DHA in the diet is cold-water fish (in a range of 0.2-1.1 gm DHA/100 gm fish). DHA intake in the USA in 1994 was reported at 92 mg/day among fish eaters, and 34 mg/day among non-fish eaters (cited in Becker and Kyle, 1998). Greenland Eskimos living on their traditional marine diet ingest 5-10 gm/day of polyunsaturated fatty acids of the n-3 family, mainly as eicosapentaenoic acid (EPA) and DHA (cited in Bonaa *et al.*, 1992). In Europe, the intake of long chain n-3 fatty acids (including DHA) is estimated to be approximately 0.1-0.5 gm/day (Sanders, 2000).

For Australian and New Zealand breast-fed infants, the estimated exposure to DHA based on the DHA levels in breast milk is 1.5 g per day (ANZFA, 2002).

DHA-rich oil and biomass from the marine micro-algae, *Schizochytrium* sp. are permitted novel foods ingredients in the Code. For that safety assessment (A428), a dietary exposure assessment was conducted to estimate the likely dietary exposure of Australians and New Zealanders to *Schizochytrium* derived DHA added to foods at the maximum proposed level of use. When background DHA exposure was taken into account, exposure of the highest 95th percentile consumer group to all sources of DHA was 950 mg per day (ANZFA, 2002).

The Code also has specifications for DHA-rich oil from the algae *Crypthecodinium cohnii*.

A dietary exposure assessment has been conducted for this application and is at Attachment 3.

2. Safety of Source Organism

DHA-rich oil is derived from a fermentation process using micro-alga, *Ulkenia* sp., which has no history of use in food.

Ulkenia sp. is a member of the kingdom Chromista, which includes golden algae, diatoms, yellow-green algae and thraustochytrids but not the toxic blue-green or dinoflagellate micro-algae. *Ulkenia* sp. is a thraustochytrid and was originally found in the Pacific Ocean. Current molecular biological techniques have demonstrated that thraustochytrids are not fungi and they are related to the heterokont algae (Cavalier-Smith *et al*, 1994).

There is no information in the scientific literature that would indicate that *Ulkenia* species (or their class Labyrinthulea) are pathogenic to humans or exhibit any other health risks. Detailed analysis of several batches of DHA-rich oil and of the used *Ulkenia* biomass for algal toxins including amnesic, diarrhetic, paralytic, and neurotoxic shellfish poisoning toxins, cyanobacterial toxins, and substances with haemolytic activity confirmed that neither the oil nor the non-extracted biomass contained any of these toxins (Luckas, 2003 unpublished study).

An acute oral toxicity study in mice and a bacterial mutation assay were performed with *Ulkenia* micro-algae. These studies are detailed below. *Ulkenia* was shown to be non-toxic, non-pathogenic and non-mutagenic under the conditions of the study.

There are no reports of human consumption of *Ulkenia* sp., however, filter feeders (e.g. clams and mussels) feed on *Ulkenia* and are part of the normal diet. Furthermore, the production organism is not present in DHA-rich oil.

2.1 Toxicity studies of *Ulkenia* sp.

Two studies were supplied by the Applicant in support of the safety of the *Ulkenia* micro-algae used as a source of DHA-rich oil. One of these studies was an acute oral toxicity study in mice and the second was a mutation test in bacteria.

Neither of these studies had a statement of GLP nor did they comply fully with the appropriate OECD guideline. However, they were both considered as relevant supporting information.

An acute oral toxicity study of *Ulkenia* micro-algae. Fujii and Suwa, Institute for Fundamental Research Suntory Ltd. *Report No. HGT-96-37. 13 April 1999*.

Test material:	*Ulkenia* micro-algae (no further details were given about method of purification)
Test Species:	Mice (ICR) 5 males per test dose, administration via gavage.
Dose:	0 and 2000 mg/kg bw/day for 14 days.
Guidelines:	None

Study conduct

Mice were given 2000 mg/kg bw/day *Ulkenia* micro-algae by gavage in distilled water. The control group received an equivalent dose of water.

Mice were observed for clinical signs including mortality and moribundity before treatment, 1 and 6 hours following the first dose and then once daily for the following 14 days. Mice were provided with rodent diet *ad libitum.* Body weights were recorded before the first dose then once daily for 14 days. All the mice were sacrificed on day 14 and macroscopically examined.

Histopathological examinations were not performed as no macroscopic abnormalities were observed.

Results

There were no deaths in either the control or test group. Each group showed similar body weight gains and there were no gross necropsy findings.

Conclusions

It was concluded that *Ulkenia* micro-algae had low acute toxicity and the LD_{50} was greater than 2000 mg/kg body weight in male mice under the conditions employed in this study.

Bacterial reverse mutation test with *Ulkenia* micro-algae (Ames test). Fujii and Suwa. Institute for Fundamental Research Suntory Ltd, *Report No. HMS-96-14, 13 April 1999.*

Test material:	*Ulkenia* micro-algae (no further details were given about method of purification)
Test object:	*Salmonella typhimurium* strains TA97, TA98, TA100 and TA102
Dose:	0, 500, 1250, 2500, 3750, and 5000 µg/plate
Guidelines:	none

Study conduct

The mutagenicity *Ulkenia* sp. was examined using Ames/*Salmonella* test strains TA97, TA98, TA100, and TA102 in the presence or absence of a metabolic activation system (S-9 mix) at a concentration of 0, 500, 1250, 2500 and 5000 µg/plate in experiments.

Two replicate plates only were prepared for each strain/S-9/dose level along with positive and negative controls. Plates were examined after 2 days at 37^{o} C.

Results

In the absence and presence of the S9 activation and in all strains, *Ulkenia* micro-algae did not cause a two-fold or greater increase in the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative controls. Nor was there any evidence of a dose-response relationship. The positive controls gave the expected increase in the mean number of revertant colonies.

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse mutation (*In vitro*)	*Ulkenia* micro-algae	0, 1250, 3500 and 5000 µg/plate, with and without S9 mix	*Salmonella strains* TA97, TA98, TA100 and TA102	-ve

Conclusions

The results indicate that the test substance is not mutagenic under the conditions of this study, towards any of the *S. typhimurium* strains used in the in the presence or absence of a metabolic activation system (S-9 mix).

3. Review of available safety studies on DHA-rich oil

3.1 Animal studies

3.1.1 Acute toxicity

An acute oral toxicity study of DHA-rich oil in rats (Limit test). Neda K. Nippon Experimental Medical Research Institute Co. Ltd. *Study number H-99141. December 15 1999.*

Test material:	DHA-rich oil (from *Ulkenia* sp.) DHA content 46.3% > 98% triacylglycerols, colourless to pale yellow, fluid.
Test Species:	Rats (Sprague-Dawley, Crj/CD (SD)IGS) 5 males and 5 females per test dose, administration via gavage.
Dose:	Single acute doses at 2000 mg/kg bw.
GLP:	OECD GLP
Guidelines:	OECD guideline 401

Study conduct

Rats were given a single dose of 2000 mg/kg body weight DHA-rich oil by gavage. The control group received an equivalent dose of water. Rats were observed for clinical signs including mortality and moribundity before treatment, at 15 and 30 minutes, 1, 3, and 6 hours post dosing and then once daily for the following 14 days. Rats were provided with rodent diet *ad libitum* except for overnight fasting prior to dosing and for 3 hours post dosing. Body weights were recorded on days 0, 1, 2, 3, 5, 7, 10 and 14. Animals were sacrificed on day 14 and the body surface, and tissues/organs in the cranium, thorax, and abdomen were macroscopically examined.

Histopathological examinations were not performed as no macroscopic abnormalities were observed.

Results

There were no deaths in either the control or test group. Watery diarrhoea was observed at 6 hours post-treatment in 2 males of the test group. This finding was considered to have no toxicological significance; rather is attributable to the lipid characteristic and the large volume orally given leading the test substance to be excreted without absorption. No changes were observed from the next day of treatment to the end of the observation period. No changes were observed in any of the other male rats or the female rats. Each group showed similar body weight gains and there were no gross necropsy findings.

Conclusions

It was concluded that DHA-rich oil has low acute toxicity, with the approximate LD_{50} greater than 2000 mg/kg body weight in male and female rats under the conditions employed in this study.

3.1.2 Sub-chronic toxicity

A 90-day repeated oral dose toxicity study of DHA-rich oil in rats. Neda K. Nippon Experimental Medical Research Institute Co Ltd. *Project Number H-99142. 4 September 2000.*

Test material:	DHA-rich oil from *Ulkenia* sp. (DHA-rich oil containing 45% DHA).
Reference material	DHA-27 (fish oil containing 27% DHA)
Control material	Distilled Water

Test Species:	Sprague-Dawley Crj;CD(SD)IGS rats. 15/sex/dose. A recovery period of 4 weeks was set for an additional 5 animals/sex/dose in the control, reference and highest dose groups
Dose:	0/0 (control), 0/2000 (reference), 500/1500, 1000/1000, and 2000/0 mg/kg bw/day of DHA-rich oil/DHA-27
Exposure:	90 days (by gavage)
GLP:	OECD 1997
Guidelines:	OECD 408

Test article and control material

Analyses confirmed the DHA content of the DHA-rich oil and the DHA-27 oil at the beginning and end of the treatment period.

Study conduct

The study consisted of five groups of rats receiving a combination of DHA-rich oil and DHA27 (fish oil) to equalise the lipid content across the groups or water as a control.

Table 1: Dosage of DHA-rich oil, DHA27 (fish oil), and total DHA dose administered in the 90-day sub chronic toxicity study

Group Number	DHA-rich oil (mg/kg bw/day)	DHA-27 (mg/kg bw/day)	Total dose of DHA (mg/kg bw/day)[1]
Control	0	0	0
1	0	2000	540
2	500	1500	630
3	1000	1000	720
4	2000	0	900

[1] The total dose of DHA from both DHA-rich oil (45% DHA) and DHA27 (27% DHA)

Animals were dosed from 6 weeks of age. Clinical observations were recorded daily and bodyweight, food and water intake, and neurological observations were recorded weekly. Functional tests (reaction to stimuli, grip strength, and spontaneous movement) were performed on 10 rats of each sex in each group in week 13. Eye examination was performed once before treatment, in week 13 and in week 4 of recovery. Haematology and urinalysis were performed at the end of the treatment period and at the end of the recovery period.

At the end of the treatment and recovery period the animals scheduled for necropsy were killed. The brain, pituitary gland, thyroid (including parathyroid), thymus, salivary gland (submandibular and sublingual glands), heart, lung, liver, spleen, kidneys, adrenals, testes, epididymis, prostate, seminal vesicle, ovary, and uterus were removed and weighed. In addition to the organs being weighed, histopathology was performed on target organs and on any lesions observed macroscopically.

Results

No deaths occurred in any groups of either sex throughout the treatment and recovery period. Statistically significant observations are recorded in Table 2.

There was some increase in body weights in the male and female rats in Groups 1-4 compared to the control group. This change tended to be slightly greater for the DHA-27 group than the DHA-rich oil group but the difference was not statistically significant (increased body weight in comparison with the control on day 89: 2.3% for males and 6.5% for females in the 2000 mg DHA-rich oil/kg bw group; 7.4% for males and 9.5% for females in the 2000 mg DHA-27 group). The increase in body weight was less than 10% and was not considered to have any adverse effects on the organism.

At the end of the treatment period, a statistically significantly lower or a tendency to lower total cholesterol, phospholipids and free fatty acid levels were recorded for males and females in each group compared to the control group.

Increased liver weight (absolute and relative) was reported in females of all groups compared to the water control. However, the increase was greatest in Group 1. Also, when Group 4 females were compared to Group 1 females, liver weights were significantly lower in Group 4 females. In the males, there were no significant differences in absolute liver weight between the control, Group 1, and Groups 2 – 4. No significant differences were found between the relative liver weights of the control group and the treatment groups, however, Groups 3 and 4 had significantly lower relative liver weight compared to Group 1.

Table 2: Summary of results of sub-chronic toxicity study

Dose (mg DHA-rich oil/kg bw)	500 (Group 2)		1000 (Group 3)		2000 (Group 4)		DR
	m	f	m	f	m	f	
Mortality	No treatment related findings						
Clinical signs	No treatment related findings						
Body weight	No treatment related findings						
Food consumption	No treatment related findings						
Water intake	No treatment related findings						
Ophthalmoscopy	No treatment related findings						
Neurobehavioral & functional tests	No treatment related findings						
Urinalysis[1] - sodium excretion -potassium excretion			 dc dc		 dc dc		
Haematology - lymphocyte ratio - segmented neutrophil ratio - RBC count				 dc	 ic dc		
Clinical chemistry - total cholesterol /phospholipid[2] - free fatty acids - urea nitrogen - alkaline phosphatase (ALP) [2] - A/G ratio[2] - albumin fraction ratio[2] - α-2-globulin fraction ratio[2] - β-globulin fraction ratio[2] - total bilirubin	 dc dc ic ic ic dc dc 	 dc dc dc	 dc dc ic ic ic	 dc dc dc	 dc ic dc 	 dc 	
Organ weights - liver - heart[2] - lung[2] - salivary gland[2] - spleen[2] - kidneys - right adrenal[2]	 dc[r] dc[r] 	 ic[a,r] ic[a] ic[a] ic[a]	 	 in[a,r] ic[a] ic[a] ic[a] 	 	 ic[a,r] ic[a] ic[a] ic[a] ic[a]	
Pathology - Macroscopy - Microscopy	No treatment related findings No treatment related findings						

DR = dose related
dc/ic = statistically significantly decreased/increased compared to the controls
m/f = male/female
[1] No relevant significant differences were found in urinalysis parameters when compared to the reference control group DHA-27, except for the urine volume and total excretion value of potassium in the recovery period of females in the 2000 mg/kg bw/day group
[2] The reference control group DHA-27 showed similar effects.
a/r absolute/relative

Conclusion

The administration of both DHA-27 and DHA-rich oil resulted in a number of similar changes when compared with the control group. The observed decreases in cholesterol, phospholipids, and free fatty acids in the DHA-27 and DHA-rich oil groups are treatment related and can be ascribed to the lowering effects of polyunsaturated fatty acids on blood lipids (Harris, 1989), but may also be partly due to the administration of additional fat in comparison to the control group (see Hempenius *et al*., 2000).

The increased or a tendency to increased absolute and relative liver weights was not accompanied by histopathological effects which would indicate liver toxicity, and were instead attributed to the high lipid intake. All other changes in organ weights were considered to be of no toxicological significance either because they were slight, inconsistent and not accompanied by changes in relative organ weight or histopathology and/or not dose related.

On the basis of this study, there was no evidence of adverse effects in rats following administration of DHA rich oil at 2000 mg/kg bw/day (equivalent to 900 mg/kg bw/day of DHA).

2.1.3 Reproduction study

An oral one generation reproduction study with DHA-rich oil in rats. Kuilman and Waalkens-Berendsen. TNO Nutrition and Food Research. *Project No. 41089. 22 January 2001.*

Test material:	DHA-rich oil from *Ulkenia* micro-algae (44% DHA)
Control material:	Corn oil (7.5% w/w in control group)
Test Species:	Wistar outbred (Crl:(WI)WU BR) rats, four groups of 28 male rats and 28 female rats.
Dose:	0 (control), 1.5, 3.0, 7.5% (w/w) in diet.
GLP:	OECD
Guidelines:	OECD 415

Test article and control material

Analysis confirmed the stability, homogeneity, and content of the test substance in the diet. Corn oil was added to the diet of the control group at 75000 mg/kg (7.5% w/w).

Study conduct

Groups of 28 male and 28 female Wistar rats were treated with DHA-rich oil in the diet at 0, 1.5, 3.0 or 7.5% (w/w).

F_0 males were treated for 10 weeks before mating and throughout mating. Shortly after mating the males were sacrificed. F_0 females were treated for 10 weeks before mating and throughout gestation and lactation to post-natal day 21. F_0 females were then sacrificed. Pups were weaned and sacrificed on post-natal day 21 after being checked for overt signs of ill-health and abnormalities.

A clinical examination was performed daily, food consumption was calculated weekly (except during pairing) and bodyweights measured weekly (pre-mating, gestation and lactation for females). Water consumption was not measured. Reproductive parameters measured included:

- pre-coital time, mating performance, male and female fertility, gestation length, parturition and gestation index, number of females with stillborn pups and post implantation loss for F_0 parents;
- litter size, offspring weights, offspring viability indices, sex ration and physical development were assessed for F_1 generation.

At necropsy, samples of the following tissues and organs of all parent animals were preserved; ovaries, uterus (with cervix), vagina, testes, epididymides, seminal vesicles with coagulating glands, prostate, pituitary, liver, spleen, and organs and tissues showing macroscopic abnormalities. The following organs were also weighed; ovaries, uterus (with cervix), testes, epididymides, seminal vesicles with coagulating glands, prostate, pituitary, liver and spleen.

Results
Daily clinical observations of the animals during the premating, mating, gestational and lactation periods did not reveal remarkable findings in the animals' appearance, general condition or behaviour that could be related to DHA-rich oil treatment. No mortalities occurred. The test substance intake is shown in Table 3.

Table 3: Test substance intake range (g/kg body weight/day)

	Low dose group 1.5% DHA-rich oil (15 000 mg/kg feed)	Mid dose group 3.0% DHA-rich oil (30 000 mg/kg feed)	High dose group 7.5% DHA-rich oil (75 000 mg/kg feed)
Pre-mating males	0.8 – 1.0	1.5 – 2.0	3.4 – 4.7
Pre-mating females	0.9 – 1.1	1.7 – 2.2	4.0 – 5.2
Gestation	1.8 – 2.2	3.4 – 4.3	7.9 –9.7
Lactation[1]	1.8 – 2.7	3.7 – 5.3	7.8 – 11.2

[1] Post-natal day 1 – 14, after post-natal day 14 the pups start eating.

Some statistically significant differences were observed in body weight, body weight gain, food consumption, organ weights and pathology results of the F_0 males and females. In the pups, statistically significant differences were observed for some clinical signs, body weigh and body weight gains. All the statistically significant findings are summarised in Table 4.

No effects of DHA-rich oil were observed on any reproductive variables examined.

