DHA-rich algal oil from *Schizochytrium* sp.ONC-T18

A Submission to the UK Food Standards Agency requesting consideration of Substantial Equivalence to DHA-rich algal oil from *Schizochytrium* sp. authorised in accordance with Regulation (EC) No 258/97

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Purpose of the submission

Commission Decision 2003/427/EC of 5 June 2003 authorised the use of oil rich in DHA (docosahexaenoic acid) from the micro-algae *Schizochytrium sp.* as a novel food ingredient under Regulation (EC) No 258/97 in a number of foodstuffs on the EU market. Commission Decision 2009/778/EC of 22 October 2009 authorised the extension of the use of oil rich in DHA from *Schizochytrium sp.* to an additional range of foodstuffs. The Commission Decisions were made in response to submissions relating to a commercial algal oil product obtained from an improved strain of the original wild-type culture, *Schizochytrium sp.* ATCC 20888.

The present submission provides information on the composition, nutritional value, metabolism and intended use of a DHA-rich oil obtained by Ocean Nutrition Canada Limited from the related strain of *Schizochytrium sp.* ONC-T18, and on the level of undesirable substances it contains, and requests an opinion on the substantial equivalence of the oil from *Schizochytrium sp.* ONC-T18 to that presently authorised from *Schizochytrium sp.* in accordance with Article 3.4 of Regulation (EC) No 258/97.

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1. Introduction

Oils in rich in omega-3 fatty acids, predominantly docosahexaenoic acid (DHA), derived from micro-algal sources have been the subject of four authorisation decisions and/or notifications under the EU Novel Food Regulation 258/97.

The first such measure was Commission Decision 2003/427/EC in June of 2003 authorising the use of DHA-rich oil from the thraustochytrid micro-algae *Schizochytrium sp.* in a range of foodstuffs and establishing a specification for the material. This was followed in December 2003 by a notification under Article 5 of the novel food regulation for the placing on the market of a DHA-rich oil derived from a second thraustochytrid micro-algae *Ulkenia sp.* on the grounds of its substantial equivalence with the oil from *Schizochytrium sp.* In 2009 Commission Decisions 2009/777/EC and 2009/778/EC authorised extensions to the approved food uses of the oils from *Ulkenia sp.* and *Schizochytrium sp.* respectively.

A third DHA-rich oil derived from the micro-algae *Crypthecodinium cohnii* was already on the EU market before the Novel Food Regulation came into effect and is therefore legally in use without the need for explicit approval.

Ocean Nutrition Canada (ONC) is a world-wide distributor of fish oil, and the largest manufacturer and refiner of Omega-3 from fish oil in North America. It has the largest independently operated marine research facility focused on oil refining technologies in North America and is a founding member of the Global Organization for EPA and DHA Omega-3 (GOED), an industry association dedicated to advancing industry standards especially in relation to the quality of Omega-3 EPA/DHA oils. ONC has developed a DHA-rich oil, which is extracted from a non-GMO strain of *Schizochytrium sp*. designated as ONC-T18 isolated off the coast of Nova Scotia, Canada.

The *Thraustochytriaceae* are a family of marine micro-algae comprising several related genera and including *Schizochytrium sp.* and *Ulkenia sp.*, DHA-rich oils from both of which are currently authorised for use in foods on the EU market by measures in place under the Novel Regulation as described above. The authorisations for these oils followed submissions made in relation to commercial oil products obtained from an improved variant of wild-type *Schizochytrium sp*. strain ATCC 20888 and from *Ulkenia sp*. strain SAM 2179 respectively (OmegaTech, 2001; Nutrinova, 2005). The close taxonomic relationship between these species of micro-algae and ONC's schizochytrid strain ONC-T18, together with the close compositional similarity of the oil products derived from them, provides the basis for considering DHA-rich oil from *Schizochytrium sp.* ONC-T18 and the presently authorised DHA-rich oil from *Schizochytrium sp.* as substantially equivalent.

This submission characterises Ocean Nutrition Canada's oil product derived from *Schizochytrium sp.* ONC-T18 in terms of its method of production and specification, and provides information on its composition, nutritional value, metabolism, intended use and levels of undesirable substances in comparison

with that for the authorised oil from *Schizochytrium sp.* in support of the case for substantial equivalence under Article 3.4 of the Novel Food Regulation.

2. Characterisation of DHA-rich oil from Schizochytrium sp. ONC-T18

2.1 Description

Common or usual name

The product that is the subject of this submission is extracted and refined oil from the wild-type heterotrophic micro-algae *Schizochytrium sp*. ONC-T18. It is a mixture of triglycerides containing polyunsaturated fatty acids (PUFA) in which the predominant fatty acid (>35%) is docosahexaenoic acid (DHA). There are a number of common or usual names for oils extracted from closely related micro-algae including but not limited to:

- DHA-rich algal oil
- Algal oil
- Omega-3 algal oil

Empirical formula and chemical structure of DHA

The empirical formula for docosahexaenoic acid (DHA) is $C_{22}H_{32}O_2$. The systematic name is 4,7,10,13,16,19-docosahexaenoic acid, and is often written in short-hand form as 22:6n-3 where these numbers indicate the number of carbon atoms in the molecule(22), the number of double bonds (6), and the number of carbon atoms from the methyl terminus to the first double bond (3). The structural formula for DHA is represented below in Figure 1.

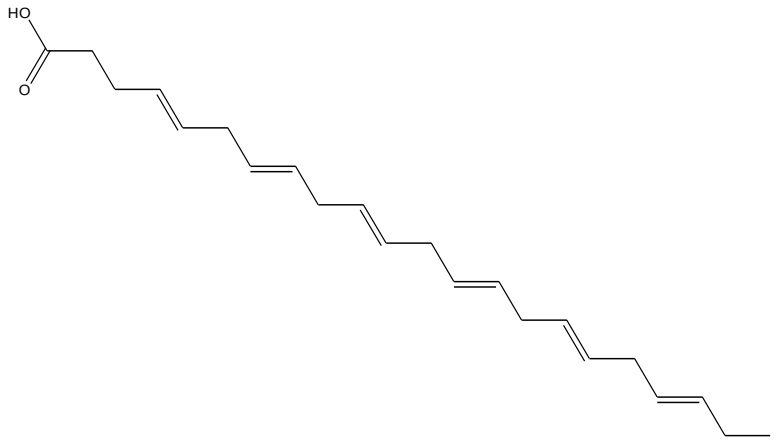


Figure 1: Docosahexaenoic Acid (DHA)

2.2 Method of manufacture

An oil rich in PUFA is produced by a heterotrophic fermentation process with a single cell marine micro-algae of the genus *Schizochytrium,* in particular, *Schizochytrium sp*. ONC-T18. This organism can be grown to a high cell density using a carbon-based substrate. Operating parameters such as

temperature, aeration, agitation and pH are controlled throughout the process to ensure that results, in terms of cell growth and oil production, are reproducible. The components of the fermentation medium are listed at Annex 1.