An increase in spleen weights of both male (low, mid, and high dose) and female (high dose) was observed. In addition, microscopic examination revealed increased extramedullury haematopoiesis in the spleen of the treated animals. Furthermore, yellow discolouration of abdominal adipose tissue was observed macroscopically in some males and the majority of females in the high dose group. Microscopically, lipogranulomata were observed in all DHA-rich oil treated males and in females of the mid and high dose groups.

The other macroscopic and microscopic observations did not reveal treatment related changes and were found only in one or a few animals and are common findings in rats of this ages and strain.

Table 4: Summary of results of reproductive toxicity study

Dose (g/kg food)	**15**		**30**		**75**		**dr**
	Male	Female	Male	Female	Male	Female	
F_0 animals							
Mortality	None						
Clinical signs	No treatment-related findings						
Body weight		ic[1]		ic[1]	ic	ic	
Body weight gain		ic[2]		ic[2]	ic[3]	ic	
Food consumption	ic	ic	ic	ic		dc[4]	
Mating/fertility/gestation	No treatment-related findings						
Organ weight - liver - spleen - pituitary - seminal vesicles[5]	ic[r]	ic[a] dc[r]	ic[a,r] ic[a,r]	ic[a]	ic[a] ic[a,r] dc[r]	ic[a] ic[a,r] dc[r]	m,f
Pathology Macroscopy *Abdominal cavity* - yellow spots in adipose tissue Microscopy *Abdominal cavity* - lipogranulomas[6] *Spleen* - increased extramedullary haematopoiesis[6]	++ ++		++ ++	++	+ ++ ++	++ ++	m,f m
F_1 pups							
Litter size	No treatment-related findings						
Survival index	No treatment-related findings						
Sex ratio	No treatment-related findings						
Clinical signs - increased no. of cold pups - increased no. of pups with no milk in stomach - increased no. of sparsely haired pups			+[8]	+[8]	+[7] +[7]	+[7] +[7]	
Body weight	dc[8]	dc[8]			dc[9]	dc[9]	
Body weight gain	dc[9]	dc[9]	dc[10]	dc[10]	dc[10]	dc[10]	
Pathology Macroscopy	No treatment-related findings						

dr dose related
dc/ic statistically significantly decreased/increased compared to the control
m/f male/female
a/r absolute/relative
+/++ present in one/a few animals / present in most/all animals
1 on day 21 of gestation and days 7-21 of lactation
2 on day 14-21 of gestation
3 statistically significant on day 7-28 only
4 on the first week of the premating period, gestation days 14-21, and lactation days 7-21
5 statistically significant at premating only
6 increased incidences and severity. All control animals showed very slight extramedullary haematopoiesis
7 on postnatal day 1
8 on postnatal day 21
9 between postnatal days 14-21 for males and females
10 between postnatal days 7-14 for males and females

Discussion and conclusion
The effects seen on body weight were not dose related and were incidental in time, except for the effects in the highest group. The effects on food consumption were inconsistent and not dose related. Except for the increased spleen weight (both relative and absolute), the changes organ weights were not dose related, not accompanied by microscopic changes, and were mostly considered to be fortuitous findings.

The yellow spots (lipogranulomas) in the abdominal cavity fat tissue were considered to be associated with 'yellow fat disease' or steatitis, a nutritional imbalance known to occur in certain species of animals consuming diets rich in n-3 poly unsaturated fatty acids and which are deficient in vitamin E. The presence of lipogranulomas is indicative of ongoing inflammatory responses and may have been associated with the increased weight and extramedullary haematopoiesis of the spleen since this is a common response to inflammation. Steatitis is a recognised consequence of peroxidation of administered lipid and appears naturally and experimentally in many different species fed diets that are high in unsaturated fatty acids, including DHA and DHA-containing fish oils and are relatively low in antioxidants, particularly vitamin E and selenium.

This condition of nutritional imbalance has never been reported in humans and the dose levels used in this study greatly exceed any anticipated human intake of the test substance. Therefore effects observed in adipose tissue and spleen are not considered to be related to the source of the test substance.

The clinical signs observed in the pups are normal for pups of this age, and were only significant on pup basis and not on litter basis. The lower body weight of the pups in the highest dose group most likely results from lower food consumption by the maternal animals due to low palatability of the food. No reproductive and developmental effects were observed.

From the data in the study it can be concluded that dietary administration of DHA-rich oil up to concentrations of 7.5% (w/w) for in one generation of rats had no effect on the reproductive parameters or on the development of the pups.

3.2 Mutagenicity studies

Bacterial reverse mutation test with DHA-oil 45. Bruijntjes-Rozier T. and van Ommen B. TNO Nutrition and Food Research, *Report No.V2505/08, 3 April 2001.*

Test material:	DHA-oil 45 from *Ulkenia* sp.
Test object:	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* strain WP2 *uvrA*
Dose:	0, 62, 185, 556, 1667, and 5000 µg/plate
Guidelines:	OECD guideline 471 (1997) and GLP

Study conduct

The mutagenicity of oil derived from *Ulkenia* sp. was examined using *Salmonella* strains TA1535, TA1537, TA98, TA100, and *E. coli* strains WP2 *uvr* in the presence or absence of an Aroclor 1254-induced rat metabolic activation system (S-9 mix) at a concentration of 0, 62, 185, 556, 1667, and 5000 µg/plate in experiments.

Toxicity was not expected, therefore a preliminary test to assess the toxicity was not performed, rather this was incorporated into the first mutagenicity assay. Three replicate plates were prepared for each strain/S-9/dose level along with positive and negative controls. Plates were examined after 3 days at 37^{o} C.

Results

In the absence and presence of the S9 activation and in all strains, DHA-oil 45 did not cause a two-fold or greater increase in the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative controls. Nor was there any evidence of a dose-response relationship. The mean number of his^{+} and trp^{+} revertant colonies on the negative control plates were within the acceptable range and the positive controls gave the expected increase in the mean number of revertant colonies.

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse mutation (*In vitro*)	DHA-rich oil	0, 62, 185, 556, 1667, and 5000 µg/plate, with and without S9 mix	*Salmonella strains* TA1535, TA1537, TA98, TA100, and *E. coli* WP2 *uvrA*	-ve

Conclusions

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

Bacterial reverse mutation test with DHA-oil 45. Fuji and Suwa. Institute for Fundamental Research Suntory Ltd, *12 October 1998.*

Test material:	DHA-oil 45 from *Ulkenia* sp.
Test object:	*Salmonella typhimurium* strains TA97, TA98, TA100, and TA102
Dose:	0, 500, 1250, 2500, 3750, and 5000 µg/plate
Guidelines:	OECD guideline 471 (not in full compliance), no GLP

Study conduct

The mutagenicity of oil derived from *Ulkenia* sp. was examined using *Salmonella* test strains in the presence or absence of rat liver microsomal fraction (S-9 mix). The study was not performed in full compliance with OECD guideline 471, but was considered acceptable as a supplementary study.

Results

In the absence and presence of the S9 activation and in all strains, DHA-oil 45 did not cause a marked increase in the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative controls.

Nor was there any evidence of a dose-response relationship. The mean number of revertant colonies on the negative control plates were within the acceptable range and the positive controls gave the expected increase in the mean number of revertant colonies.

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse mutation (*In vitro*)	DHA-rich oil	0, 500, 1250, 2500, 3750, and 5000 µg/plate, with and without S9 mix	*Salmonella strains* TA97, TA98, TA100, and TA102	-ve

Conclusions

The results indicate that the test substance is not mutagenic under the conditions of this study.

Chromosome aberration test of DHA-rich oil in cultured Chinese hamster cells. Sarwar, M. Nippon Experimental Medical Research Institute Co. Ltd. *Project No.H-99144, 13 March 2000.*

Test material:	DHA-rich oil from *Ulkenia* sp.
Negative control	1% Carboxymethyl cellulose sodium salt
Positive control	Mitomycin C at 0.001 mg/mL and benzo (a) pyrene at 0.02 mg/mL
Test object:	Cultured Chinese hamster cells (CHL/IU, a fibroblast cell line)
Dose:	1.25, 2.5 and 5 mg/mL
Guidelines:	OECD Guideline 473, GLP

Study conduct

The potential for DHA-rich oil to induce chromosome aberrations in mammalian cells was tested in cultured Chinese hamster cells (CHL/IU) with and without metabolic activation. Selected dose levels were based on a range finding study using doses of 0.007, 0.021, 0.062, 0.185, 0.556, 1.667, and 5 mg/mL. Cells were incubated with DHA-rich oil for 6 hours (with and without S9 activation) or for 24 and 48 hours (without S9 activation). However, none of the tested doses reduced the cell growth to 50%. Cell growth at 5 mg DHA-rich oil/mL was 69-64% after short treatment and 78-82% of the controls after continuous treatment. Therefore, the highest dose (5 mg/mL) was chosen and the lower doses of 2.5 and 1.25 mg/mL at a nominal ratio of 2 were selected and used for the chromosome aberration test.

Cultured cells were treated with DHA-rich oil at the designated doses with and without S9 activation for 6 hours followed by 18 hours expression time. Continuous treatment was also carried out for each dose without S9 activation for 24 and 48 hours. A confirmation experiment was also done with S9 activation, a treatment time of 6 hours in combination with an expression time of 24 hours.

Results

Test	*Test material*	*Concentration*	*Test object*	*Result*
Chromosomal aberration test (*In vitro*)	DHA-rich oil	0, 1250, 2500, and 5000 mg/ mL 6 hours with and without S9 24 and 48 hours without S9	Chinese hamster cells	-ve

The incidence of DHA-rich oil-treated cells with structural or numerical chromosomal aberrations was similar to that of the negative controls. Due to the negative result, a confirmation test was conducted for 6 hours in the presence of S9 mix with an expression period of 24 hours. This test result was also negative.

The incidence of cells with structural chromosomal aberrations (gap, break and exchange) was significantly increased in the positive controls and both the negative and positive controls were within the range of historical data.

Conclusion

DHA-rich oil did not induce chromosomal aberrations in cultured Chinese hamster cells under the conditions of this study.

3.3 Human Studies

3.3.1 Adult intervention studies

As no human studies have been conducted using DHA-rich oil from *Ulkenia* sp., the safety in humans is based on the scientific literature in which the effects of DHA or DHA containing foods are presented. The main component of DHA-rich oil from *Ulkenia* sp. is DHA, and there are a large number of published studies in which the effects of DHA in the human diet have been studied. The parameters examined included blood pressure, plasma triglycerides, plasma free fatty acids, total cholesterol, and LDL and HDL cholesterol.

Some research indicates that consumption of high amounts of long chain n-3 polyunsaturated fatty acids (PUFA) leads to longer bleeding times (NHMRC 1992 cited in ANZFA 2000). The human studies available were conducted primarily for efficacy purposes but there were no reports of adverse effects at doses levels up to 6 g/day for 90 days in adults. Clinical trials using low to moderate doses of fish oil (0.5 g to 2.0 g per day of n-3 PUFA) did not increase bleeding time significantly (Connor, 1994). The USFDA have stated that consumption of up to 3 g/day of EPA plus DHA has been considered to have no effect on bleeding times. Animal studies using up to 2 g DHA-rich oil/kg body weight/day showed no increase in bleeding times (equivalent to 900 mg DHA/kg body weight/day).

In general, the occurrence of adverse events associated with the use of DHA is rare and the events reported are mild. For example, Davidson *et. al.* (1997) reported a few mild effects (e.g. burping, indigestion, sinus congestion) but there were no significant differences in frequency of reported side effects between the placebo and treatment (2.5 g DHA/day for 6 weeks) groups.

Inis and Hansen (1996) reported a significant increase in burping in the treatment groups (0.6 g, 1.7 g and 2.9 g DHA/day for 14 days) compared to the control group, however, the author suggested that this could be reduced or avoided by using deodorised oils at the lowest dose reported in this study (0.6 g DHA). No clinically significant dose-related effects became apparent from physical examination or from routine laboratory tests.

On the whole, no clinically significant adverse effects were observed in adults consuming diets supplemented with DHA (with and without EPA) (Agren *et. al.*, 1996; Conquer and Holub, 1996; Davidson *et. al.*, 1997; Hansen *et. al.*, 1997; Innis and Hansen, 1996; Nelson *et. al.*, 1997a,b; Vanderhoof *et. al.*, 1997; also reviewed by Becker and Kyle, 1998).

No adverse effects were observed in diabetic individuals receiving up to 3 g/day omega-3 fatty acids (including 1.2g/day DHA) (Friedberg *et. al.*, 1998).

3.3.2 Infant development

DHA is present in breast milk, however, the levels vary depending on the mother's diet. Vegan women have lower levels of DHA in their breast milk than women on omnivorous diets, while women who eat fish regularly have the highest levels of DHA in their milk (Makrides and Gibson, 2000).

Studies have shown benefits to preterm infants fed with formula supplemented with both DHA and an n-6 long chain fatty acid, arachidonic acid (AA) (cited in Makrides and Gibson, 2002). Based on these findings supplementation of preterm infant formulas with both DHA and AA has been recommended (Aggett *et. al.*, 1991; FAO, 1994) and, in Australia, all available preterm formulas are supplemented.

A number of randomised trials of supplementing infant formula with long chain polyunsaturated fatty acids (including DHA) at levels equivalent to those found in breast milk have been undertaken. No trials have consistently shown a negative effect on either growth or developmental indices (Gibson *et. al.*, 2001).

In weaning infants, including n-3-enriched eggs in the diet has been shown to substantially increase the DHA intake of young children without changing their growth rates (Makrides *et. al.*, 2002).

No studies have been done on infants using DHA-rich oil derived from *Ulkenia* sp.

3.3.3 Conclusion

In clinical trials, algal oil rich in DHA from a variety of sources (not including DHA-rich oil derived from *Ulkenia* sp) has been shown to be safe for consumption by infants and adults, and not led to any clinically significant adverse effects even at high doses up to 6 g DHA/day for 90 days in adults (Agren *et. al.*, 1996; Conquer and Holub, 1996; Davidson *et. al.*, 1997; Hansen *et. al.*, 1997; Innis and Hansen, 1996; Nelson *et. al.*, 1997a,b; Vanderhoof *et. al.*, 1997; also reviewed by Becker and Kyle, 1998). The FDA (2000) stated that the consumption of EPA and DHA omega-3 fatty acids is safe, provided that combined daily intakes for EPA and DHA does not exceeded 3 g/person/day from both conventional foods and dietary supplements.

4. Overall Conclusion

The safety assessment of DHA-rich oil from *Ulkenia* sp. concluded that:

- Although the source organism does not have a history of safe use in food, information from the scientific literature, toxicity studies, and the pathogenicity study indicates that *Ulkenia* sp. is non-pathogenic;
- Acute toxicity studies in rats indicate that the LD_{50} for DHA-rich oil is more than 2000 mg/kg bw in rats;
- There was no evidence of toxicity in the sub-chronic toxicity study in rats fed DHA-rich oil for 90 days at the highest dose tested (2000 mg/kg bw/day). This is the equivalent of 900 mg/kg bw/day of DHA;
- A reproductive study showed no DHA-rich oil related adverse effects on reproductive parameters;
- The DHA-rich oil preparation produced no evidence of genotoxic potential in *in vitro* assays; and
- There is no evidence of adverse effects in humans from the consumption of DHA from other sources (such as fish oil or other micro-algae) at low to moderate dose levels.

References

Aggett PJ, Haschke F, Heine W, Hernell O, Koletzko B, Launiala K, Rey J, Rubino A, Schoch, Senterre J, *et. al*. (1991) Comment on the content and composition of lipids in infant formulas. Acta Paediatr Scand; 80: 887-896.

Agren JJ, Hanninen O, Julkenen A, Fogelholm L, Maliranta H, Schwab U, Pynnonen O, Uusitupa m (1996) Fish diet, fish oil and docosahexaenoic acid triglyceride (DHASCO oil). Food and chemical toxicology 38: 35-49.

ANZFA (2002) Final Assessment Report, Application A428 – DHA-rich dried marine micro-algae (Schizochytrium sp.) and DHA-rich oil derived from *Schizochytrium* sp. as novel food ingredients. http://www.foodstandards.gov.au/standardsdevelopment/applications/applicationa428marinemicroalgaeasanovelfood/index.cfm

Becker CC and Kyle DJ (1998) Developing functional foods containing algal docosahexaenoic acid. Food Technology, *52*: 68-71.

BNF (1999) Briefing paper: n-3 Fatty Acids and Health. The British Nutrition Foundation, July 1999.

Bonaa KH, Bjerve KS, Nordoy A (1992) Habitual fish consumption, plasma phospholipids fatty acids, and serum lipids: the Tromso Study. Am J Clin Nutr 55(6): 1126-1134.

Carneielli VP, Verlato G, Pederzini F, Luijendijk I, Boerlage A, Pedrotti D, Sauer PJ (1998) Intestinal absorption of long-chain polyunsaturated fatty acids in pre-term infants fed breast milk or formula. Am. J. Clin. Nutr., *67(1)*, 97-103.

Cavalier-Smith T, Allsop MTEP, Chao EE (1994) Thraustochytrids are chromists, not Fungi: 18s rRNA signatures of Heterokonta. Phil. Trans R. Soc. Lond. B.:346, 387-397

Connor WE (1994) Omega-3 fatty acids and heart disease. *Kritchevsky D. Carrol KK eds. Nutrition and Disease Update: Heart Disease. Champaign, Il: AOCS Press. pp1-42.*

Conquer JA and Holub BJ (1996) Supplementation with an algal source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. J Nutr 126: 3032-3039.

Croset M, Brossard N, Pachiaudi C, Normand S, Lecerf J, Chirouze V, Riou JP, Tayot JL, Lagarde M (1996) In Vivo compartmental metabolism of 13C docosahexaenoic acid studied in gas chromatography combustion isotope ratio mass spectrometry. Lipids, *31*, 109-115.

Davidson MH, Maki KC, Kalkowski J, Schaefer EJ, Torri SA, Drennan KB (1997) Effects of docosahexaenoic acid on serum lipoproteins in patients with combined hyperlipidemia: A randomised, double-blind, placebo-controlled trial. J Am college Nutr 16(3): 236-243.