Cells (biomass) from the liquid fermentation medium are concentrated and dried prior to extraction of the crude oil with propan-2-ol (an EU-permitted extraction solvent). Biomass is separated from the crude oil-solvent mixture by filtration and the solvent is evaporated from the crude oil under vacuum. The crude oil is subsequently refined using processes and techniques common in the edible oil refining industry including acid and alkali treatment, water washing, an optional winterization step, and bleaching. Steam deodorization is the last refining step prior to the addition of EU permitted antioxidants to ensure stability, and packaging in airtight containers. The process is represented schematically in Figure 2. It is essentially the same as that described for the production of the currently authorised oil from *Schizochytrium sp*. (OmegaTech 2001).

Figure 2: Production of DHA-rich oil from *Schizochytrium sp*. ONC-T18.

Production of ONC-T18 DHA-rich oil is in accordance with Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices including quality control (QC) checks at every stage of the production process. Upstream (fermentation) processing includes the sterilization of growth media and all vessels/containers/fermenters used to grow ONC-T18 cells. The fermentation is carried out in the absence of light under axenic conditions. Cells containing oil are dried and exposed to extraction with an organic solvent. Both bleaching

and deodorization use high temperatures under vacuum. All of these steps (from fermentation to deodorization) provide conditions that minimize the risk of contamination with foreign microorganisms.

2.3 Specification

The specification for DHA-rich oil from *Schizochytrium sp.* ONC-T18 manufactured by the above method is set out in Table 1:

Table 1: Specification for DHA-rich oil from *Schizochytrium sp*. ONC-T18

Physical and Chemical Tests		
	Specification	**Test Method**
Colour	Report Actual	Gardner colour
Acid Value	Max. 0.5 mg KOH/g	AOCS CD 3D-63
Peroxide Value (PV)	Max. 5 meq/kg	AOCS Cd 8-53
Moistureand Volitiles	Max 0.01%	AOCS Ca 2d-25
Unsaponifiables	Max 3.5%	AOCS Ca 6a-40
Trans-fatty acids	Max 1%	AOAC 996.06
DHA (Area %)	Min 35%	EP 2003:1352 Method 2.4.29
mg/g	Min 350 mg/g	
Residual propan-2-ol	Max 1 mg/kg	POS SOP IN-LS-113
Elemental Analysis		
Arsenic	<0.1 mg/kg	US EPA 200.8
Copper	<0.05 mg/kg	ISO 8294 Equivalent
Iron	<0.2 mg/kg	ISO 8294 Equivalent
Mercury	<0.04 mg/kg	US EPA 245.6
Lead	<0.01 mg/kg	US EPA 200.8

Certificates of analysis of three batches of oil demonstrating the consistency of compliance with the above specification are presented at Annex 2.

3. Comparison of DHA-rich oils from Schizochytrium sp. ONC-T18 (present submission) and Schizochytrium sp. (Commission Decisions 2003/427/EC and 2009/778/EC)

3.1 Compositional equivalence

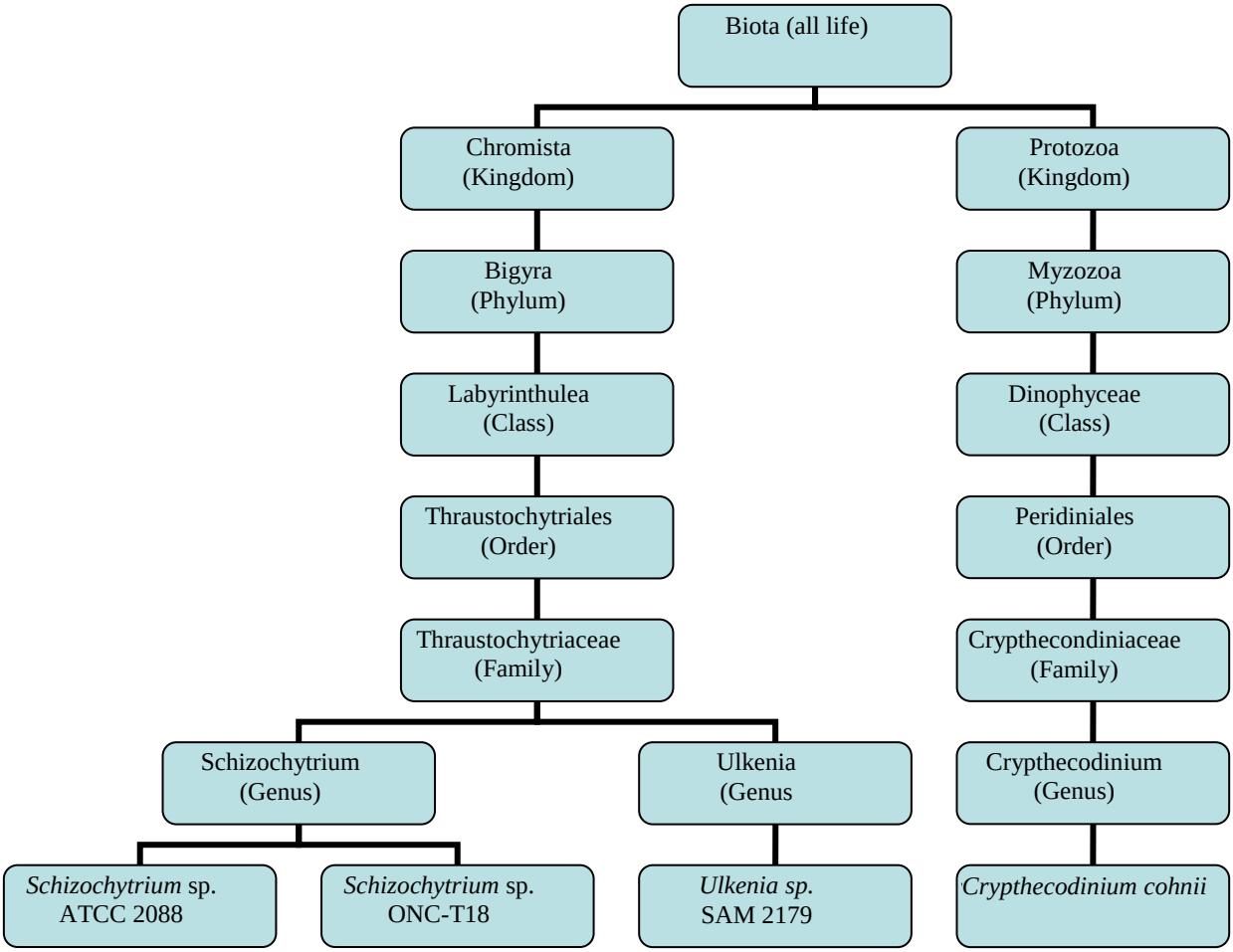
Source organism

The micro-algal family *Thraustochytriaceae* has historically comprised seven genera, *Japanochytrium, Schizochytrium, Ulkenia, Althornia, Diplophrys, Aplanochytrium* and *Thraustochytrium,* all of which are referred to as thraustochytrids. Under this classificatory scheme ONC's strain ONC-T18 has previously been assigned to the genus *Thraustochytrium* (Burja *et al.,* 2006).The genera *Thraustochytrium* , *Schizochytrium* and *Ulkenia* , oils from the latter two of which are the subject of previous authorisations under the EU novel food regulation, comprise marine protists commonly found in marine and estuarine environments.

In recent times the taxonomic structure of the family *Thraustochytriaceae* has been the subject of discussion and the redistribution of some of the component organisms into a broader suite of genera has been proposed, in particular in relation to members of the genus *Schizochytrium* (Yokoyama and Honda, 2007) and the genus *Ulkenia* (Yokoyama, Salleh and Honda 2007). In the light of the on-going debate, ONC commissioned an expert review of the relationship between its thraustochytrid strain ONC-T18 and *Schizochytrium sp.* ATCC 20888, the parent wild-type strain which is the basis of Commission authorisation decision 2003/427/EC. The review has concluded on the basis of their morphological characteristics, their pigment and fatty acid profiles and a comparison of small subunit ribosomal DNA (SSU-rDNA) sequences that, notwithstanding the on-going scientific debate about the taxonomy of the family *Thraustochytriaceae* as a whole, these two organisms are closely related, so much so that strain ONC-T18 is more appropriately to be considered as falling within the genus *Schizochytrium* sensu lato. The report of this study is attached as Annex 3. This conclusion has been supported by an additional independent expert review attached as Annex 4.

The taxonomic relationship between strain ONC-T18 and the source organisms of the micro-organism-derived DHA-rich oils currently on the market can thus be represented as set out in Figure 3.

Figure 3. Taxonomic relationship between source organisms of DHA-rich oils



Ref. World Register of Marine Species (WORMS):
http://www.marinespecies.org/users.php

Specification

DHA-rich oil from *Schizochytrium sp.* ONC-T18 complies with the specification for the oil from *Schizochytrium sp.* as set out in the Annex to Commission Decision 2003/427/EC. The identity of the specifications for the two oils is demonstrated in Table 2.

Table 2: specifications for DHA-rich oils from *Schizochytrium sp.* as per Commission Decision 2004/427/EC and *Schizochytrium sp.* ONC-T18

	oil from *Schizochytrium sp.* (Commission Decision 2003/427/EC)	oil from *Schizochytrium sp.* ONC-T18 (Ocean Nutrition Canada Ltd)
Acid value	0.5mg KOH/g max.	0.5mg KOH/g max.
Peroxide value	5.0 meq/kg oil max.	5.0 meq/kg oil max.
Moisture and volatiles	0.05% max.	0.01% max.
Unsaponifiables	4.5% max.	3.5% max.
Trans-fatty acids	1% max.	1% max.
DHA content	32% min.	35% min.

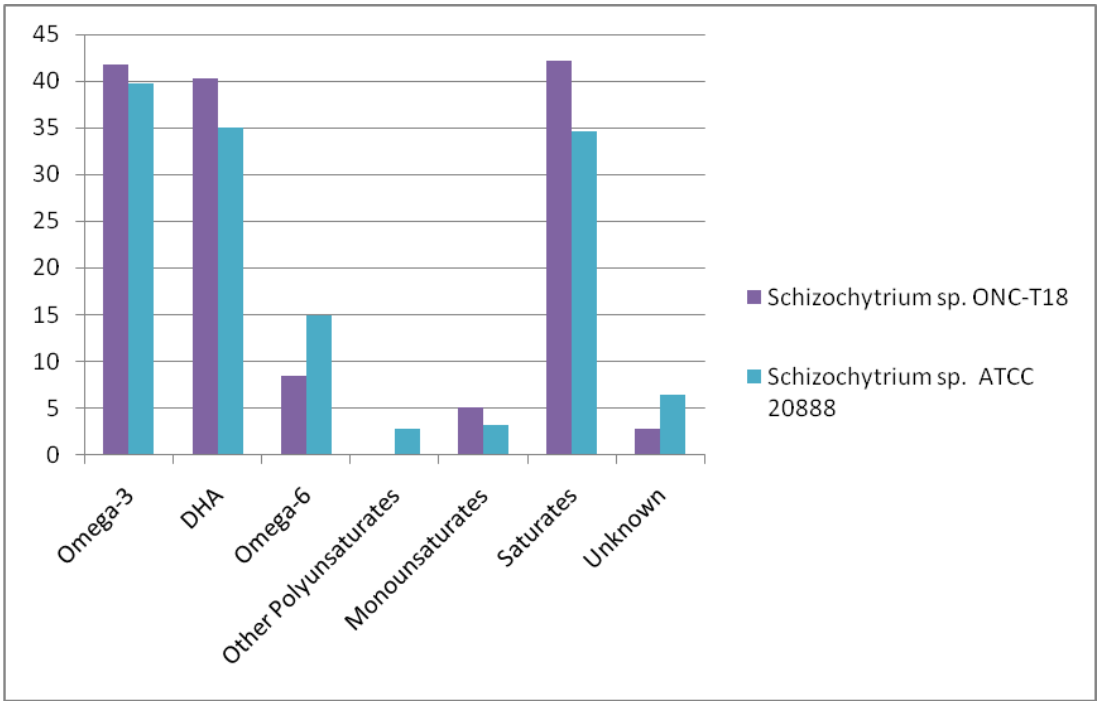
Proximate analysis

Proximate analysis shows Ocean Nutrition Canada's product, like the oil from *Schizochytrium sp.* as presently authorised, to be free from protein and carbohydrate (limit of detection of 0.1%, certificate of analysis Annex 2).