Food and Agricultural Organization (FAO) (1994) Fats and oils in human nutrition. FAO Food and Nutrition Paper 57. Rome: World Health Organization.

Friedberg CE, Janssen MJ, Heine RJ, Grobbee DE (1998) Fish oil and glycemic control in diabetes. A meta-analysis. Diabetes Care 21(4): 494-500.

Gibson RA, Chen W, Makrides M. (2001) Randomized trials with polyunsaturated fatty acid interventions in preterm and term infants: functional and clinical outcomes. Lipids; 36: 873-883.

Hansen J, Schade D, Harris C, Merkel K, Adamkin D, Hall R, Lim M, Moya F, Stevens D, Twist P (1997) Docosahexaenoic acid plus arachidonic acid enhance preterm infant growth. Prostaglandins Leukotrienes Essential Fatty Acids 57(2): 196.

Harris WS (1997) N-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 65(5 Suppl): 1645S-1654S.

Hempenius RA, Lina BAR and Haggitt RC (2000) Evaluation of a subchronic (13-week)oral toxicity study, preceeded by an in utero phase, with arachidonic acid derived from Mortierella alpine in rats. Food and chemical toxicology, 38, 127-139.

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

Jensen, R.G. (1996). The lipids in human milk", Prog Lipid Res, *3,* 53.

Kalivianakis M (1998) Mechanisms involved in malabsorption of dietary lipids. Thesis.

Luckas B (2003). Scientific expert opinion concerning the possible occurrence of algal toxins in microalgal oil (supplied as DHA45-oil) and dry algal biomass, September 2003. Unpublished report.

Makrides M, Neumann MA and Gibson RA (1996) Effect of maternal DHA supplementation on breast milk composition. Eur. J. Nutr., 50(6), 352-357

Makrides M and Gibson RA (2000) Long chain polyunsaturated fatty acid (LCPUFA) requirements during pregnancy and lactation. Am J Clin Nutr; 71: 307S-311S.

Makrides M and Gibson RA (2002) *The role of fats in the lifecycle stages; Pregnancy and the first year of life.* Medical Journal of Australia; 176 (11 Suppl): S111-S112

Makrides M, Hawkes JS, Neumann MA and Gibson RA (2002). Nutritional effect of including egg yolk in the weaning diet of breast-fed and formula-fed infants: a randomized controlled trial. Am J Clin Nutr. Jun;75(6):1084-92.

Nelson GJ, Schmidt PS, Bartolini GL, Kelley DS and Kyle D (1997a) The effect of dietary docosahexaenoic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. Lipids 32(11): 1129-1136.

Nelson GJ, Schmidt PS, Bartolini GL, Kelley DS and Kyle D (1997b) The effect of dietary docosahexaenoic acid on plasma lipoproteins and tissue fatty acid composition in humans. Lipids 32(11):1137-1146.

NHMRC Working Party (1992) The role of polyunsaturated fats in the Australian diet. Australian Government Publishing Service, Canberra.

Sanders TA (2000) Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 71(Suppl): 176S-178S.

Sinclair HM (1984) Essential fatty acids in perspective. Hum Nutr Clin Nutr. *38,* 245-60.

Vanderhoof J, Gross S, Hegyi T, Clandinin T, Porcelli P, DeCristofaro J, Rhodes T, Tsang R, Shattuck K, Cowett R, Adamkin K, McCarton C, Heird W, Hook B, Pereira G, Pramuk K, and Euler A (1997) A new arachidonic acid (ARA) and docosahexaenoic acid (DHA) supplemented preterm formula: Effect on growth and safety assessment. Ped Res 41(4):242A

Attachment 3

Dietary Intake Assessment Report

An Application was received by FSANZ requesting the *Australia New Zealand Food Standards Code* (the Code) be amended to allow the use of DHA-rich oil derived from the marine micro-algae *Ulkenia* sp. (hereafter referred to as DHA-rich oil (*Ulkenia* sp.)) as a novel food ingredient, under Standard 1.5.1 – Novel Foods – for use in a variety of food products.

Summary

A dietary intake assessment was undertaken to determine the impact of allowing DHA-rich oil (*Ulkenia* sp.) to be added to a variety of foods. The oil extracted from the *Ulkenia* sp. is rich in long chain polyunsaturated fatty acids, particularly the omega-3 fatty acid docosahexaenoic acid (DHA). The assessment took into account naturally occurring levels of DHA in foods (the 'naturally occurring' scenario – Scenario 1). The requested uses of DHA-rich oil (*Ulkenia* sp.) from the current application were also considered in a separate scenario (the 'A522' scenario – Scenario 2). Under Standard 1.5.1 there is an existing permission to add DHA derived from *Schizochytrium* sp. to various foods. Consequently, naturally occurring DHA concentrations were considered in conjunction with the current and proposed permissions for DHA addition from either micro-algae sp (the 'naturally occurring plus micro algal DHA' scenario – Scenario 3).

Dietary intakes of DHA were calculated for the Australian and New Zealand populations, and for the population sub-groups of infants aged 9 months, children aged 2-6 years and females aged 16-44 years. An intake assessment was conducted on the population group of children aged 2-6 years because children generally have higher intakes due to their smaller body weight and because they consume more food per kilogram of body weight compared to adults. An intake assessment was also conducted for women of childbearing age (assumed to be females aged 16-44 years) as it is possible that women in this age group may be more likely than other population sub-groups to consume foods fortified with DHA due to its possible link to foetal brain development. As there are no data available from either of the NNSs for children under two years of age, a diet was constructed to estimate dietary intake of DHA for infants of 9 months of age. This was thought necessary due to the proposed addition of DHA to infant formula and follow on formula, infant cereal products, infant foods and infant drinks. The methodology used for the construction of the infant diet was the same as that used for the Australian Total Diet Survey (ATDS) (FSANZ, 2003).

When naturally occurring and all proposed food sources of added DHA were considered (Scenario 3), the estimated mean dietary intakes of DHA were lowest for Australian infants aged 9 months (310 mg/day) and highest for the whole New Zealand population aged 15 years and above (498 mg/day). The estimated 95th percentile dietary intakes were lowest for Australian children aged 2-6 years (785 mg/day) and highest for the whole New Zealand population aged 15 years and above (1 150 mg/day). The major contributors to intake of DHA were breads and related products, oil emulsions and liquid milk for all population groups assessed, except infants where infant formula and follow on formula and bread and related products were the major contributors.

Background

DHA is a component of the oil derived from the micro-algae *Ulkenia* sp. The DHA content of the micro algal oil is typically 45%. It is intended that DHA-oil can be added to food as an ingredient with the aim of increasing consumer intake of omega-3 polyunsaturated fatty acids (PUFA).

The Applicant has indicated that two levels of addition of DHA-rich oil (*Ulkenia* sp.) are proposed: either 30 mg or 60 mg of DHA per serve (as provided by the Applicant) of nominated food, depending on the food category.

It is proposed that DHA-rich oil (*Ulkenia* sp.) be added to the foods identified in Table 1 at the concentrations listed.

Table 1: Proposed uses of DHA-rich oil (Ulkenia sp.) in foods, as provided by the Applicant

Food Name	DHA Concentration (mg/serve)	Serve Size (g)
Regular breads and rolls	60	36
Breakfast cereals (plain, single & mixed source)	60	60
Savoury Biscuits	60	35
Cake	60	30
Sweet biscuits and cookies	60	35
Polyunsaturated, monounsaturated table margarines & spreads, reduced fat margarines and other spreads	30	10
Modified milk (different types)	30	250 ml
Fruit and vegetable drinks	30	250 ml
Non-carbonated water based beverages	30	250 ml
Sour cream based dips	30	15
Cream cheese based products	30	10
Yoghurt products	60	200 ml
Salad dressings/mayonnaise	30	15
Meal replacement bars	60	150
Meal replacements drinks	60	250 ml
Infant formula & follow-on formula (Food Code NNS 311)	60	230
Infant cereal products (Food Code NNS 312)	60	25
Infant foods (Food Code NNS 313)	60	75
Infant drinks (Food Code NNS 314)	60	125 ml

DHA is an 'essential' fatty acid (EFA) because it cannot be made by the body and therefore needs to be provided in the diet. DHA is naturally occurring in the food supply, the main source of which is cold-water fish. DHA-rich oil derived from dried marine micro-algae (*Schizochytrium* sp.) (Application A428) has previously been approved by FSANZ (then ANZFA) for use in various food products.

Dietary intake assessment provided by the Applicant

The Applicant did not provide new data relating to dietary intakes of DHA in Australia or New Zealand. Instead, the Applicant referred to a previous dietary intake assessment conducted by FSANZ (Application A428), which showed that the highest DHA intake at the mean level would occur in Australian children 13-18 years, and in New Zealand adults (19 years and above). High consumers (95^{th} percentile) had intakes of micro-algae derived DHA between 400 mg/day (Australia 2-6 years) and 750 mg/day (New Zealanders 15-18 years).

When naturally occurring DHA was considered, as well as derived DHA, the population with the highest potential DHA intake was Australian adults aged 19 years and above (950 mg/day).

The dietary intake assessment provided by the Applicant was not sufficiently detailed to draw conclusions about the projected intake of DHA should approval for the requested permissions be given. FSANZ therefore conducted a dietary intake assessment to estimate potential intake of DHA from naturally occurring and derived sources if the proposed permissions are approved.

Dietary modelling

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

Dietary intake = food chemical concentration x food consumption

The intake was estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data, with naturally occurring and/or proposed levels of use of DHA in foods.

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 2 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.

Additional food consumption data or other relevant data

No further information was required or identified for the purpose of refining the dietary intake estimates for this Application.

Population groups assessed

The products containing added DHA are not targeted towards a specific group(s) of consumers, therefore consumption by the whole population is a possibility. The dietary intake assessment was conducted for both Australian and New Zealand populations. An assessment was conducted for the whole population, as well as for infants 9 months old (Australia only), children 2-6 years (Australia only) and females 16-44 years (Australia and New Zealand).

Dietary intake assessments were conducted for the whole population as a proxy for intake over a lifetime. A dietary intake assessment was conducted for infants aged 9 months based on the constructed diet used in the Australian Total Diet Survey (ATDS, 2003). A dietary intake assessment was deemed necessary for this population group due to the proposed addition of DHA-rich oil (*Ulkenia* sp.) to infant formula and follow on formula, infant cereal products, infant foods and infant drinks.

A dietary intake assessment was conducted for children 2-6 years because children generally have higher intakes due to their smaller body weight, and they consume more food per kilogram of body weight compared to adults. It is important to note that, while children aged (2-6 years) have been assessed as a separate group, this group has also been assessed in the whole population's dietary intake assessment. A dietary intake 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 more likely than other population sub-groups to consume foods fortified with DHA due to its possible link to being beneficial for foetal brain development.

DHA concentration levels

The levels of DHA in foods that were used in the dietary intake assessment were derived from the permissions already present in Standard 1.5.1 – Novel Foods – of the Code – 1.5.1 (the Novel Food Standards), those provided in the application, and from Australian (ANZFA, 1998) and New Zealand (Crop and Food Research, 2000) food composition data. The foods and the DHA concentrations assigned to them for dietary modelling purposes are shown below in Table 2 for naturally occurring levels and Table 3 for the levels proposed in the Application.

FSANZ has already approved the use of DHA from the micro algal species *Schizochytrium* in certain foods. The current Application is seeking the approval of DHA from the micro-algae *Ulkenia* sp. in some of the food products already approved to contain DHA derived from *Schizochytrium*. For the purpose of this assessment, it is assumed that these foods contain added DHA at the proposed levels regardless of which species of micro-algae the DHA is derived from.

Concentrations of DHA were assigned to food groups using DIAMOND food classification codes. These codes are based on the Australian New Zealand Food Classification System (ANZFCS) used in Standard 1.3.1 Food Additives (for example 7.1.1 represents regular breads and rolls). The foods proposed by the Applicant to contain DHA (as shown in Table 1) were matched to the most appropriate ANZFSC code(s) for dietary modelling purposes.

Scenarios for dietary modelling

Three different scenarios were used in the assessment of dietary DHA intake:

- Scenario 1 is based on naturally occurring levels of DHA in foods ('naturally occurring' scenario);
- Scenario 2 is based on the proposed addition of DHA-rich oil (*Ulkenia* sp.) to foods ('A522' scenario); and
- Scenario 3 based on naturally occurring levels in food as well as existing and proposed permissions for the addition of DHA (*Schizochytrium* sp. and *Ulkenia* sp.) to foods ('naturally occurring plus micro algal DHA' scenario).

Table 2: Naturally occurring levels of DHA in foods for Australia and New Zealand used for the dietary intake assessment

DIAMOND Food Code	Food	DHA Concentration Level used in modelling (mg/kg)		Source of Data	
		Australia	New Zealand	Australia	New Zealand
4.3.1.4	Dried vegetables	67	67	1	1
6.4.1	Hotplate products	460	460	1	1
7.2.2	Cakes & muffins	43	43	1	1
8.1	Raw meat, poultry & game	510	95	1	2
8.1.1	Fresh poultry	36	123	1	2
8.2	Processed meat, poultry & game products	45	95	1	2
8.3	Processed comminuted meat, poultry & game products	321	95	1	2
9.1	Unprocessed fish & fish fillets	3 478	3 774	1	2
9.1.2	Unprocessed crustacea	300	930	1	2
9.1.3	Unprocessed molluscs	2 233	1768	1	2
9.1.4	Roe	10 500	100	1	2
9.2	Processed fish & fish products	3 575	2 597	1	2
9.2.2	Processed crustacea	425	1 107	1	2
9.2.3	Processed molluscs	1 750	2 890	1	2
9.3	Semi preserved fish & fish products	2 367	11 430	1	2
9.4	Fully preserved fish including canned fish products	7 211	2 351	1	2
10	Eggs and egg products	720	436	1	2

(1) Supplement to NUTTAB95 (ANZFA, 1999); (2) New Zealand fatty acid data (Crop and Food Research, 2000)

Table 3: Proposed uses of DHA in foods and levels of use used in the dietary intake assessment

DIAMOND Food Code	Food	Serve size (g)	Proposed level of DHA per serve (mg/serve)	Concentration Level used in modelling (mg/kg)
1.1.1	Modified milk (different types)	250	30	120
1.2	Yoghurt products	200	60	300
2.2	Oil emulsions (oil in water)	10	30	3 000
6.3	Breakfast cereals (plain, single & mixed source)	60	60	1 000
7.1	Breads and related products	36	60	1 660
7.2.1	Sweet biscuits and cookies	35	60	1 710
7.2.1.1	Savoury Biscuits	35	60	1 710
7.2.2	Cakes & muffins	30	60	2 000
13.1	Infant formula & follow on formula (Food Code NNS 311)	230	60	260
13.2	Infant cereal products (Food Code NNS312)	25	60	2 400
13.2	Infant foods (Food Code NNS 313)	75	60	800
13.2	Infant drinks (Food Code NNS 314)	125	60	480
13.3.1	Meal replacement bars	150	60	400
13.3.2	Meal replacements drinks	250	60	240
14.1.2.2	Fruit and vegetable drinks	250	30	120
14.1.3.4	Cordial only	250	30	120
14.1.3.5	Electrolyte/sports drinks	250	30	120
20.2.1.1	Desserts, dairy only	10	30	3 000
20.2.2	Cereal products (commercial)	60	60	1 000
20.2.4.2	Mayonnaise and salad dressings	15	30	2 000
20.2.8	Fat based dips and other fat based products	10	30	3 000

How were the estimated dietary intakes calculated

1. All population groups, excluding the infants aged 9 months

The DIAMOND program allows DHA concentrations to be assigned to food groups. Each individual's intake of DHA was calculated using his or her individual food records from the dietary survey. The DIAMOND program multiplies the specified concentration of DHA by the amount of food that an individual consumed from that group in order to estimate the DHA intake from each food. Once this 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 and 95th percentile intakes) are then derived from the individuals' ranked intakes.

Food consumption amounts for each individual take into account where each food in a classification code is consumed alone and as an ingredient in mixed foods. For example, milk consumed as a glass of milk, milk consumed with breakfast cereal, and milk used in cakes are all included in the consumption of milk. Where a higher-level food classification code (e.g. 7.2.1 Biscuits) is given a DHA concentration, as well as a sub-category (e.g. 7.2.1.1 Savoury biscuits), the consumption of the foods in the sub-classification is not included in the higher-level classification code.

In DIAMOND, all mixed foods in classification codes 20 and 21 have a recipe. Recipes are used to break down mixed foods into component ingredients which are in classification codes 1-14. The data for consumption of the ingredients from the recipe are then used in models and multiplied by the DHA concentrations in each of the raw ingredients. This only occurs if the *Mixed food* classification code (classification code 20), or a sub-code in classification 20 (e.g. 20.2.1), is not assigned its own DHA permission. If the *Mixed foods* classification is assigned a DHA concentration, the total consumption of the mixed food is multiplied by the proposed level, and the recipes are not used for that food group. If only a sub-code of classification 20 is assigned a DHA concentration, (i.e. category 20 itself is not assigned a DHA concentration), the recipes are used for all other mixed foods in category 20 that are not in the sub-code.

When a food that does not have a recipe is classified in two food groups in classification codes 1-14, and these food groups are assigned different permissions, DIAMOND will assume the food is in the food group with the highest assigned DHA level to assume a worst-case scenario. If the food groups have the same permitted DHA level, DIAMOND will assume the food is in the food group that appears first, based numerically on the ANZFCS.

Percentage contributions of each food group to total estimated intakes are calculated by summing the intakes for a food group from each individual in the population group who consumed a food from that group and dividing this by the sum of the intakes of all individuals from all food groups containing DHA, and multiplying this by 100.