Lipid profile

The fatty acid and sterol contents of the two oils show a high degree of similarity. The fatty acid profiles of both oils are summarised in Figure 4 and presented in detail in Table 3

Figure 4: Fatty acid profiles of oils from *Schizochytrium sp. ex* OmegaTech and *Schizochytrium sp.* ONC-T18



- values for oil from *Schizochytrium sp.* taken from OmegaTech novel food submission
- values for oil from *Schizochytrium sp.* ONC-T18: average analysis of 3 batches

Table 3: Fatty acid profiles of oil from *Schizochytrium sp.* ONC-T18

The analysis of ONC lots 2269, 22630, 22740 and a sample of the currently authorised oil (Martek) were done simultaneously and run on the same instrument sequentially to make data comparison as consistent as possible.

		ONC Lot Analysis			ONC Analysis of Commercial lot of Martek DHA-S	Information from Omega-Tech Novel Food Application Table I-3 (Average Weight %)
Fatty Acid (Composition by Area %)	Formula	22629	22630	22740		
Laurate	12:0	1.1	1.0	1.2	Trace	0.40
Myristate	14:0	13.9	13.2	14.2	4.5	10.11
Palmitate	16:0	26.1	27.0	26.6	13.5	23.68
Palmitoleate	16:1n7	2.0	1.7	3.7	0.2	1.76
Stearate	18:0	0.8	0.8	0.8	0.9	0.45
Oleate	18:1n9	0.7	0.3	0.3	17.1	Not Reported
Vaccenate	18:1n7	1.9	1.5	2.9	0.3	Trace -1.36
Linoleate	18:2n6	0.2	Trace	Trace	1.4	Not Reported
Octadecatetraenoate	18:4n3	0.2	0.2	0.2	0.3	Trace – 0.85
Dihomo-gamma Linolenate (Martek designates as coeluting with Eicosatetraenoate –20:4n-7)	20:3n6	0.1	0.1	Trace	0.3	2.21
Arachidonate	20:4n6	0.2	0.3	0.2	1.0	0.94
Eicosatetraenoate	20:4n3	0.5	0.5	0.4	0.8	0.87
EPA	20:5n3	0.8	1.0	0.8	1.2	2.63
Docosapentaenoate	22:5n6	8.0	8.2	7.5	15.9	13.50
DHA	22:6n3	40.8	41.3	38.6	39.6	35.00
Other	N/Ap	2.8	3.0	2.6	3.3	6.24

3.2 Nutritional value and metabolism

The oil from *Schizochytrium sp.* ONC-T18 has an identical proximate composition and a closely similar lipid (fatty acid and sterol) profile to that of the presently authorised oil from *Schizochytrium sp*. At the intended levels of use (60 – 600 mg DHA-equivalent/100g of food, see Section 3.3), the small differences in their lipid profiles will have no significance for their relative nutritional value or metabolic impact.

3.3 Intended use

The use of the existing oil from *Schizochytrium sp.* is limited by the terms of Commission Decisions 2003/427/EC and 2009/778/EC to specified foods at up to specified maximum levels expressed on an added DHA basis. It is intended that the oil from *Schizochytrium sp.* ONC-T18 will be used in the same way and will therefore replace, rather than add to, intake from the currently authorised oil.

The list of specified foods and maximum levels of use defined by the two Commission Decisions is reproduced in Table 5.

Table 5: authorised uses of DHA-rich oil from *Schizochytrium sp.*[1]

Food	Maximum content of DHA
Dairy products except milk-based drinks	200 mg/100 g or for cheese products 600 mg/100 g
Dairy analogues except drinks	200 mg/100 g or for analogues to cheese products 600 mg/100 g
Spreadable fat and dressings	600 mg/100 g
Breakfast cereals	500 mg/100 g
Food supplements	200 mg per daily dose as recommended by the manufacturer
Dietary foods for special medical purposes	In accordance with the particular nutritional requirements of the persons for whom the products are intended
Foods intended for use in energy-restricted diets for weight reduction	200 mg/meal replacement
Bakery products (breads and bread rolls)	200 mg/100 g
Cereal bars	500 mg/100 g
Non-alcoholic beverages (including milk based beverages)	60 mg/100 ml

[1] as defined by Commission Decisions 2003/427/EC and 2009/778/EC

3.4 Levels of undesirable substances

DHA-rich oil from *Schizochytrium sp.* ONC-T18 is produced from micro-algae grown by controlled fermentation. Operating parameters such as temperature, aeration, agitation and pH are controlled throughout the process to ensure that results, in terms of cell growth and oil production, are reproducible. The end product (refined DHA-rich oil) is tested to ensure compliance with a specification which includes maximum limits for arsenic, copper, iron, mercury, lead, and trans-fatty acids.

The product is a highly refined oil and the absence of protein and carbohydrate as revealed by proximate analysis down to a limit of detection of 0.1% makes it highly unlikely that any allergens will be present.

Upstream (fermentation) processing includes the sterilization of growth media and all vessels/containers/fermenters used to grow ONC-T18 cells and produce oil. Fermentation takes place in the absence of light. Cells containing oil are dried and exposed to extraction with an organic solvent. Both bleaching and deodorization use high temperatures under vacuum. All of these steps (from fermentation to deodorization) provide conditions that minimize the risk of growth of foreign microorganisms. Microbiological testing is nevertheless a routine part of the final QC testing prior to release of the oil to ensure compliance with the following limits:

coliforms	max 10 MPN/g
E. coli	negative
Aerobic Plate Count	<1000 CFU/g
Yeasts and Molds	<100 CFU/g
Salmonella	Negative/25g
Staphylococcus aureus	<10 CFU/g

These procedures ensure that the quality of DHA-rich oil from ONC-T18 is the same as, or better than the oil from *Schizochytrium sp*. presently commercially available.