2. Infants aged 9 months

As there are no data available from the NNS on children under two years, the theoretical infant diet from the 20th ATDS was used to estimate the dietary intake of DHA by infants aged 9 months. The theoretical infant diet was constructed to estimate dietary exposure to the food chemicals of interest in the ATDS for infants at 9 months of age. The recommended energy intake for a nine-month-old boy at the 50th percentile weight was used as the basis for the model diet (WHO 1983). Boys' weights were used because boys tend to be heavier than girls at the same age and therefore have higher energy and food requirements. It was assumed that 50 per cent of the energy intake was derived from milk (infant formula) and 50 per cent from solid foods (Hitchcock et al. 1986). The patterns of consumption for all two-year-old respondents from the NNS were scaled down and used to determine the solid portion of the nine-month old's diet. Certain foods such as nuts, coffee and alcohol were removed from the infant diet. The consumption of breakfast cereals was assumed to be in the form of either infant cereal or single grain breakfast cereals, excluding bran-based cereals. All milk consumption was assumed to be in the form of infant formula. Breast milk consumption was not considered in the theoretical infant diet.

The infant formula referred to in this theoretical diet was assumed to contain no DHA for Scenario 1. Although many infant formula manufacturers were adding DHA to some infant formula products prior to the approval of DHA derived from *Schizochytrium* sp., there is no data available to indicate what proportion of infant formula products had DHA added or at what level. Therefore, it is not possible to accommodate this use of DHA in infant formula in this theoretical diet for Scenario 1.

Assumptions in the dietary modelling

The aim of the dietary intake assessment was to make as realistic an estimate of dietary intake as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the dietary intake assessment did not underestimate intake.

Assumptions made in the dietary modelling include:

- where a permission is given to a food classification, all foods in that group contain DHA;
- all the foods within the group contain DHA at the levels proposed in either Table 2, Table 3 or a combination of both Table 2 and 3;
- consumption of foods as recorded in the NNS represent current food consumption patterns;
- consumers always select the products containing DHA;
- consumers do not alter their food consumption habits besides to substitute non-DHA or lower-DHA containing products with higher-DHA containing products;
- where foods are fortified with DHA derived from micro-algae it is assumed they will only be fortified with DHA from one species of micro-algae;
- consumers do not increase their consumption of foods/food groups upon foods/food groups containing DHA becoming available;
- where foods have naturally occurring DHA levels and they have been proposed to contain DHA-rich oil (*Ulkenia* sp.) in A522, the DHA concentration in the food is equal to the sum of the DHA from DHA-rich oil (*Ulkenia* sp.) and the naturally occurring DHA;
- where a food was not included in the intake assessment, it was assumed to contain a zero concentration of DHA;
- butter and butter products will not have DHA-rich oil (*Ulkenia* sp.) added;
- rolled oats will not have DHA-rich oil (*Ulkenia* sp.) added;
- where a food has a specified DHA concentration, this concentration is carried over to mixed foods where the food has been used as an ingredient e.g. (for example milk used in cakes);
- there are no reductions in DHA concentrations from food preparation or due to cooking;
- for the purpose of this assessment, it is assumed that 1 millilitre is equal to 1 gram for all liquid and semi-liquid foods (e.g. milk, yoghurt); and
- there is no contribution to DHA intake through the use of complementary medicines (Australia) or dietary supplements (New Zealand). While FSANZ acknowledges that complementary medicines/dietary supplements containing DHA are available on the market, it is not possible to consider this use in the dietary intake assessment.

Overall, these assumptions are likely to lead to a conservative estimate for DHA dietary intake.

Limitations of the dietary modelling

A limitation of estimating dietary intake 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 intakes are likely to be higher than actual high percentile intakes over a lifetime.

Daily food consumption amounts for occasionally consumed foods based on 24 hour food consumption data would be higher than daily food consumption amounts for those foods based on a longer period of time. This specifically affects the food groups in this assessment such as cream cheese based products.

Over time, there may be changes to the ways in which manufacturers and retailers make and present foods for sale. Since the data were collected for the Australian and New Zealand NNSs, there have been significant changes to the Code to allow more innovation in the food industry. As a consequence, another limitation of the dietary modelling is that some of the foods that are currently available in the food supply were either not available or were not as commonly available in 1995/1997.

The NNSs did not collect data on the use of complimentary medicines (Australia) or dietary supplements (New Zealand). Consequently, these could not be included in the dietary intake assessment as a potential source of DHA.

While the results of national nutrition surveys can be used to describe the usual intake of groups of people, they cannot be used to describe the usual intake of an individual (Rutishauser, 2000). In particular, they cannot be used to predict how consumers will change their eating patterns as a result of an external influence such as the availability of a new type of food.

FSANZ does not apply statistical population weights to each individual in the NNSs in order to make the data representative of the population. This prevents distortion of actual food consumption amounts that may result in an unrealistic intake estimate. Maori and Pacific Islanders were over-sampled in the 1997 New Zealand NNS so that statistically valid assessments could be made for these population groups for the survey. As a result, there may be bias towards this population group in the dietary intake assessment because population weights were not used.

Results

Estimated dietary intakes of DHA

All population groups, except infants
The estimated dietary intakes for consumers of DHA for each scenario are shown in Figures 1 and 2 for Australia and Figures 3 and 4 for New Zealand (full results in Table A1.1-A1.3 in Appendix 1).

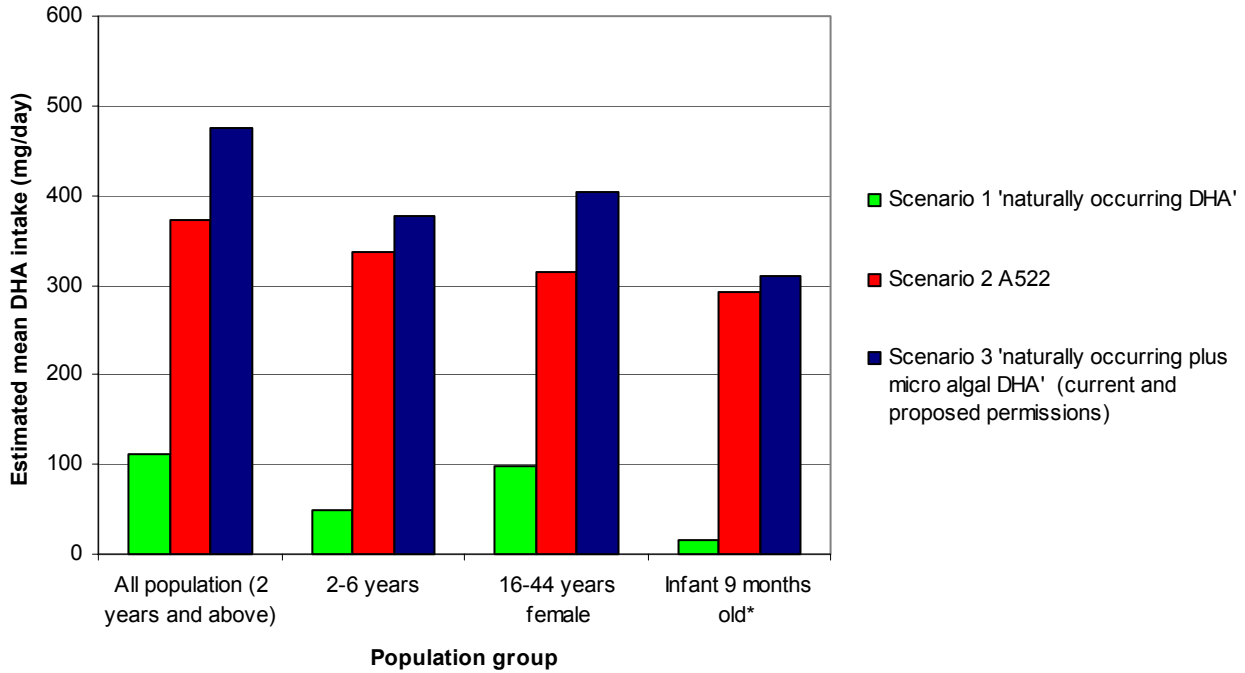
For consumers of naturally occurring DHA (Scenario 1), estimated mean intakes of DHA were the lowest for Australian children aged 2-6 years at 48 mg/day and were highest for Australians aged 2 years and above at 112 mg/day. Estimated 95th percentile intakes of DHA were the lowest for Australian children aged 2-6 years and highest for Australians aged 2 years and above at 467 mg/day.

When intake of DHA from DHA-rich oil (*Ulkenia* sp.) only was considered (Scenario 2), estimated mean intakes of DHA for consumers were the lowest for Australian females aged 16-44 years at 315 mg/day and highest for New Zealanders aged 15 years and above at 406 mg/day. Estimated 95th percentile intakes of DHA were the lowest for Australian females aged 16-44 years at 685 mg/day and highest for New Zealanders aged 15 years and above at 932 mg/day.

Based on the current and proposed permissions for the addition of DHA to foods as well as naturally occurring levels (Scenario 3), estimated mean consumer intakes of DHA were the lowest for Australian children aged 2-6 years at 378 mg/day and highest for New Zealanders aged 15 years and above at 498 mg/day. Estimated 95th percentile intakes were the lowest for Australian children aged 2-6 years at 785 mg/day and highest for all New Zealanders aged 15 years and above at 1 150 mg/day.

Infants – 9 months of age
Based on the theoretical infant diet, the estimated mean dietary intake of DHA was 16 mg/day from naturally occurring sources (Scenario 1), 293 mg/day from DHA-rich oil (*Ulkenia* sp.), and 310 mg/day from all sources (Scenario 3). Ninety-fifth percentile intakes cannot be derived for infants based on the constructed diet, as the same pattern of food consumption can be assumed to be found for all average and high consuming infants.

Figure 1: Estimated mean dietary intakes for consumers* of DHA for different scenarios and population groups for Australia



*For all population groups except infants (9 months old), consumer mean intakes are presented. Mean intakes for infants (9 months old) are based on a constructed diet for that population.

Figure 2: Estimated 95th percentile dietary intakes of DHA for different scenarios and population groups for Australia

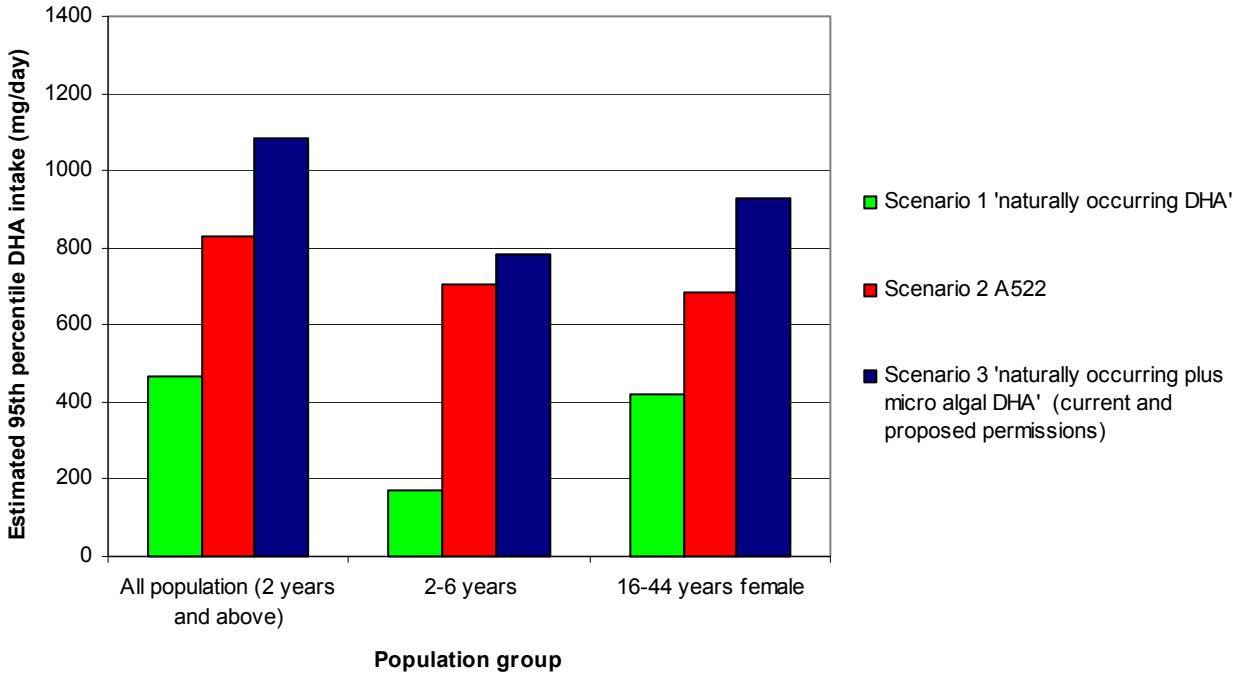


Figure 3: Estimated mean dietary intakes for consumers DHA for different scenarios and population groups for New Zealand

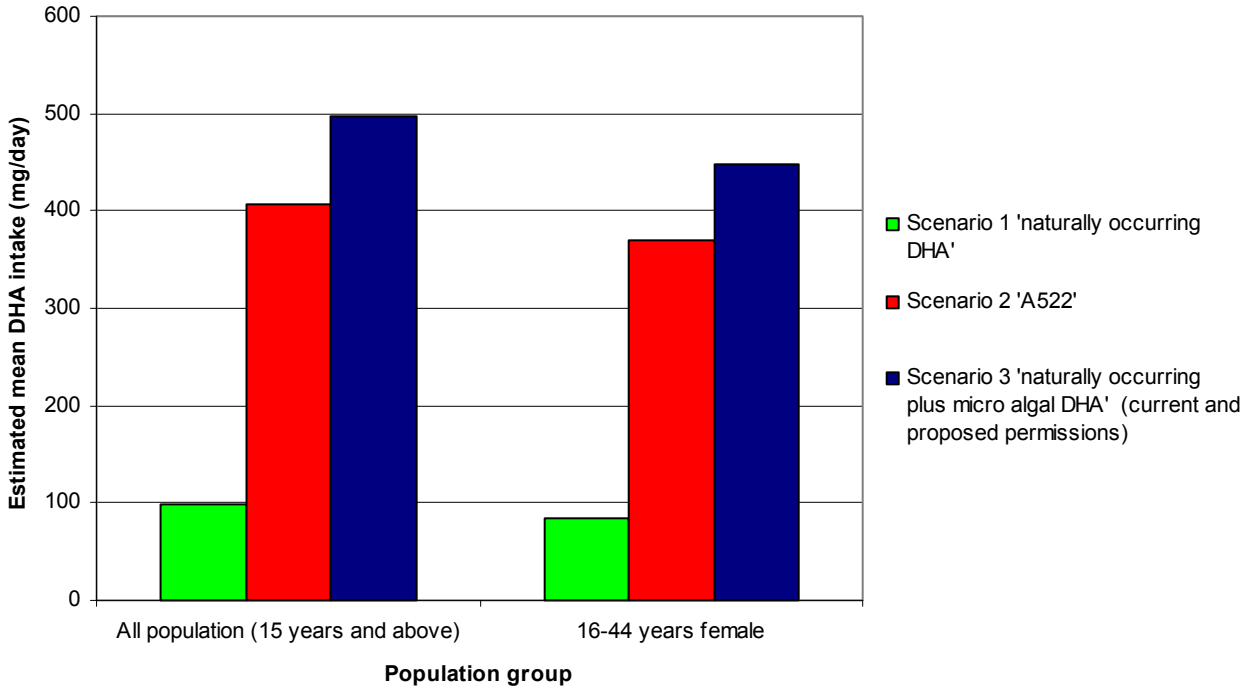
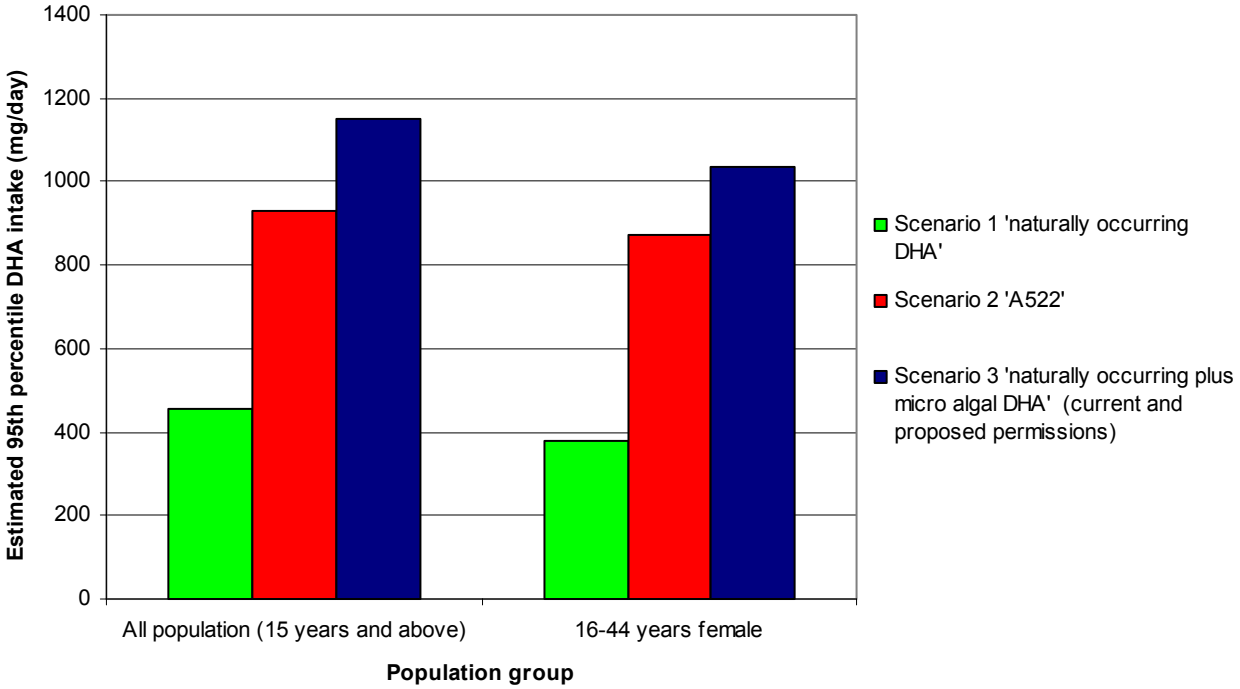


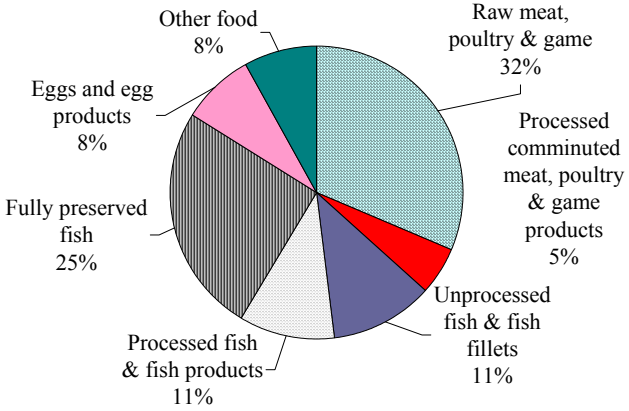
Figure 4: Estimated 95th percentile dietary intakes of DHA for different scenarios and population groups for New Zealand



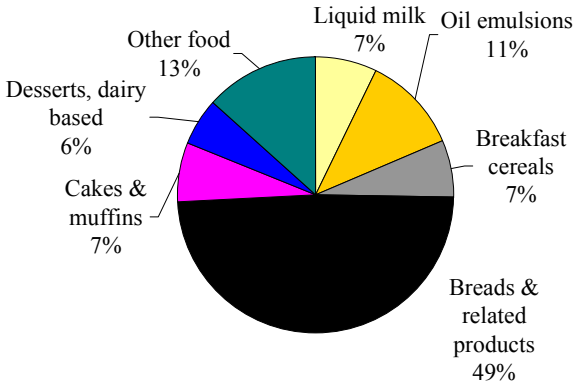
Major contributing foods to total estimated dietary intakes

The major contributors (>5%) to total DHA dietary intakes are shown in Figures 5-8 for Australia and Figures 9-10 for New Zealand for all population groups assessed and for all scenarios. The major contributors to intake of DHA were breads and related products, oil emulsions and liquid milk for all population groups assessed, except infants where infant formula and follow on formula and bread and related products were the major contributors. A full list of all the food groups and their contributions to total dietary intake of DHA for each scenario can be found in Table A1.4, A.1.5 and A.1.6 for Australia and Table A.1.7, A.1.8 and A.1.9 for New Zealand in Appendix 1.