Toxin production by *Schizochytrium* ONC-T18 is unlikely since there are no reports of toxin production by any of the *Thraustochytriaceae*, the family of which *Schizochytrium* is a member. Nevertheless, samples of ONC-T18 oil and the biomass (freeze-dried) from which it is obtained have been screened for the following algal toxins:

toxin	*m/z*
Domoic Acid	312.1447
Gymnodimine	508.3427
Desmethylspirolide C	692.4526
Azaspiracid-1	842.5055
Azaspiracid-2	856.5211
Azaspiracid-3	828.4898
Pectenotoxin-2	881.4663
Okadaic Acid	803.4582
Dinophysistoxin-1	817.4738
Dinophysistoxin-2	803.4582
Yessotoxin	1141.4706
Prymnesisn-2	1968.8037
Prymnesisn-1	2262.8988

None of the toxins were detected in extracts of the oil or the freeze-dried biomass (report at Annex 5).

4. Other relevant data

4.1 Consumer information

The Commission Decisions authorising the use DHA-rich oil from *Schizochytrium sp.* require that its presence be declared on the labelling of foods containing it. Similar provisions would apply in the case of the oil from *Schizochytrium sp.* ONC-T18. Consumers will therefore be able to identify any foods in which it has been used.

5. Summary and conclusions

- DHA-rich oil from *Schizochytrium sp*. ONC-T18 is a well-characterised product produced under controlled conditions to a reproducible and consistent standard.

- Its source organism is closely related to *Schizochytrium sp*. ATCC 20888, the wild-type parent of the source organism of the DHA-rich oil currently authorised under Novel Food Regulation (EC) No 258/97.

- Analysis shows it to be compositionally closely similar to the presently authorised oil from *Schizochytrium sp*.

- It is intended to be used in the same foods and under the same conditions as the presently authorised oil.

- When used at the intended levels, the nutritional value and metabolism of the oil from *Schizochytrium sp.* ONC-T18 will be indistinguishable from that of the presently authorised oil.

On the basis of the above rationale, this submission requests an opinion on whether the DHA-rich oil from *Schizochytrium sp.* ONC-T18, when produced,

refined and used as described, can be considered substantially equivalent to the DHA-rich oil from *Schizochytrium sp.* currently authorised by Commission Decisions 2003/427/EC and 2009/778/EC.

References

Burja, Radianingtyas, Windust and Barrow, 2006. Isolation and characterization of polyunsaturated fatty acid producing *Thraustochytrium* species: screening of strains and optimization of omega-3 production. Appl. Microbiol. Biotechnol. 72: 1161 – 1169.

Nutrinova 2005. As referred to in Committee Paper ACNFP/72/1, Advisory Committee on Novel Foods and Processes – DHA rich microalgal oil: extension of use. May 2005.

OmegaTech 2001. Application for the Approval of DHA-rich Oil under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients.

Yokoyama and Honda 2007. Taxonomic rearrangement of the genus *Schizochytrium* sensu lato based on morphology, chemotaxonomic characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for *Schizochytrium* an erection of*Aurantiochytrium* and *Oblongichytrium* gen. nov., Mycoscience 48: 199 – 211.

Yokoyama, Salleh, and Honda 2007. Taxonomic rearrangement of the genus *Ulkenia* sensu lato based on morphology, chemotaxonomical characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for *Ulkenia* and erection of *Botryochytrium*, *Parietichytrium*, and *Sicyoidochytrium* gen. nov., Mycoscience 48: 329-341.

ANNEX 1

Annex 1 – Fermentation Ingredients to Prepare Media for ONC-T18

Fermentation Ingredients to Prepare Media for ONC-T18

[redacted]	[redacted]
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[redacted]	[redacted]

ANNEX 2

Annex 2 – Certificates of analysis

Bulk oil (3 Lots)	[redacted]	[redacted]
Proximate analysis	[redacted]	[redacted]
Trans-fatty acids	[redacted]	[redacted]
Metals	[redacted]	[redacted]
Cu and Fe	[redacted]	[redacted]
Microbiol.	[redacted]	[redacted]
Microbiol. (Salmonella, Staph. aureus)	[redacted]	[redacted]
Residual solvents	[redacted]	[redacted]
Moisture & Volatiles	[redacted]	[redacted]

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Bulk Oil
Certificate of Analysis

Product Description: ONC-T18 Algal Oil
O.N.C. Lot #: 22629 **Product/Document Code:** DHA3T4505.03/111001
[redacted] **Retest Date:** Sep. /2011

ANALYSIS	SPECIFICATIONS	RESULTS
Colour	Report Actual (Gardner)	4
Free Fatty Acid (as % Oleic)	Max. 0.25%	0.09
Acid Value	Max. 0.5 mg of KOH/g	0.2
p-Anisidine Value	Max. 15 (at time of release)	8
Peroxide Value	Max. 5 meq/kg (at time of release)	3
Moisture + Volatiles (%)	< 0.01%	< 0.01
Residual Solvent	Max. 1.0 ppm	< 1.0
TOTOX	Max. 23 (duration of shelf-life)	14
Unsaponifiable Matter	Max. 3.5%	1.8
Trans Fat (g/100g)	Report Actual	0.106
Fatty Acid Profile		
DHA (A%)	Report Actual (Target Min. 35%)	42
DHA mg/g (as TG)	Report Actual (Target Min. 350)	390
DHA mg/g (as FFA)	Report Actual	376
Total Omega 3 (A%)	Report Actual	44
Total Omega 3 mg/g (as TG)	Report Actual	409
Total Omega 3 mg/g (as FFA)	Report Actual	395
Antioxidants (based on input oil)		
"IP" Mixed Natural Tocopherol	Min. 3 mg/g	Pass
Ascorbyl Palmitate	Min. 250 ppm	Pass
Microbiological Data		
Coliform	Max 10 MPN/g	< 3
E. coli	Negative	Negative
Salmonella	Negative/25g	Negative
Staphlococcus aureus	< 10 CFU/g	< 10
Total Plate Count	< 1000 CFU/g	< 10
Yeast & Mold	< 100 CFU/g	< 10
Contaminant Data		
Dioxins & Furans (PCDDs and PCDFs)	Max. 1.0 pg WHO-PCDD/FTEQ/g	0.328
Dioxin-Like PCBs*	Max. 3 pg WHO-Dioxin Like PCBs-TEQ/g	0.153
Sum Dioxins & Furans + Dioxin Like PCBs	Max. 4.0 pg WHO-PCDD/F + Dioxin Like PCBs-TEQ/g	0.481
PAHs: Benzo(a)pyrene	Max. 2.0 ppb (µg/kg)	< 0.1
PCBs**	< 0.09 ppm	0.0001778
Total PCBs (Canada only)	Max. 0.1 ppm (mg/kg)	0.000195
Arsenic	< 0.1 ppm (mg/kg)	< 0.01
Iron	< 0.2 ppm (mg/kg)	< 0.1
Lead	< 0.01 ppm (mg/kg)	< 0.01
Copper	< 0.05 ppm (mg/kg)	< 0.05
Cadmium	< 0.01 ppm (mg/kg)	< 0.01
Mercury	< 0.04 ppm (mg/kg)	0.007
Phosphorus	< 10 ppm (mg/kg)	< 5
Strontium (Canada only)	Max. 0.5 ppm (mg/kg)	< 0.2