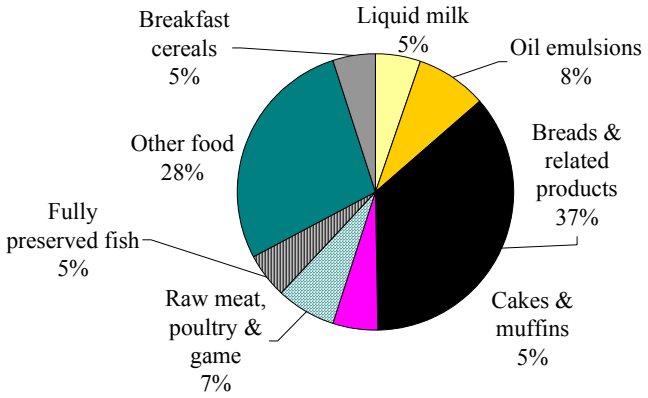
Figure 5: Contributors to DHA mean intakes for the Australian population aged 2 years and above



Scenario 1
(112 mg/day)



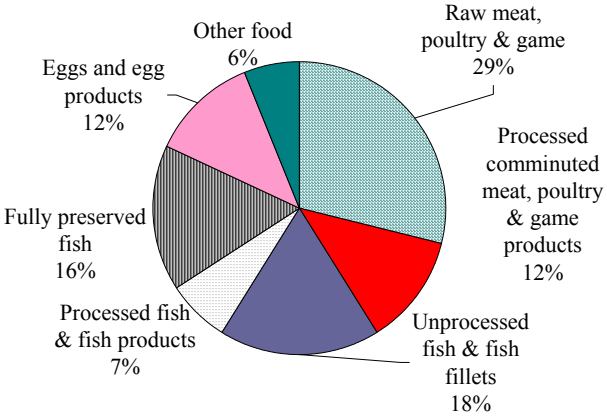
Scenario 2
(372 mg/day)



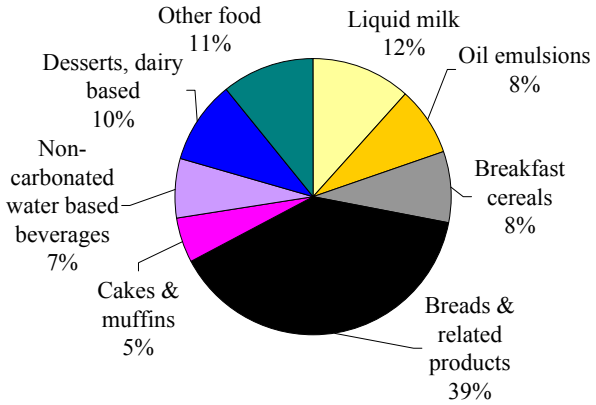
Scenario 3
(475 mg/day)

Note: In the above diagrams, Cereal products and Processed meal & cereal products are referred to as 'Breakfast cereals'.

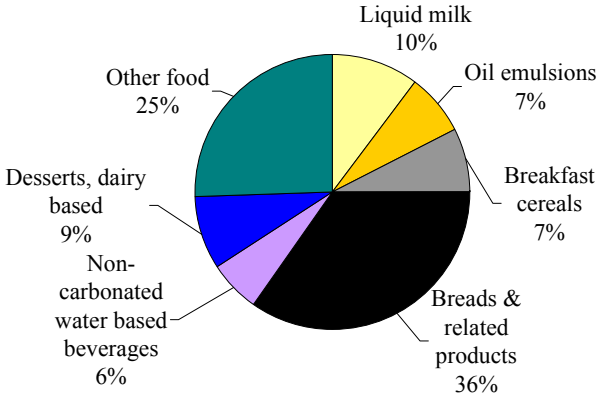
Figure 6: Contributors to total DHA mean intakes for the Australian population aged 2-6 years



Scenario 1
(48 mg/day)



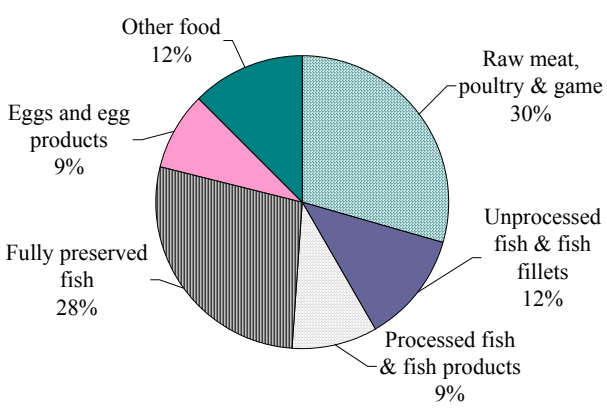
Scenario 2
(336 mg/day)

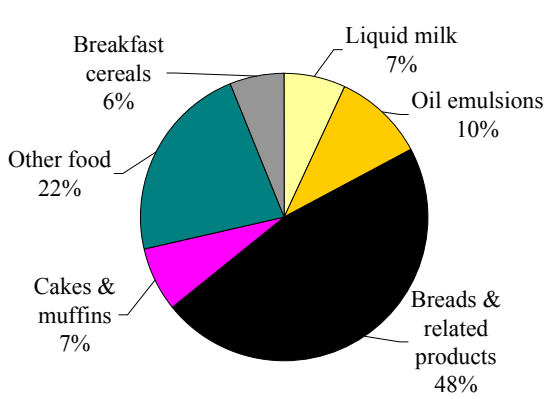


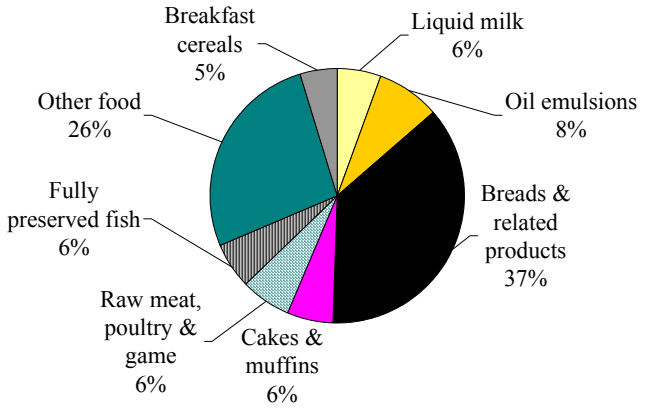
Scenario 3
(378 mg/day)

Note: In the above diagrams, Cereal Products and Processed meal & cereal products are referred to as 'Breakfast cereals' and Electrolyte/sports drinks and Cordials are referred to as 'Non-carbonated water based beverages'.

Figure 7: Contributors to total DHA mean intakes for the Australian population of females 16-44 years



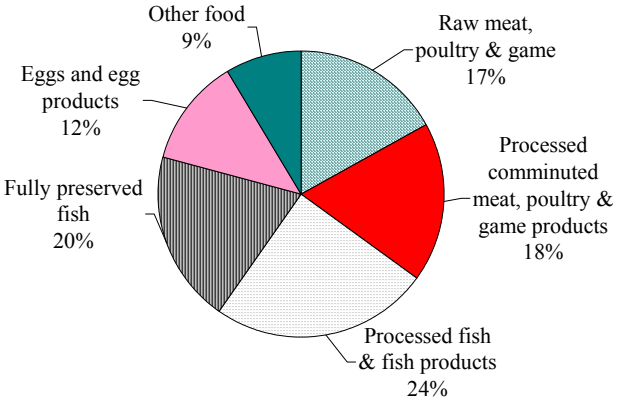




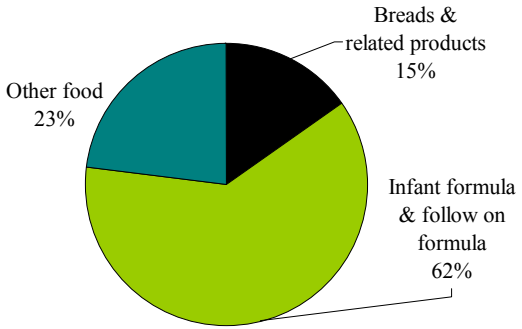
Scenario 1 (99 mg/day)	Scenario 2 (315 mg/day)	Scenario 3 (404 mg/day)

Note: In the above diagrams, Cereal products and Processed meal & cereal products are referred to as 'Breakfast cereals'.

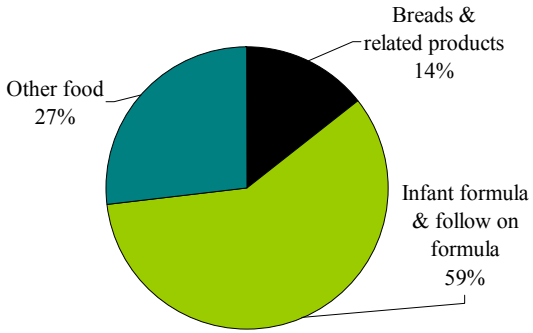
Figure 8: Contributors to total DHA mean intakes for the Australian population infants 9 months old



Scenario 1
(16 mg/day)

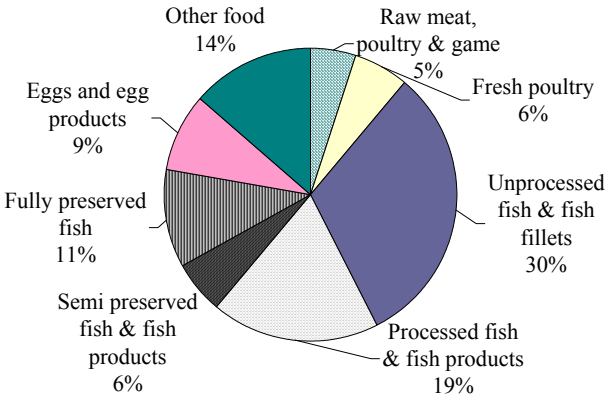


Scenario 2
(293 mg/day)



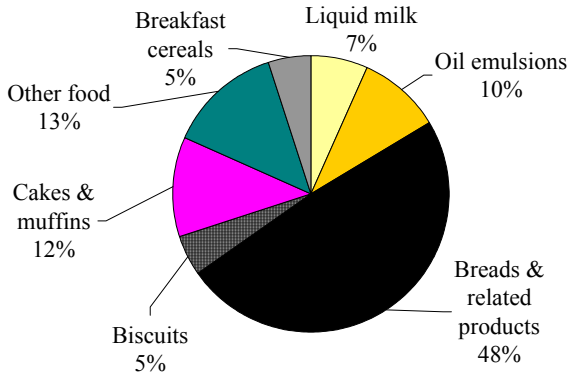
Scenario 3
(310 mg/day)

Figure 9: Contributors to total DHA mean intakes for the New Zealand population aged 15 years and above



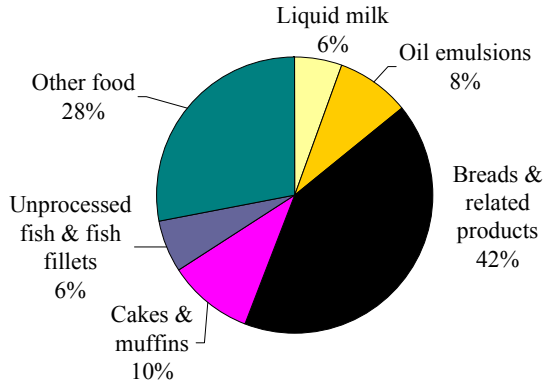
Scenario 1

(99 mg/day)



Scenario 2

(406 mg/day)

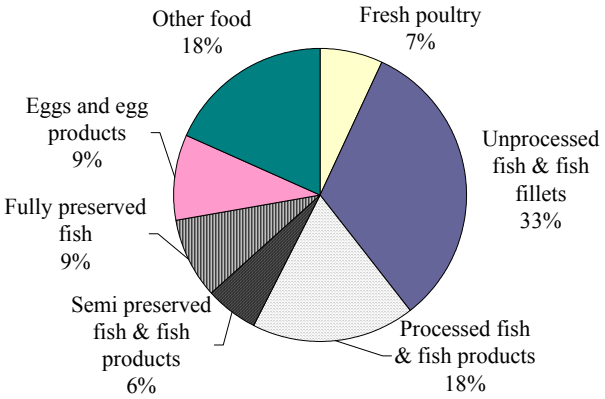


Scenario 3

(498 mg/day)

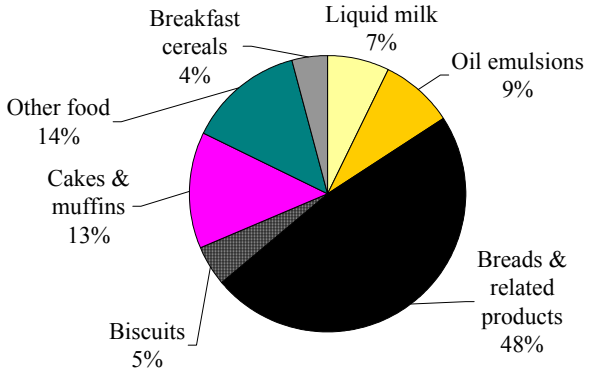
Note: In the above diagrams, Cereal products and Processed meal & cereal products are referred to as 'Breakfast cereals'.

Figure 10: Contributors to total DHA mean intakes for the New Zealand population of females 16-44 years



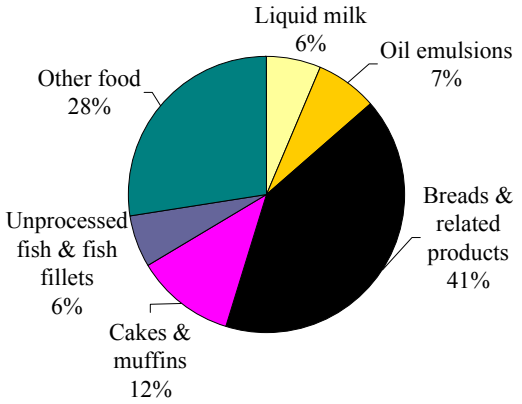
Scenario 1

(85 mg/day)



Scenario 2

(369 mg/day)



Scenario 3

(447 mg/day)

Note: In the above diagrams, Cereal products and Processed meal & cereal products are referred to as 'Breakfast cereals'.

REFERENCES

Australia New Zealand Food Authority, 1999, *Supplement to the NUTTAB95 database*, Australia New Zealand Food Authority, Canberra.

Crop and Food Research, 2000, New Zealand Fatty Acid data provided to ANZFA in 2000.

Food Standards Australia New Zealand, 2003, *The 20th Australian Total Diet Survey*, www.foodstandards.gov.au/_srcfiles/Final_20th_Total_Diet_Survey.pdf

Hitchcock, N.E., Gracey, M., Gilmour, A.I., Owler, E.N., 1986, 'Nutrition and growth in infancy in infancy and early childhood: a longitudinal study from birth to five years', *Monographs in Paediatrics*, 19, pp 1–92.

Institute of Medicine, National Academy of Sciences, 2000, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, National Academy Press, Washington, DC.

National Health and Medical Research Council, 2001, *National Health and Medical Research Council Website (www.health.gov.au/nhmrc/publications/diet)*

Rutishauser I. 2000. *Getting it right:- how to use the data from the 1995 National Nutrition Survey*. Commonwealth of Australia: Canberra.

World Health Organization, 1983, *Measuring Change in Nutritional Status*, WHO, Geneva.

World Health Organization, 2001, *Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956-2001) (First through fifty-seventh meetings) Internet Edition*, ILSI Press International Life Sciences Institute, Washington DC.

Appendix 1

Complete Information on Dietary Intake Assessment Results

Table A1.1: Scenario 1 - estimated dietary intakes of DHA from naturally occurring sources

Country	Population group	Number of consumers of DHA	Consumers♦ as a % of total respondents#	Mean all respondents (mg/day)	Mean consumers (mg/day)	95th percentile consumers (mg/day)
Australia	Whole population (2 years+)	12 907	93	105	112	467
	2-6 years	881	89	43	48	170
	16-44 years female	2 897	91	90	99	422
	Infants 9 months old	-	-	16	-	-
New Zealand	Whole population (15 years+)	4 436	96	95	99	455
	16-44 years female	1 437	95	81	85	381

Total number of respondents for Australia: whole population = 13 858, 2-6 years = 989, 16-44 years females = 3 178; New Zealand: whole population = 4 636, 16-44 years females = 1 509. Respondents include all members of the survey population whether or not they consumed a food that contains DHA.