*Sum of IUPAC No. 81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189

**Sum of IUPAC No. 28, 52, 101, 118, 138, 153 and 180

[redacted]

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Bulk Oil
Certificate of Analysis

Product Description: ONC-T18 Algal Oil
O.N.C. Lot #: 22630 **Product/Document Code:** DHA3T4505.03/111001
[redacted] **Retest Date:** Sep. /2011

ANALYSIS	SPECIFICATIONS	RESULTS
Colour	Report Actual (Gardner)	5
Free Fatty Acid (as % Oleic)	Max. 0.25%	0.14
Acid Value	Max. 0.5 mg of KOH/g	0.3
p-Anisidine Value	Max. 15 (at time of release)	13
Peroxide Value	Max. 5 meq/kg (at time of release)	2
Moisture + Volatiles	< 0.01%	< 0.01
Residual Solvent	Max. 1.0 ppm	< 1.0
TOTOX	Max. 23 (duration of shelf-life)	17
Unsaponifiable Matter	Max. 3.5%	2.3
Trans Fat (g/100g)	Report Actual	0.116
Fatty Acid Profile		
DHA (A%)	Report Actual (Target Min. 35%)	42
DHA mg/g (as TG)	Report Actual (Target Min. 350)	393
DHA mg/g (as FFA)	Report Actual	379
Total Omega 3 (A%)	Report Actual	44
Total Omega 3 mg/g (as TG)	Report Actual	411
Total Omega 3 mg/g (as FFA)	Report Actual	397
Antioxidants (based on input oil)		
"IP" Mixed Natural Tocopherol	Min. 3 mg/g	Pass
Ascorbyl Palmitate	Min. 250 ppm	Pass
Microbiological Data		
Coliform	Max 10 MPN/g	< 3
E. coli	Negative	Negative
Salmonella	Negative/25g	Negative
Staphlococcus aureus	< 10 CFU/g	< 10
Total Plate Count	< 1000 CFU/g	< 10
Yeast & Mold	< 100 CFU/g	< 10
Contaminant Data		
Dioxins & Furans (PCDDs and PCDFs)	Max. 1.0 pg WHO-PCDD/FTEQ/g	0.300
Dioxin-Like PCBs*	Max. 3 pg WHO-Dioxin Like PCBs-TEQ/g	0.154
Sum Dioxins & Furans + Dioxin Like PCBs	Max. 4.0 pg WHO-PCDD/F + Dioxin Like PCBs-TEQ/g	0.454
PAHs: Benzo(a)pyrene	Max. 2.0 ppb (µg/kg)	0.2
PCBs**	< 0.09 ppm	0.0001845
Total PCBs (Canada only)	Max. 0.1 ppm (mg/kg)	0.0001319
Arsenic	< 0.1 ppm (mg/kg)	< 0.01
Iron	< 0.2 ppm (mg/kg)	< 0.1
Lead	< 0.01 ppm (mg/kg)	< 0.01
Copper	< 0.05 ppm (mg/kg)	< 0.05
Cadmium	< 0.01 ppm (mg/kg)	< 0.01
Mercury	< 0.04 ppm (mg/kg)	< 0.005
Phosphorus	< 10 ppm (mg/kg)	< 5
Strontium (Canada only)	Max. 0.5 ppm (mg/kg)	< 0.2

*Sum of IUPAC No. 81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189

**Sum of IUPAC No. 28, 52, 101, 118, 138, 153 and 180

[redacted]

101 Research Drive, Dartmouth, N.S., Canada, B2Y 4T6
Telephone (902) 480-3200 Fax (902) 480-3199

OCEAN NUTRITION CANADA
wellness through innovation

Bulk Oil
Certificate of Analysis

Product Description: ONC-T18 Algal Oil
O.N.C. Lot #: 22740 **Product/Document Code:** DHA3T4505.03/111001
[redacted] **Retest Date:** Sep. /2011

ANALYSIS	SPECIFICATIONS	RESULTS
Colour	Report Actual (Gardner)	4
Free Fatty Acid (as % Oleic)	Max. 0.25%	0.14
Acid Value	Max. 0.5 mg of KOH/g	0.3
p-Anisidine Value	Max. 15 (at time of release)	10
Peroxide Value	Max. 5 meq/kg (at time of release)	3
Moisture + Volatiles (%)	< 0.01%	< 0.01
Residual Solvent	Max. 1.0 ppm	< 1.0
TOTOX	Max. 23 (duration of shelf-life)	16
Unsaponifiable Matter	Max. 3.5%	2.1
Trans Fat (g/100g)	Report Actual	0.110
Fatty Acid Profile		
DHA (A%)	Report Actual (Target Min. 35%)	39
DHA mg/g (as TG)	Report Actual (Target Min. 350)	367
DHA mg/g (as FFA)	Report Actual	354
Total Omega 3 (A%)	Report Actual	41
Total Omega 3 mg/g (as TG)	Report Actual	382
Total Omega 3 mg/g (as FFA)	Report Actual	369
Antioxidants (based on input oil)		
"IP" Mixed Natural Tocopherol	Min. 3 mg/g	Pass
Ascorbyl Palmitate	Min. 250 ppm	Pass
Microbiological Data		
Coliform	Max 10 MPN/g	< 3
E. coli	Negative	Negative
Salmonella	Negative/25g	Negative
Staphlococcus aureus	< 10 CFU/g	< 10
Total Plate Count	< 1000 CFU/g	< 10
Yeast & Mold	< 100 CFU/g	< 10
Contaminant Data		
Dioxins & Furans (PCDDs and PCDFs)	Max. 1.0 pg WHO-PCDD/FTEQ/g	0.300
Dioxin-Like PCBs*	Max. 3 pg WHO-Dioxin Like PCBs-TEQ/g	0.154
Sum Dioxins & Furans + Dioxin Like PCBs	Max. 4.0 pg WHO-PCDD/F + Dioxin Like PCBs-TEQ/g	0.454
PAHs: Benzo(a)pyrene	Max. 2.0 ppb (µg/kg)	< 0.1
PCBs**	< 0.09 ppm	0.0001336
Total PCBs (Canada only)	Max. 0.1 ppm (mg/kg)	0.000206
Arsenic	< 0.1 ppm (mg/kg)	< 0.01
Iron	< 0.2 ppm (mg/kg)	< 0.1
Lead	< 0.01 ppm (mg/kg)	< 0.01
Copper	< 0.05 ppm (mg/kg)	< 0.05
Cadmium	< 0.01 ppm (mg/kg)	< 0.01
Mercury	< 0.04 ppm (mg/kg)	< 0.005
Phosphorus	< 10 ppm (mg/kg)	< 5
Strontium (Canada only)	Max. 0.5 ppm (mg/kg)	< 0.2