♦ Consumers only – This only includes the people who have consumed a food that contains DHA.

Table A1.2: Scenario 2 – estimated dietary intakes of DHA from DHA-rich oil (*Ulkenia* sp.)

Country	Population group	Number of consumers of DHA	Consumers♦ as a % of total respondents#	Mean all respondents mg/day (mg/day)	Mean consumers mg/day (mg/day)	95th percentile consumers mg/day (mg/day)
Australia	Whole population (2 years+)	13 798	99.6	370	372	829
	2-6 years	987	99.8	335	336	703
	16-44 years female	3 156	99.3	313	315	685
	Infants 9 months old	-	-	293	-	-
New Zealand	Whole population (15 years+)	4 607	99.4	403	406	932
	16-44 years female	1 497	99.2	366	369	873

Total number of respondents for Australia: whole population = 13 858, 2-6 years = 989, 16-44 years females = 3 178; New Zealand: whole population = 4 636, 16-44 years females = 1 509. Respondents include all members of the survey population whether or not they consumed a food that contains DHA.
♦ Consumers only – This only includes the people who have consumed a food that contains DHA.

Table A1.3: Scenario 3 – estimated dietary intakes of DHA from naturally occurring plus micro-algae fortified sources (current and proposed permissions)

Country	Population group	Number of consumers of DHA	Consumers♦ as a % of total respondents#	Mean all respondents mg/day (mg/day)	Mean consumers mg/day (mg/day)	95th percentile consumers mg/day (mg/day)
Australia	Whole population (2 years+)	13 843	99.9	475	475	1 082
	2-6 years	989	100	378	378	785
	16-44 years female	3 172	99.8	403	404	930
	Infants 9 months old	-	-	310	-	-
New Zealand	Whole population (15 years+)	4 630	99.9	498	498	1 150
	16-44 years female	1 507	99.9	446	447	1 037

Total number of respondents for Australia: whole population = 13 858, 2-6 years = 989, 16-44 years females = 3 178; New Zealand: whole population = 4 636, 16-44 years females = 1 509. Respondents include all members of the survey population whether or not they consumed a food that contains DHA.
♦ Consumers only – This only includes the people who have consumed a food that contains DHA.

Table A1.4: Scenario 1 - contribution of each food group to total DHA mean dietary intake for Australian population groups

Food Name	% Contribution to DHA dietary intake			
	All population 2 years and above	**2-6 years**	**Females 16-44 years**	**Infants 9 months old**
Dried vegetables	0.1	0.05	0.1	4.8
Hotplate products	1.0	2.2	1.2	0.0
Cakes & muffins	0.5	0.9	0.6	0.8
Raw meat, poultry & game	**31.5**	**29.4**	**29.5**	**16.9**
Fresh poultry	1.1	1.2	1.4	1.7
Processed meat, poultry & game products	0.5	0.5	0.5	1.2
Processed comminuted meat, poultry & game products	**5.3**	**11.8**	3.9	**18.1**
Unprocessed fish & fish fillets	**11.2**	**17.6**	**12.1**	0.0
Unprocessed crustacea	0.3	-	0.3	0.0
Unprocessed molluscs	1.2	0.6	1.1	0.0
Roe	0.03	-	0.05	0.0
Processed fish & fish products	**10.7**	**7.4**	**9.5**	**24.8**
Processed crustacea	0.6	0.2	0.6	0.2
Processed molluscs	1.2	0.3	1.9	0.0
Semi preserved fish & fish products	1.4	0.1	0.8	0.0
Fully preserved fish including canned fish products	**25.3**	**16.4**	**27.9**	**19.5**
Eggs and egg products	**8.1**	**11.5**	**8.6**	**12.0**

Table A1.5: Scenario 2 - contribution of each food group to total DHA mean dietary intake for Australian population groups

Food Name	% Contribution to DHA dietary intake			
	All population 2 years and above	**2-6 years**	**Females 16-44 years**	**Infants 9 months old**
Liquid milk	**7.3**	**11.5**	**7.4**	0.0
Fermented & rennetted milk products	1.4	2.1	1.8	0.0
Oil emulsions	**11.2**	**7.9**	**10.9**	3.1
Processed cereal & meal products	**5.1**	**6.1**	4.4	1.3
Breads & related products	**48.7**	**38.5**	**49.9**	**15.2**
Biscuits	4.0	4.7	3.4	1.7
Savoury biscuits	2.0	2.5	2.2	1.1
Cakes & muffins	**7.1**	**5.1**	**7.9**	2.0
Infant formula & follow on formula	0.00	0.01	-	**61.8**
Infant cereal products	-	-	-	4.0
Infant foods	0.03	0.3	0.02	3.5
Infant drinks	0.01	0.06	-	0.0
Solid formula meal replacements & supplements	0.00	-	0.01	0.0
Liquid formula meal replacements & supplements	0.1	0.1	0.1	0.0
Fruit and vegetable juice products	1.1	2.3	1.1	2.8
Cordial	2.6	**6.6**	2.1	3.6
Electrolyte/sports drinks	0.1	0.03	0.05	0.0
Desserts, dairy based	**5.6**	**9.7**	4.0	0.0
Cereal products	1.7	2.0	2.1	0.0
Mayonnaise & salad dressings	1.6	0.5	2.0	0.0
Fat based dips & other fat based products	0.3	0.3	0.6	0.0

Table A1.6: Scenario 3 - contribution of each food group to total DHA mean dietary intake for Australian population groups

Food Name	% Contribution to DHA dietary intake			
	All population 2 years and above	**2-6 years**	**Females 16-44 years**	**Infants 9 months old**
Liquid milk	**5.7**	**10.2**	**5.8**	0.0
Fermented & rennetted milk products	1.1	1.9	1.4	0.0
Oil emulsions	**8.8**	**7.0**	**8.5**	2.9
Dried vegetables	0.02	0.01	0.03	0.3
Processed cereal & meal products	4.0	**5.4**	3.4	1.2
Hotplate products	0.2	0.3	0.3	0.0
Breads & related products	**38.0**	**34.2**	**38.8**	**14.4**
Biscuits	3.1	4.1	2.7	1.6
Savoury biscuits	1.5	2.2	1.7	1.0
Cakes & muffins	**5.7**	4.6	**6.3**	1.9
Raw meat, poultry & game	**7.1**	3.5	**6.7**	0.9
Fresh poultry	0.2	0.1	0.3	0.1
Processed meat, poultry & game products	0.1	0.06	0.1	0.1
Processed comminuted meat, poultry & game products	1.2	1.3	0.9	1.0
Unprocessed fish & fish fillets	2.5	2.0	2.7	0.0
Unprocessed crustacea	0.05	-	0.07	0.0
Unprocessed molluscs	0.3	0.07	0.2	0.0
Roe	0.01	-	0.01	0.0
Processed fish & fish products	2.4	0.8	2.1	1.3
Processed crustacea	0.1	0.02	0.1	0.0
Processed molluscs	0.3	0.03	0.4	0.0
Semi preserved fish & fish products	0.3	0.01	0.2	0.0
Fully preserved fish including canned fish products	**5.6**	1.9	**6.2**	1.0
Eggs and egg products	1.6	1.1	1.8	0.6
Infant formula & follow on formula	0.00	0.01	-	**58.6**
Infant cereal products	-	-	-	3.8
Infant foods	0.02	0.2	0.01	3.3
Infant drinks	0.01	0.05	-	0.0
Solid formula meal replacements & supplements	0.00	-	0.01	0.0
Liquid formula meal replacements & supplements	0.1	0.1	0.1	0.0
Fruit and vegetable juice products	0.8	2.0	0.8	2.6
Cordial	2.0	**5.9**	1.7	3.4
Electrolyte/sports drinks	0.09	0.02	0.04	0.0
Desserts, dairy based	4.3	**8.6**	3.1	0.0
Cereal products	1.4	1.8	1.6	0.0
Mayonnaise and salad dressings	1.3	0.4	1.6	0.0
Fat based dips and other fat based products	0.3	0.2	0.5	0.0

Table A1.7: Scenario 1 - contribution of each food group to total DHA mean dietary intake for New Zealand population groups

Food Name	% Contribution to DHA dietary intake	
	All population 15 years and above	Females 16-44 years
Dried vegetables	0.02	0.03
Hotplate products	1.1	1.4
Cakes & muffins	1.1	1.4
Raw meat, poultry & game	**5.0**	4.6
Fresh poultry	**6.2**	**6.9**
Processed meat, poultry & game products	1.4	1.2
Processed comminuted meat, poultry & game products	2.1	1.8
Unprocessed fish & fish fillets	**31.3**	**32.5**
Unprocessed crustacea	0.4	0.6
Unprocessed molluscs	2.0	1.7
Roe	0.01	0.0
Processed fish & fish products	**18.6**	**18.1**
Processed crustacea	0.8	0.7
Processed molluscs	4.7	4.9
Semi preserved fish & fish products	**5.9**	**5.9**
Fully preserved fish including canned fish products	**10.7**	**8.9**
Eggs	**8.7**	**9.5**

Table A1.8: Scenario 2 - contribution of each food group to total DHA mean dietary intake for New Zealand population groups

Food Name	% Contribution to DHA dietary intake	
	All population 15 years and above	Females 16-44 years
Liquid milk	**7.0**	**7.7**
Fermented & rennetted milk products	1.0	1.1
Oil emulsions	**10.4**	**9.0**
Processed cereal & meal products	2.8	2.5
Breads & related products	**51.5**	**50.1**
Biscuits	**5.0**	**5.1**
Savoury biscuits	1.5	1.3
Cakes & muffins	**12.2**	**14.1**
Infant formula & follow on formula	-	-
Infant cereal products		-
Infant foods		-
Infant drinks		-
Solid formula meal replacements & supplements	0.00	-
Liquid formula meal replacements & supplements	0.06	0.06
Fruit and vegetable juice products	0.3	0.4
Cordial	0.7	1.0
Electrolyte/sports drinks	0.1	0.2
Desserts, dairy based	3.4	3.8
Cereal products	2.6	2.0
Mayonnaise & salad dressings	1.1	1.3
Fat based dips & other fat based products	0.4	0.6

Table A1.9: Scenario 3 - contribution of each food group to total DHA mean dietary intake for New Zealand population groups

Food Name	% Contribution to DHA dietary intake	
	All population 15 years and above	Females 16-44 years
Liquid milk	**5.7**	**6.3**
Fermented & rennetted milk products	0.8	0.9
Oil emulsions	**8.5**	**7.4**
Dried vegetables	0.00	0.00
Processed cereal & meal products	2.3	2.0
Hotplate products	0.2	0.3
Breads & related products	**41.7**	**41.0**
Biscuits	4.0	4.1
Savoury biscuits	1.2	1.1
Cakes & muffins	**10.1**	**11.8**
Raw meat, poultry & game	1.3	1.2
Fresh poultry	0.7	0.8
Processed meat, poultry & game products	0.3	0.2
Processed comminuted meat, poultry & game products	0.4	0.3
Unprocessed fish & fish fillets	**6.0**	**5.9**
Unprocessed crustacea	0.08	0.1
Unprocessed molluscs	0.4	0.3
Roe	0.00	0.00
Processed fish & fish products	3.6	3.3
Processed crustacea	0.2	0.1
Processed molluscs	0.9	0.9
Semi preserved fish & fish products	1.1	1.1
Fully preserved fish including canned fish products	2.0	1.6
Eggs and egg products	1.6	1.7
Infant formula & follow on formula	-	-
Infant cereal products	-	-
Infant foods	-	-
Infant drinks	-	-
Solid formula meal replacements & supplements	0.00	-
Liquid formula meal replacements & supplements	0.05	0.05
Fruit and vegetable juice products	0.2	0.3
Cordial	0.6	0.8
Electrolyte/sports drinks	0.1	0.1
Desserts, dairy based	2.8	3.1
Cereal products	2.1	1.7
Mayonnaise and salad dressings	0.9	1.1
Fat based dips and other fat based products	0.3	0.5

ATTACHMENT 4

Nutrition Assessment

The purpose of this report is to address any nutritional issues related to the proposed use of docosahexaenoic acid (C22:6) (DHA) produced from a novel source in a variety of foods.

FSANZ has considered two DHA-rich micro-algal oils, one derived from *Crypthecodinium cohnii* (branded as DHASCO), and the other derived from *Schizochytrium* sp. A safety assessment for DHA derived from *Schizochytrium* sp. determined it to be safe for consumption by the general population.

Similar to DHA-rich micro-algal oil *(Schizochytrium sp)*, DHA-rich oil (*Ulkenia sp*.) is proposed for use in foods consumed by the general population. This nutritional assessment includes a discussion of:

- the nutritional role of DHA;
- the potential interaction between DHA-rich oil (*Ulkenia sp*.) and other nutrients;
- a comparison of the ratio of eicosapentaenoic acid (C20:5) (EPA) to DHA in DHA-rich oil (*Ulkenia* sp.) with other natural sources in the food supply; and
- a potential increase in the number of foods bearing omega-3 claims.

1. Background Information

1.1 The Nutritional Role of DHA

There are two main families of polyunsaturated fatty acids: the omega-6 (or n-6) family, and the omega-3 (or n-3) family. The omega-6 family is derived from linoleic acid (C18:2), and the omega-3 family is derived from alpha-linolenic acid (C18:3) (ALA). Linoleic acid and alpha-linolenic acid are referred to as essential fatty acids, as they cannot be made in the human body and must be obtained from foods.

DHA is an omega-3 fatty acid, and is an important structural element of cell membranes and new tissues. DHA is mostly obtained in the human diet from fish, which has a highly variable DHA content in the range of 20 to 2020 mg/100g[1,2]. The human body aged greater than 6 months can readily manufacture DHA from dietary ALA[3]. DHA accumulates rapidly in foetal and infant neural tissue during periods of most rapid growth and development; that is, during the last months of gestation and the first months of postnatal life[4,5]. Without breast milk or suitable substitutes, dietary ALA intake appears to be sub-optimal for pre-term infants and inadequate to maintain normal DHA blood levels[6,7]. Infants can typically meet their DHA requirement via the DHA that naturally occurs in breast milk (breast milk substitutes need to add DHA to achieve the same outcome).

Australia and New Zealand governments have not set a recommended dietary intake for either DHA or omega-3 fatty acids. Recommended intakes have, however, been set by the following agencies:

- a FAO/WHO expert consultation has recommended a daily omega-3 fatty acid intake of 1-2% of total energy intake[8];

- the United States Institute of Medicine has recommended a minimum daily ALA intake of 1.6 g/day (men) and 1.1 g/day (women)[9];

- the Dutch Government has set an adequate intake of omega-3 fatty acids at 0.2 g/day[10]; and

- a British Nutrition Foundation taskforce on unsaturated fatty acids has suggested that the desirable omega-3 fatty acid daily intake is 0.5% of total energy intake[11].

1.2 The Role of DHA in Chronic Illnesses

Consumption of omega-3 fatty acids has been identified as a means of preventing the development of coronary heart disease (CHD). FSANZ has identified six studies[12-18], summarised in the Appendix to this Attachment, that have assessed the intake of omega-3 fatty acids (usually EPA and DHA in combination, or as an intake of fish/fish oil) specifically against the risk of developing CHD. While the relationship between CHD and omega-3 fatty acids has been observed with the consumption of fish and fish oils containing DHA, scientific studies to date have not investigated whether DHA intake by itself mitigates the risk of developing CHD.

Current literature also indicates that the consumption of omega-3 fatty acids produces no noticeable improvement in the prevention and management of diabetes[19], and only speculative benefits in the prevention and management of inflammatory illnesses[20].

2. Interaction Between DHA-rich oil (*Ulkenia sp.*) and Other Nutrients

There is very little data on the impact of DHA on the bioavailability other nutrients. It is mentioned in the small amount of information that can be found, that increased DHA intakes can impact on vitamin E dietary intake requirements[21]. Because of its particular antioxidant role in the human body, vitamin E often acts to counter the oxidative metabolites produced from the consumption of polyunsaturated fatty acids. An increased intake of polyunsaturated fatty acids (such as DHA) places an increased demand on available vitamin E stores, thus increasing the physiological requirement for vitamin E.

Oils containing high quantities of polyunsaturated fatty acids (e.g. sunflower oil) are consumed by the general population and are permitted to contain added vitamin E for technological (i.e. antioxidant) purposes or for nutrient restoration in accordance with Standard 1.3.2 – Vitamins and Minerals. Therefore, the risk to vitamin E status through the addition of DHA-rich oil (*Ulkenia* sp.) is small.

Although the DHA-rich oil (*Ulkenia sp.*) has been derived from a novel micro-algal source, its macro-composition is similar to other oils (i.e. primarily a complex mixture of triglycerides). As triglycerides are the main form of fat ingested by humans, it is expected that the digestion and absorption of DHA will be identical to that from other non-novel DHA-containing oils.

3. The EPA to DHA ratio

DHA and EPA are both omega-3 fatty acids and are often present in the same foods. The ratio of EPA to DHA in the DHA micro-algal oil (*Ulkenia sp.*), and in recognised dietary sources of omega-3 fatty acids is compared in this section.

The Applicant has supplied FSANZ with analyses of the DHA-rich oil (*Ulkenia sp.*) undertaken in accordance with methods validated by the American Oil Chemists Society (AOCS) and the German Federation for Fat Science. These analyses do not specify the level of EPA in the DHA micro-algal Oil (*Ulkenia sp.*), although 'other fatty acids' were quantified, which may include EPA. If so, EPA would be present in the range of 0-2.8% of total fatty acids ('other fatty acids' were present at 2.8%); when assessed against DHA at 45% of total fatty acids, this range produces a minimum EPA:DHA ratio of 1:16.1. If the ratio is calculated in accordance with the minimum DHA content given in the specifications for DHA-rich oil (*Ulkenia* sp.) of 32%, the resulting EPA:DHA ratio is 1:11.4.

Some data on the levels of DHA and EPA in Australian foods are available, however New Zealand data is not available. FSANZ has reviewed the Australian data on fatty acids[1], which indicates that the EPA:DHA ratio is highly variable, ranging from approximately 1:0.1 in lamb to 1:16 in coral trout. The mean dietary intake of EPA and DHA is in the ratio of 1:1.8, with meat, fish and eggs as the main dietary sources of EPA and DHA[2]. Some indicative ratios for individual foods are provided in Table 1 below and illustrate the range of EPA:DHA ratios found across the food supply.

Table 1: Ratio of EPA to DHA in Australian Foods

Food	Ratio EPA:DHA
Lamb, excl offal	1:0.1
Kangaroo	1:0.2
Prawns	1:0.6
Beef, excl offal	1:1
Scallops	1:1.4
Tuna, canned in brine	1:2
Chicken, lean only	1:3
Pork, Snapper	1:4
Taylor, Ling, Flathead, Blue Grenadier, Bream	1:5
Barramundi, Mackeral, King Fish	1:6
Shark, battered, fried	1:9
Trumpeter	1:12
Trout, coral	1:16

Many foods do not contain either of these fatty acids (e.g. eggs, nuts, dairy foods) and some foods contain only low levels of EPA. At a representative EPA:DHA ratio of 1:16.1, DHA-rich oil (*Ulkenia sp.*) is comparable with some fish such as coral trout.

4. Nutrition Claims

Should this Application be approved, the DHA-rich oil (*Ulkenia sp.*) could be taken into account when determining a food's eligibility to bear an omega-3 fatty acid claim.

To qualify for an omega-3 fatty acid claim on a food label or in advertising, the food must meet the following criteria under Clause 13 of Standard 1.2.8 – Nutrition Information Requirements:

A claim must not be made in relation to the omega-3 fatty acid content of a food, other than fish or fish products that have no added saturated fatty acids, unless the –

(a) total of saturated fatty acids and trans fatty acids is less than 28 per cent of the total fatty acid content of the food; or
(b) food contains no more than 5 g of saturated fatty acids and trans fatty acids per 100 g of the food.

A nutrition claim must not be made in relation to the omega-3 fatty acid content of a food, unless the food satisfies the requirements of subclause (2) and contains no less than –

(a) 200 mg alpha-linolenic acid per serving; or
(b) 30 mg total eicosapentaenoic acid and docosahexaenoic acid per serving.

A nutrition claim must not be made that a food is a 'good source' of omega-3 fatty acid or words of similar import, unless the food satisfies the requirements of subclause (2) and contains no less than 60 mg total eicosapentaenoic acid and docosahexaenoic acid per serving.

4.1. Potential for Foods Containing DHA Micro-Algal Oil (*Ulkenia* sp.) to make Omega-3 Claims

If DHA-rich oil (*Ulkenia sp.*) is permitted for addition to foods as a novel ingredient, then food manufacturers may have an increased opportunity to add DHA to foods and make omega-3 claims on product labels, depending on any conditions of use imposed. Therefore, the number of food products claiming to contain omega-3 fatty acids (a content claim), or describing the benefits of omega-3 fatty acids (a function claim) could possibly increase in Australia and New Zealand as a result of this Application.

However, it should also be recognised that the type of foods that qualify to make omega-3 claims are already regulated by the mandatory eligibility criteria of Clause 13 of Standard 1.2.8. Furthermore, in December 2003, the Australia New Zealand Food Regulation Ministerial Council (ANZFRMC) released policy guidelines for nutrition, health and related claims. FSANZ is commencing implementation of this new policy within the Code via Proposal P293 – Nutrition, Health and Related Claims, and it is intended that the eligibility criteria (and any other relevant regulatory aspects) for nutrition claims will be reviewed and updated where necessary.

The relationship between omega-3 fatty acids and cardiovascular disease is also under review as a possible high-level claim (i.e. a claim that will require permission in the Code under the proposed new nutrition, health and related claims standard); this diet disease relationship may promote use of DHA-rich oil (*Ulkenia* sp.) if approved, although such an outcome will depend on any criteria that are set in relation to use of the claim.

5. Conclusion

Approval of DHA-rich oil (*Ulkenia* sp.) for use as a novel food ingredient will give manufacturers increased opportunities to add DHA to foods that have not traditionally contained significant levels of this fatty acid.

The potential nutritional impact posed by the permission to add DHA-rich oil (*Ulkenia sp.*) to foods is no greater than that posed by the use of other non-novel oils.

Interaction Between DHA-rich oil (Ulkenia sp.) and Other Nutrients

- There is evidence linking an increased intake of polyunsaturated fatty acids (such as DHA) to increasing population vitamin E requirements. However:

    - DHA is only one of several polyunsaturated fatty acids that can influence vitamin E requirements; and
    - the use of other polyunsaturated-rich oils is not limited by their impact on the vitamin E requirements.

    Vitamin E is often added to oils for technological (i.e. antioxidant) purposes or for nutrient restoration. Therefore, the potential consequences for vitamin E requirements are very minor, and do not constitute a significant public health problem.

- There is no evidence to suggest that DHA-rich oil (*Ulkenia* sp.) will impact on the bioavailability of other nutrients, or will be digested any differently to other sources of fat.

The EPA to DHA ratio of DHA-rich oil (*Ulkenia *sp.)

Traditionally rich sources of DHA (e.g. marine oils) are also the primary source of EPA in the diet. At a representative EPA:DHA ratio of 1:16.1, DHA-rich oil (*Ulkenia sp.*) is comparable with some fish such as coral trout.

Nutrition Claims

- The potential for the approval of DHA-rich oil (*Ulkenia* sp.) to increase the number of foods carrying omega-3 fatty acid claims within Australian and New Zealand markets is already managed by mandatory eligibility criteria. The eligibility criteria for omega-3 fatty acid claims prevent these claims from appearing on the labels of nutritionally inappropriate foods, by addressing inappropriate nutritional characteristics (e.g. saturated fatty acid content). It is expected that omega-3 fatty acid claims will be reviewed in the near future as part of the development of a health, nutrition and related claims standard.

7. Reference List

1. Mann NJ, Sinclair AJ, Percival P, Lewis JL, Meyer BJ, Howe P (2003); *'Development of a database of fatty acids in Australian foods'*; Nutrition & Dietetics, 60(1): 42-45.
2. Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ, Howe PR (2003); *'Dietary Intakes and Food Sources of Omega-3 Polyunsaturated Fatty Acids'*; Lipids, 38(4): 391-398.
3. Linscheer WG and Vergroesen AJ (1994), *'Lipids'* in: Shils ME (ed), Olson JA (ed), Shike M (ed), *'Modern Nurtition in Health and Disease'*, 8th Ed, Lea & Febiger, Sydney.
4. Martinez M (1992); *'Tissue levels of polyunsaturated fatty acids during early human development'*; J Paediatr, 120(Suppl): 129 – 138.
5. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA (1994); *'Fatty acid composition of brain, retina and erythrocytes in breast- and formula-fed infants'*; Am J Clin Nutr, 60: 189 – 194.
6. Carlson SE, Rhodes PG, Ferguson MG (1986); *'Docosahexanoic acid status of preterm infants at birth and following feeding with human milk or formula'*; Am J Clin Nutr, 44: 798 – 804.
7. Koletzko B, Schmidt E, Berner HJ, Haug M, Harzer G (1989); *'Effects of dietary long chain polyunsaturated fatty acids on the essential fatty acid status of premature infants'*; Eur J Ped, 148: 669 – 675.
8. World Health Organization (2003); *'Diet, nutrition and the prevention of chronic diseases: Report of the joint WHO/FAO expert consultation'*; WHO Technical Report Series, 916: 81-94.
9. United States Institute of Medicine (2002); *'Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids'*; National Academies Press, Washington DC, 8-34 – 8-40.
10. Health Council of the Netherlands (2002); *'Dietary Reference Intakes: Energy, Protein, Fats, and Digestible Carbohydrates'*; The Hague: Health Council of the Netherlands, publication no. 2001/19ER.
11. British Nutrition Foundation (2000); *'n-3 Fatty Acids and Health'*; British Nutrition Foundation Briefing Paper Series, http://www.nutrition.org.uk/publications/briefingpapers/n3fatty.htm
12. Albert CM, Hennekens CH, O'Donnell CJ, Ajami UA; Carey VJ, Willett WC, Ruskin JN, Manson JE (1998); *'Fish Consumption and Risk of Sudden Cardiac Death'*; J Am Med Assoc, 279:23-28.
13. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM (1989); *'Effects of changes in fat fish and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART)'*; Lancet, 2(8666): 757-761.
14. Burr ML, Sweetnam PM, Fehily AM, Elwood PC (1994); *'Letters to the editor'*; European Heart Journal, 15:1152-1154.
15. GISSI-Prevenzione Investigators (1999); *'Dietary Supplementation with n-3 Polyunsaturated Fatty Acids and Vitamin E after Myocardial Infarction: results of the GISSI-Prevenzione trial'*; Lancet, 354:447-455.
16. Hu FB, Bronner L, Willet WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D, Manson JE (2002); 'Fish and Omega-3 Fatty Acid Intake and Risk of Coronary Heart Disease in Women'; J Am Med Assoc, 287(14): 1815-1821.
17. Singh RB, Niaz MA, Sharma JP, Kumar R, Rastiogi V, Moshiri M (1997); *'Randomized, double-blind, placebo controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the indian experiment of infarct survival-4'*; Cardiovascular Drugs and Therapy, 11:485-491.

18. Siscovick D, Raghunathan TE, King I, Weinmann S, Bovbjerg VE, Kushi L, Cobb LA, Copass MK, Psaty BM, Lemaitre R, Retzlaff B, Knopp RH (2000); *'Dietary intake of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest'*; Am J Clin Nutr, 71(1): 208-212.

19. Friedberg CE, Janssen MJ, Heine RJ, Grobee DE (1998); *'Fish Oil and Glycaemic Control in Diabetes'*; Diabetes Care, 21(4): 494-500.

20. Simopoulos AP (2003); *'Omega-3 fatty acids in inflammation and autoimmune diseases'*; J Am Coll Nutr, 21(6): 495-505.

21. Valk EE and Hornstra G (2000); *'Relationship between vitamin E requirement and polyunsaturated acid intake in man: a review'*; Int J Vitam Nutr Res, 70(2): 31-42.

22. Simopoulos AP (1999); *'Essential fatty acids in health and chronic disease'*; Am J Clin Nutr, 70(Suppl): 560S-569S.

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

Appendix 1

Table 1: Omega-3 Fatty Acid Intake and the Risk of Coronary Heart Disease

Study	Study Design	Study Period	No. and type of Subjects		Intake level	Results: Relative risk total CHD	Results: Relative risk fatal CHD (except where indicated)	Results: Relative risk non-fatal CHD	Significant Difference? ($p<0.05$)
Albert *et al* (1998)[8]	RCT, double blind, cohort	11 years	20551 male physicians. Subjects stratified by fish intake (serve = 84-112 g fish)		< 1 serve / month	-	1.00	1.00	Yes for trend over intake groups for non-fatal CHD, but not for fatal CHD.
					1-3 serves / month	-	0.96	0.64	
					1 serve / week	-	0.79	0.47	
					2-4 serves / week	-	0.84	0.51	
					≥ 5 serves / week	-	0.81	0.39	
Burr *et al* (1989) and Burr *et al* (1994) (two articles on same study)[13]	RCT, no blinding	2 years	2033 males and females with recent CHD diagnosis, equal group numbers	Received no advice to eat fish	0g fish/week	1.00	-	-	No
				Received advice to eat fish	200-400g fish/week	0.84	-	-	
			454 male and female subset of 2033 subjects, equal group numbers	Received no fish oil capsules	0 g fish oil	-	9.3 (% incidence of death in group)	-	Yes, between the two groups, although authors indicated there may be selection bias on results
				Received fish oil capsules	3 g fish oil	-	3.5 (% incidence of death in group)	-	
GISSI-Prevenzione Investigators (1999)[15]	RCT, double-blinding	42 months	11324 males and females with recent CHD diagnosis	Control (placebo)	0 mg	1.00	1.00	1.00	Yes, the omega-3 group results were significantly lower except for non-fatal CHD. Other groups did not experience a
				Omega-3 group	859-882 mg EPA and DHA / day	0.80	0.70	0.96	
				Vitamin E group	300 mg Vitamin E /day	0.88	0.80	1.02	

Study	Study Design	Study Period	No. and type of Subjects		Intake level	Results: Relative risk total CHD	Results: Relative risk fatal CHD (except where indicated)	Results: Relative risk non-fatal CHD	Significant Difference? ($p<0.05$)
				Omega-3 and Vitamin E group	859-882 mg EPA and DHA/day + 300 mg Vitamin E /day	0.88	0.80	1.01	significant lowering in the relative risk of CHD in comparison to the control, except for fatal CHD results.
Hu *et al* (2002)[16]	RCT, double blind, cohort	16 years	1513 female nurses	Subjects stratified by fish intake (serve = 168-224 g fish)	< 1 serve / month	1.00	1.00	1.00	Yes, significant trend with increasing intake for total, fatal and non-fatal CHD events
					1-3 serves / month	0.79	0.81	0.78	
					1 serve / week	0.71	0.66	0.74	
					2-4 serves / week	0.69	0.73	0.68	
					≥ 5 serves / week	0.66	0.55	0.73	
				Subjects stratified by omega-3 fatty acid intake	0.03% of energy intake	1.00	1.00	1.00	Yes, significant trend with increasing intake for total, fatal and non-fatal CHD events
					0.05% of energy intake	0.93	0.94	0.91	
					0.08% of energy intake	0.78	0.61	0.79	
					0.14% of energy intake	0.68	0.41	0.66	
					0.24% of energy intake	0.67	0.42	0.57	
Singh *et al* (1997)[17]	RCT, single blinding	1 year	360 patients with suspected infarction	Group A – fish oil (n=122)	1.08 g EPA/day, and 0.72 g DHA/day	0.70	0.52	0.51	Yes, between fish oil and placebo groups, and mustard oil and placebo groups.
				Group B – mustard oil (n=120)	20 g/day mustard oil (2.9 g ALA/day)	0.81	0.60	0.59	
				Group C – placebo (n=118)	0g of omega-3 fatty acids/day	1.00	1.00	1.00	

Siscovick *et al* (2000)[18]	Case-control, random control selection	1 month retrosp-ective dietary analysis	334 CHD case subjects, 493 control subjects	No seafood intake group	0 g omega-3 fatty acids /month	1.00	-	-	Yes, significant trend with increasing seafood intake
				Seafood intake group (stratified equally into groups - intake levels are as mean of group)	1 g omega-3 fatty acids /month	0.90	-	-	
					2.9 g omega-3 fatty acids /month	0.73	-	-	
					5.5 g omega-3 fatty acids /month	0.50	-	-	
					13.7 g omega-3 fatty acids /month	0.38	-	-	

ATTACHMENT 5

FOOD TECHNOLOGY REPORT

A522 - DHA rich micro-algal oil from *Ulkenia* sp. as a novel food ingredient

Introduction

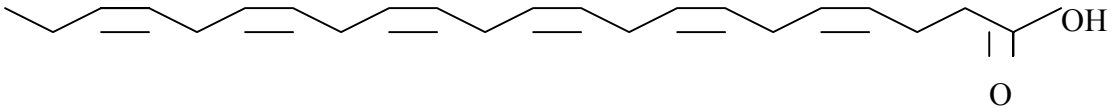
Food Standards Australia New Zealand (FSANZ) received an Application (A522) in December 2003, from Nutrinova Australasia Pty Ltd, to amend Standard 1.5.1 - Novel Foods, of the *Australia New Zealand Food Standards Code* (the Code), to permit the use of oil produced by micro-algae of the *Ulkenia* species, that is rich in the omega-3 long chain polyunsaturated fatty acid, docosahexaenoic acid (DHA), as a novel food ingredient in a 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 $C_{22}H_{32}O_2$.

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-unsaturated'). 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

Extracted DHA Rich Oil

The product covered by this application is the extracted oil from the micro-algae of the *Ulkenia* species. This oil contains a number of long chain fatty acids (C12 –C22) with DHA being the major fatty acid (typically being 45% of total fatty acids). The extracted oil is colourless to pale yellow, fluid to waxy oil, with a characteristic "bland to fish-like" odour. The oil also contains a small percentage of trans-fatty acids (less than 2%) and non-saponifiables (essentially identified and unidentified sterols, less than 2.0%).

The Applicant states the oil is stabilised by antioxidants approved within category 2 – Edible oils and oil emulsions in Schedule 1 of Standard 1.3.1 – Food Additives – of the Code. The permitted food additives for edible oils and oil emulsions in this Standard is given as follows:

160b	Annatto extracts	20	mg/kg
304	Ascorbyl palmitate	GMP	
306	Tocopherols concentrate mixed	GMP	
307	Tocopherol, d-alpha-, concentrate	GMP	
308	Synthetic gamma-tocopherol	GMP	
309	Synthetic delta-tocopherol	GMP	
310	Propyl gallate	100	mg/kg
311	Octyl gallate	100	mg/kg
312	Dodecyl gallate	100	mg/kg
319	Tertiary butylhydroquinone	200	mg/kg
320	Butylated hydroxyanisole	200	mg/kg
321	Butylated hydroxytoluene	100	mg/kg

The Application contains stability studies where tocopherols have been used as the antioxidant.

Production Process

The processes used are summarised but not detailed in the Application. A brief overview is provided here. The technology used is commonly employed for comparable processes such as fed-batch fermentations for micro-organisms and food oil extraction processes.

Fermentation process

The micro-algae are produced from a production fermentation process using dextrose as the main carbon source. 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 is 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 fermentors using GMP. Once the fermentation has reached the required mass the broth is chilled and the micro-algae separated and dried. The dried micro-algae can be further processed to extract and purify the DHA-rich oil as explained below.

DHA-rich oil extraction

Figure 1 contains a schematic of the expected DHA-rich oil extraction process. 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 oxidise 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 processing aids commonly used by the food industry for a range of applications.