*Sum of IUPAC No. 81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189

**Sum of IUPAC No. 28, 52, 101, 118, 138, 153 and 180

[redacted]

101 Research Drive, Dartmouth, N.S., Canada, B2Y 4T6
Telephone (902) 480-3200 Fax (902) 480-3199

[redacted]

CERTIFICATE OF ANALYSIS

[redacted]

Sample Matrix: FOOD
Samples Received: 3

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Method Reference
Ash	3	N/A	[redacted]	CAM SOP-00713	AOAC 923.03
Calories	3	N/A		CAM WI-00708	Calculation
Carbohydrates	3	N/A		CAM WI-00708	Calculation
Fat (total) by Gravimetric Analysis	3	2010/07/30			AOAC methodology
KJ	3	N/A		CAM WI-00708	Calculation
Moisture (Karl Fischer)	3	N/A		CAM SOP-00735	AOAC 926.08 925.10
Protein	3	N/A		CAM SOP-00711	AOAC 992.15

* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

Encryption Key

Please direct all questions regarding this Certificate of Analysis to your Project Manager.

[redacted]

[redacted]

RESULTS OF ANALYSES OF FOOD

[redacted]

	Units	DHA3T4505 LOT 22629	DHA3T4505 LOT 22630	DHA3T4505 LOT 22740	RDL	QC Batch
Nutritional Parameters						
KJ	/100g	3766	3765	3765	1	2216671
Moisture (Karl Fischer)	g/100g	ND	ND	ND	0.5	2223648
Ash	g/100g	ND	ND	ND	0.1	2222147
Fat (gravimetric)	g/100g	100	100	100	N/A	2221869
Calories	/100g	900	900	900	1	2216669
Protein	g/100g	ND	ND	ND	0.10	2223663
Carbohydrates	g/100g	ND	ND	ND	0.1	2216670

ND = Not detected
RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

[redacted]

GENERAL COMMENTS

Results relate only to the items tested.

[redacted]

QA/QC Batch Num Init	QC Type	Parameter	Date Analyzed yyyy/mm/dd	Value	Recovery	Units	QC Limits
2221869 JCH	QC Standard	Fat (gravimetric)	[redacted]		100	%	N/A
	RPD	Fat (gravimetric)		0.5		%	N/A
	Method Blank	Fat (gravimetric)		0.00130		g/100g	
	RPD [GP5696-01]	Fat (gravimetric)		0.05		%	N/A
2222147 FC	QC Standard	Ash			100	%	N/A
	Method Blank	Ash		ND, RDL=0.1		g/100g	
	RPD	Ash		0.2		%	20
2223648 TJO	QC Standard	Moisture (Karl Fischer)			101	%	N/A
	RPD [GP5696-01]	Moisture (Karl Fischer)		NC		%	N/A
2223663 OK	QC Standard	Protein			97	%	93 - 107
	Method Blank	Protein		ND, RDL=0.10		g/100g	
	RPD	Protein		2.0		%	20

N/A = Not Applicable
Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.
QC Standard: A blank matrix to which a known amount of the analyte has been added. Used to evaluate analyte recovery.
Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.
NC (RPD): The RPD was not calculated. The level of analyte detected in the parent sample and its duplicate was not sufficiently significant to permit a reliable calculation.

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

[redacted]

Sample Matrix: FOOD
Samples Received: 3

Analyses	Quantity	Date Extracted	Date Analyzed	Laboratory Method	Method Reference
Fatty Acid Profile by GC/FID (1)	3	[redacted]		CAM SOP-00702	AOAC 996.06

* RPDs calculated using raw data. The rounding of final results may result in the apparent difference.

(1) "Note: Total fatty acids and individual fatty acids are expressed as the triglycerides. Summed fatty acid groups are expressed as the fatty acids"

[redacted]

Total cover pages: 1

[redacted]

[redacted]

RESULTS OF ANALYSES OF FOOD

[redacted]		GS7519	GS7520	GS7521		
Sampling Date		[redacted]				
	Units	**LOT 22629 DHA3T4505**	**LOT 22630 DHA3TY505**	**LOT 22740 DHA3T4505**	**RDL**	**QC Batch**
Nutritional Parameters						
Trans-Fatty Acids	g/100g	0.106	0.116	0.110	0.001	2231673

RDL = Reportable Detection Limit
QC Batch = Quality Control Batch

[redacted]

RESULTS OF ANALYSES OF FOOD

Fatty Acid Profile by GC/FID: Fatty acids were detected in the method blank at a level marginally above the detection limit. Sample results have not been blank corrected. Those results at or near the detection limit may be biased high.

Results relate only to the items tested.

[redacted]

QA/QC Batch Num Init	QC Type	Parameter	Date Analyzed yyyy/mm/dd	Value	Recovery	Units	QC Limits
2231673 RBA	Method Blank	Trans-Fatty Acids	[redacted]	<0.001		g/100g	
	RPD	Trans-Fatty Acids		4.1		%	20

Duplicate: Paired analysis of a separate portion of the same sample. Used to evaluate the variance in the measurement.
Method Blank: A blank matrix containing all reagents used in the analytical procedure. Used to identify laboratory contamination.