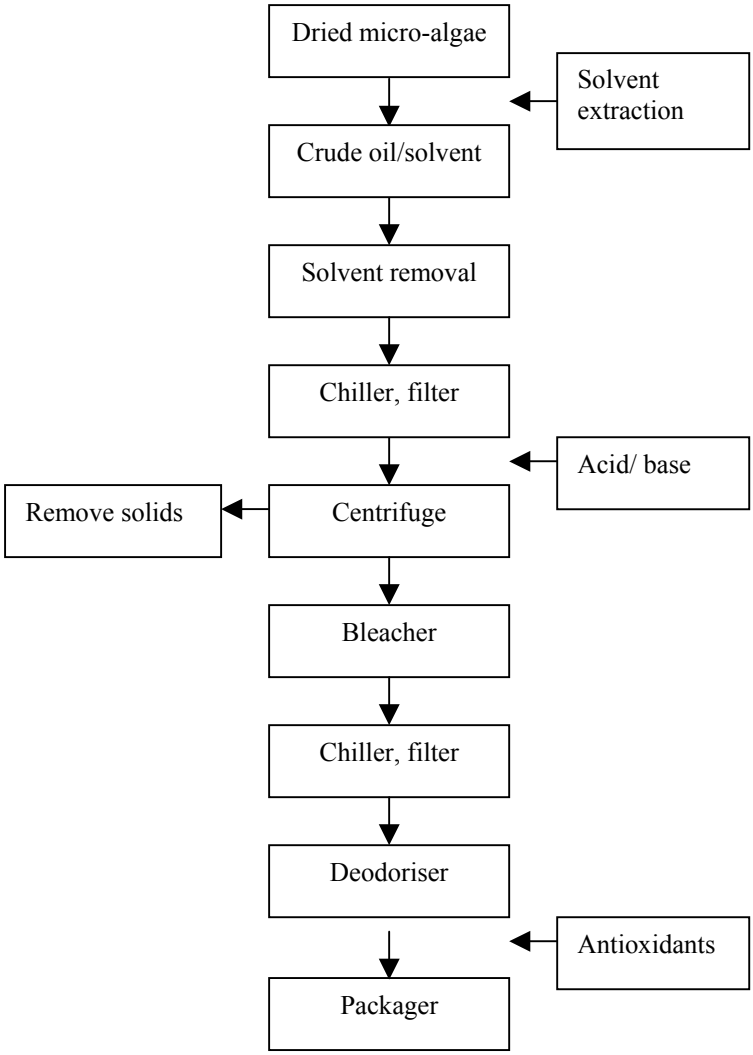


Figure 1: Schematic of the expected DHA oil extraction process

Stability studies

DHA-rich oil

Unsaturated fatty acids (fatty acids with at least one double bond) are readily oxidised by contact with oxygen which limits the quality of the extracted oil. Such oxidation limits the shelf life and quality of the oil by also causing unpleasant oil rancidity to occur. The Applicant provided stability studies that were performed to determine the shelf-life of the DHA-rich oil under various storage conditions. The studies used 0.1% tocopherols as the antioxidant, as well as packaging in containers with limited oxygen content (under a nitrogen atmosphere), preventing contact with light (in sealed drums) and storage at low temperatures. Trials reported the DHA-rich oil is stable for 12 months when stored at 5ºC (as well as –35ºC) under an inert atmosphere. The DHA-rich oil should be used within 4 weeks once sealed containers are opened.

Food products with added DHA-rich oil

Some storage shelf life studies were performed and reported in the Application where food products enriched with oil containing DHA (but not necessarily DHA-rich oil derived from *Ulkenia* sp.) were compared to controls to check the stability of food products containing DHA. The studies reported include products such as energy bars and biscuits which did not show odour differences, or the introduction of rancid or fish-like odours with ageing. Shelf-life trials for different commercial products would need to performed to determine 'best-before' date labelling as part of normal product development trials.

Labelling issues

When DHA-rich oil is added to food the normal ingredient labelling requirements as described in Standard 1.2.4 – Labelling of Ingredients – of the Code will apply. Clause 8 of Standard 1.2.4 describes the requirements for the declaration of food additives. The issue of whether antioxidants contained within the DHA-rich oil need to be labelled for foods containing the oil depends on whether the antioxidant has a technological function in the final food. This is covered and explained in clause 6 and the subsequent editorial note of Standard 1.2.4.

Specifications

The manufacturer's specifications for the extracted oil are listed in Table I, and compared to an approved oil extracted from *Schizochytrium sp*. (contained in Standard 1.3.4 – Identity and Purity, of the Code). As can be seen both are reasonably similar.

Table I: Specifications of the extracted oil

SPECIFICATION	OIL FOR THIS APPLICATION FROM *Ulkenia sp.*	EXTRACTED OIL FROM *Schizochytrium sp.*
Appearance	Fluid to waxy oil	Free flowing oil
Colour	Colourless to pale yellow	Pale to medium yellow
Odour	Characteristic bland to fish-like	Characteristic "fishy"
DHA	Minimum 32%	Minimum 35% and Maximum 45%
Docosapentaenoic acid 22:5n-6 (%)	Minimum 8%	Minimum 10% Maximum 20%
Saturated fat	Maximum 45%	Not stated
Trans fatty acids	Maximum 2.0%	Maximum 2.0%
Peroxide value	Maximum 10 meq/kg	Maximum 3.5 meq/kg
Moisture and volatiles	Maximum 0.1%	Maximum 0.05%
Non-saponifiables	Maximum 2.0%	Maximum 4.5%
Acid value	Maximum 0.5 mg KOH/g	Not stated
Lead	Maximum 0.2 ppm	Maximum 0.2 ppm
Arsenic	Maximum 0.2 ppm	Maximum 0.2 ppm
Mercury	Maximum 0.2 ppm	Maximum 0.2 ppm
Hexane	Maximum 10 ppm	Maximum 10 ppm

Composition of the extracted oil

The typical fatty acid composition of the DHA-rich oil is in Table II and sterol composition is in Table III. This oil is compared in Table II to other similar food oils that contain similar long chain fatty acid profiles, including another DHA rich oil extracted from single cell organisms, *Schizochytrium sp.*, *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[2]. Arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA 22:5n-6), and DHA are commonly found in significant amounts in meats and seafood[3].

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.

[2] 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.

[3] Hui YH, ed. (1996). Bailey's Industrial Oil and Fat Products. 5th edition v.1 New York: John Wiley & Sons. pp444-495.

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[4]. 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 Table III) in DHA-rich oil from *Ulkenia* sp. are cholesterol, 7-dehydrostigmasterol and 4-methyl-chondrillasterol. These are all common in human foods including soy bean oil and rice bran[5].

[4] 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.
[5] Fabritius D (2001) Analytical data Nutrinova PROTOS Biotech, July 25, 2001.

Table 2: Comparison of fatty acid profiles of DHA-rich oil from *Ulkenia 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]	DHA OIL[2]	DHASCO OIL[3]	*Thalassiosira pseudonana*[4]	*Pavlova lutheri*[4]	*Chroomonas salina*[4]	*Laminaria japonica*[5,6]	*Porphyridium cruentum*[7,8]
Lauric	12:0	-	0.4	4.4	Trace	0.3	Trace	-	-
Myristic	14:0	2.7	10.1	12.7	14.3	11.5	8.4	5.4	-
Palmitic	16:0	32.9	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	1.1	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	1.1	2.2	-	-	-	-		-
Arachidonic (ARA)	20:4n-6	-	1.8	-	0.3	Trace	1.0	11.8	19.8
Eicosatetraenoic n-3	20:4n-3	0.8	-	-	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	11.2	13.6	-	-	2.0	0.1	-	-
Docosahexaenoic (DHA)	22:6n-3	45.6	35.0	40.0	3.9	9.4	5.5	-	-

NOTES:

1. Derived from *Ulkenia* sp., information taken from this Application, average of 3 lots
2. Derived from *Schizochytrium* sp.; Monsanto derived 1997 analytical data from 5 bench lots.
3. Derived from *Crypthecodinium cohnii*; oil composition data from Martek Home Page, Martek Biosciences Corp., 1996.
4. 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.
5. S.V. Khtimchenko and I.V. Kulikova, Botanica Marina, *43*, 87-91, 2000.
6. The reported results are from the middle parts of the blade of the brown algae.
7. M.M. Rebolloso Fuentes, G.G. Acien Fernandez, J.A. Sandez Perez and J.L. Guil Guerrero, Food Chemistry, *70*, 345-353, 2000.
8. 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 3: Typical sterol profiles (% of sterols) of different oil sources

Sterol	DHA oil[1]	DHA oil[2]	DHASCO oil[3]	Lyprinol oil[4]	*Laminaria japonica*[5]	Tuna oil[5]
cholesterol	23.1	25	2	31.8	trace	98.5
7-dehydrostigmasterol	30.4					
4-methyl-chondrillasterol	15.7					
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			

NOTES:

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

Conclusion

The proposed use of DHA-rich oil (*Ulkenia* sp.) is as a food/food ingredient, providing a source of omega-3 fatty acids. The use of the extracted DHA-rich oil obtained from the micro-algae *Ulkenia* sp. as a food ingredient is consistent with the use of DHA derived from the micro-algae *Schizochytrium sp*. that is already approved as a novel food in Standard 1.5.1 of the Code.

As a food/food ingredient, DHA-rich oil (*Ulkenia* sp.) undergoes the normal processing and preparation requirements for the particular food to which it is added. The oil itself is prepared using commonly employed techniques of fed-batch fermentation for micro-organisms and food grade oil extraction and purification processes.

Unsaturated fatty acids are readily oxidised by contact with oxygen. The DHA-rich oil is stabilised by: the addition of antioxidants permitted in Standard 1.3.1 – Food Additives – of the Code, primarily tocopherols; packaging in containers with limited oxygen content; preventing contact with light; and storage at low temperatures.

ATTACHMENT 6

Summary of submissions

Submissions received in response to the Initial Assessment Report

A total of five submissions were received in response to the Initial Assessment Report. Only two of these nominated a preferred regulatory option, which was in both cases, Option 2 – Permit the use of DHA-rich oil (*Ulkenia sp*.) as a novel food.

Submitter	Preferred regulatory option	Specific comments
Dietitians Association of Australia (DAA)	No preferred regulatory option stated, however, support for the acceptance of the Application for draft assessment.	• Omega-3 fatty acids have been shown to have many beneficial effects. DHA in particular, has been shown to have an important role in neural development. • Since natural sources of DHA are also sources of eicosapentanoic acid (EPA), the ratio of these fatty acids in foods may be important. • DAA requests that the following be addressed: ❖ A complete analysis of the fatty acid profile of DHA-rich micro-algal oil and how the ratio of DHA to EPA compares with current sources such as meat and fish. ❖ Dietary modelling to determine the potential total intake of DHA for high consumers if DHA-rich micro-algal oil were used in all the proposed products. ❖ Safety assessment, including the safety of increasing DHA intakes considering any changes to fatty acid composition at the cellular level after feeding trials. ❖ The effect of DHA-rich micro-algal oil on the integrity and bioavailability of other nutrients in the foods to which it is added.
Food Technology Association of Victoria (FTA Victoria)	Option 2	No further comments
Department of Human Services South Australia, Food Section	General support for option 2	Concern that there may be conflicting health messages if foods such as sweet biscuits and cakes are marketed as an additional source of omega-3 fatty acids.

New Zealand Food Safety Authority (NZFSA)	No preferred regulatory option stated.	• Clarification is sought on the term 'infant drinks' for the purposes of dietary modelling. Are infant formula and follow-on formula included? • The UK Advisory Committee on Novel Foods and Processes (ACNFP) did not agree with the positive opinion of the German Competent Authority that there is sufficient evidence to support the substantial equivalence of DHA-rich micro-algal oil from *Ulkenia sp*. and the product obtained from *Schizochytrium sp*. • The safety assessment to be undertaken for this Application should not be on the basis of studies undertaken on *Schizochytrium sp*., but rather on studies of DHA-rich oil from *Ulkenia sp*. itself. • The safety assessment should include any safety issues related to use in infant formula. • Dietary modelling in infants and young children should be undertaken in addition to the general dietary modelling. • The stability of DHA-rich micro-algal oil should be investigated and any antioxidants used to limit oxidation should be named. If antioxidants continue to function in the final food, additive labelling may be required.
Australian Food and Grocery Council (AFGC)	No preferred regulatory option stated.	AFGC consider that: • DHA-rich oil is a standardised food under Standard 2.4.1 – Edible oils. • DHA is a natural constituent of the diet and Standard 1.5.1 does not specifically refer to food derived from novel sources, therefore DHA-rich oil (*Ulkenia* sp.) is not 'non-traditional'. • FSANZ has not provided justification for the decision that DHA-rich oil is novel. • If DHA-rich oil is assessed as being safe without the need for any conditions of use, there must be sufficient knowledge in the community to allow safe use and if that is the case the food cannot be novel. Therefore, AFGC assert that if DHA-rich oil is assessed as being safe without any conditions of use it should be declared to be a food, not a novel food.

A late submission was received from the Western Australian Food Advisory Committee. The issues raised in this submission could not be specifically addressed in section 5 of the Draft Assessment Report due to the late receipt. The submission requested a safety assessment be undertaken with a focus on the source organism and the fatty acid profile of the DHA-rich oil (*Ulkenia* sp.).

Submissions received in response to the Draft Assessment Report

A total of seven submissions were received in response to the Draft Assessment Report. Five of these seven submissions support Option 2 – amend the Code to permit the use of DHA-rich oil (*Ulkenia* sp.) as a novel food.

Submitter	**Preferred regulatory option**	**Specific comments**
Melrose Laboratories Pty Ltd (Geoff Steinicke)	Option 2	Support the availability of vegetarian sources of DHA for addition to foods, for vegetarians and vegans.

Food Technology Association of Victoria (FTA Victoria)	Option 2	Concerned about the draft variations to the Code which include removing the conditions of use from the Table to clause 2 for entries regarding DHA from *Schizochytrium* sp. for the following reasons: • Public comment should be specifically sought from stakeholders; and • This amendment is not the subject of this application or any other application. It was suggested that this amendment should be made through the next minor amendments omnibus proposal. FTA agree with actual amendment. The impact analysis in the draft assessment states that consumers will be provided with additional choice based on the approval of DHA-rich oil (*Schizochytrium* sp.). FTA contend that since there is no proposed requirement to label the DHA-rich oil as such, then the additional choice is really a benefit to the manufacturer.
Queensland Health (Gary Bielby)	Option 2 – on the proviso that their comments and questions are addressed in the final assessment report.	The following questions are posed in the submission: • Given that DHA-rich oil (*Ulkenia* sp.) is not stable at temperatures above 5^{o}C, is the applicant proposing to provide storage and shelf-life instructions to food manufacturers and advice on the need to carry out stability tests on finished products containing the oil? • The German Competent Authority has imposed limitations for children aged 1-3 years. Are there any similar restrictions proposed for any population sub-groups (by the applicant), given that DHA-rich oil is intended to be included in infant foods? • The EU requires that 'DHA-rich oil from the micro-algae *Schizochytrium* sp.' be displayed on the product label or in the list of ingredients of foodstuffs containing it. Has FSANZ given consideration to requiring such labels for DHA derived from either *Schizochytrium* sp. or *Ulkenia* sp.?
New Zealand Food Safety Authority (NZFSA)	No preferred option stated.	NZFSA request that FSANZ address the following points in the Final Assessment Report: • The reason why the maximum intake of DHA recommended by the US FDA has not been recommended after assessing this application? Although the toxicity tests for the DHA-rich oil (*Ulkenia* sp.) and the source material *Ulkenia* sp. itself did not indicate any safety issues, and the data from other food sources of DHA confirms the absence of risks, there was limited data overall and FSANZ should consider restrictions. • The need for an upper limit on use since the high consumption figure for children in Australia is about 50% higher than the US FDA figure and the predicted New Zealand intake figures are all higher than the Australian figures.

Australian Food and Grocery Council (AFGC)	Support for permitting the use of DHA-rich oil (*Ulkenia* sp.) based on the safety assessment, but not as a novel food.	The following points were made in the submission: • DHA-rich oil (*Ulkenia* sp.) is not non-traditional and therefore cannot be considered novel. DHA-rich oil (*Ulkenia* sp.) is traditional because DHA itself is traditional as a normal constituent of the (non-vegan) human diet and the definition of 'non-traditional food' in Standard 1.5.1 does not refer to novel sources or processes. DHA is the same whether it is obtained directly from a micro-algal source or indirectly through the food chain from fish. • Although DHA derived from marine micro-algae is regulated as a novel food in the EU, this is legitimate because the EU definition includes reference to novel sources/processes, however, this is not the case in Standard 1.5.1. • Support the conclusions of the risk assessment. • Believe that the risk management conclusions (i.e. no necessity to employ risk management strategies in conjunction with a permission for DHA-rich oil) provide support that DHA-rich oil is not a novel food. • Impact analysis – disadvantage for both industry and consumers in not approving the use of DHA-rich oil (*Ulkenia* sp.) in that it limits competition between suppliers of DHA-rich oils; potential cost to government of not approving its use as it would be necessary for enforcement agencies to ensure any food to which DHA-rich oil is added does not contain DHA-rich oil from *Ulkenia* sp.; additional advantage to manufacturers in having alternative sources of DHA-rich oil and the added competition this brings to the marketplace. • Stated that DHA-rich oil (*Ulkenia* sp.) cannot be considered novel in accordance with the definition for novel food if no conditions of use are specified. • Supports the removal of the conditions of use for DHA derived from Schizochytrium sp. from the Table to Clause 2 of Standard 1.5.1. • Support the inclusion of specifications for DHA-rich oil (*Ulkenia* sp.) in Standard 1.3.4.
NSW Food Authority (Michael Apollonov)	Option 2	Support the conclusions of the draft assessment.
University of Auckland (Michael Deo)	Option 2	Support the approval of DHA-rich oil (*Ulkenia* sp.) for a variety of reasons. Those relevant to FSANZ and this Application include: • Its approval and use in food products may increase the public awareness of the importance of omega-3 fatty acids in the diet. • DHA has an important role in cardiovascular health, inflammation and brain development. • Its approval will provide consumers with additional choice of DHA sources. • Its approval would provide an additional vegetarian source of DHA. • Additional sources of DHA may promote competition and industry innovation leading to a reduced cost for consumers.