[redacted]

[redacted]

Analysis of Samples

[redacted] Sample ID:			107954-1	107954-1 Dup	107954-2
Client Sample ID:			Lot 22630 DHA3T4505	Lab Duplicate	Lot 22629 DHA3T4505
Date Sampled:					
Analytes	**Units**	**RL**			
Aluminum	mg/kg	1	< 1	< 1	< 1
Antimony	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Arsenic	mg/kg	0.01	< 0.01	< 0.01	< 0.01
Barium	mg/kg	1	< 1	< 1	< 1
Beryllium	mg/kg	0.02	< 0.02	< 0.02	< 0.02
Bismuth	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Boron	mg/kg	1	< 1	< 1	< 1
Cadmium	mg/kg	0.01	< 0.01	< 0.01	< 0.01
Calcium	mg/kg	5	< 5	< 5	< 5
Chromium	mg/kg	1	< 1	< 1	< 1
Cobalt	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Copper	mg/kg	0.05	< 0.05	< 0.05	< 0.05
Iron	mg/kg	0.5	< 0.5	< 0.5	< 0.5
Lead	mg/kg	0.01	< 0.01	< 0.01	< 0.01
Lithium	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Magnesium	mg/kg	2	< 2	< 2	< 2
Manganese	mg/kg	0.05	< 0.05	< 0.05	< 0.05
Mercury	mg/kg	0.005	< 0.005	< 0.005	< 0.005
Molybdenum	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Nickel	mg/kg	0.01	< 0.01	< 0.01	< 0.01
Potassium	mg/kg	5	< 5	< 5	< 5
Rubidium	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Selenium	mg/kg	1	< 1	< 1	< 1
Silver	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Sodium	mg/kg	10	< 10	< 10	< 10
Strontium	mg/kg	0.2	< 0.2	< 0.2	< 0.2
Tellurium	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Thallium	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Tin	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Uranium	mg/kg	0.1	< 0.1	< 0.1	< 0.1
Vanadium	mg/kg	1	< 1	< 1	< 1
Zinc	mg/kg	0.1	< 0.1	< 0.1	< 0.1

This report relates only to the sample(s) and information provided to the laboratory.

RL = Reporting Limit

[redacted]

[redacted]

Analysis of Samples

[redacted] Sample ID:			107954-3
Client Sample ID:			Lot 22740 DHA3T4505
Date Sampled:			
Analytes	**Units**	**RL**	
Aluminum	mg/kg	1	< 1
Antimony	mg/kg	0.1	< 0.1
Arsenic	mg/kg	0.01	< 0.01
Barium	mg/kg	1	< 1
Beryllium	mg/kg	0.02	< 0.02
Bismuth	mg/kg	0.1	< 0.1
Boron	mg/kg	1	< 1
Cadmium	mg/kg	0.01	< 0.01
Calcium	mg/kg	5	< 5
Chromium	mg/kg	1	< 1
Cobalt	mg/kg	0.1	< 0.1
Copper	mg/kg	0.05	< 0.05
Iron	mg/kg	0.5	< 0.5
Lead	mg/kg	0.01	< 0.01
Lithium	mg/kg	0.1	< 0.1
Magnesium	mg/kg	2	< 2
Manganese	mg/kg	0.05	< 0.05
Mercury	mg/kg	0.005	< 0.005
Molybdenum	mg/kg	0.1	< 0.1
Nickel	mg/kg	0.01	0.01
Potassium	mg/kg	5	< 5
Rubidium	mg/kg	0.1	< 0.1
Selenium	mg/kg	1	< 1
Silver	mg/kg	0.1	< 0.1
Sodium	mg/kg	10	< 10
Strontium	mg/kg	0.2	< 0.2
Tellurium	mg/kg	0.1	< 0.1
Thallium	mg/kg	0.1	< 0.1
Tin	mg/kg	0.1	< 0.1
Uranium	mg/kg	0.1	< 0.1
Vanadium	mg/kg	1	< 1
Zinc	mg/kg	0.1	< 0.1

[redacted]

General Report Comments

Portions of the samples were prepared by Microwave Assisted Digestion in nitric acid.
The resulting solutions were analyzed for trace elements by ICP-MS.
Mercury was analysed by Cold Vapour AAS.

[redacted]

QA/QC Report

[redacted] Sample ID:			RB000642
Type:			Blank
Analytes	**Units**	**RL**	
Aluminum	mg/kg	1	< 1
Antimony	mg/kg	0.1	< 0.1
Arsenic	mg/kg	0.01	< 0.01
Barium	mg/kg	1	< 1
Beryllium	mg/kg	0.02	< 0.02
Bismuth	mg/kg	0.1	< 0.1
Boron	mg/kg	1	< 1
Cadmium	mg/kg	0.01	< 0.01
Calcium	mg/kg	5	< 5
Chromium	mg/kg	1	< 1
Cobalt	mg/kg	0.1	< 0.1
Copper	mg/kg	0.05	< 0.05
Iron	mg/kg	0.5	< 0.5
Lead	mg/kg	0.01	< 0.01
Lithium	mg/kg	0.1	< 0.1
Magnesium	mg/kg	2	< 2
Manganese	mg/kg	0.05	< 0.05
Mercury	mg/kg	0.005	< 0.005
Molybdenum	mg/kg	0.1	< 0.1
Nickel	mg/kg	0.01	< 0.01
Potassium	mg/kg	5	< 5
Rubidium	mg/kg	0.1	< 0.1
Selenium	mg/kg	1	< 1
Silver	mg/kg	0.1	< 0.1
Sodium	mg/kg	10	< 10
Strontium	mg/kg	0.2	< 0.2
Tellurium	mg/kg	0.1	< 0.1
Thallium	mg/kg	0.1	< 0.1
Tin	mg/kg	0.1	< 0.1
Uranium	mg/kg	0.1	< 0.1
Vanadium	mg/kg	1	< 1
Zinc	mg/kg	0.1	< 0.1

[redacted]

Ocean Nutrition Canada Ltd.
101 Research Drive
NS N2Y 4T6 Dartmouth
Canada

Certificate of analyses No. 201007003

[redacted]

Sample said to be	: See Marked
Packing	: Tinen Can
Sample quantity	: 30 ml
Sample temperature	: Ambient
Sample sealed	: Yes, lid seal
Marked	: Lotnr. : 22629
	Description : DHA3T4505 Algal Oil

Test Results:

Metal and Element analysis

Iron (Fe) (eq.ISO 8294)	less than 0,1	mg/kg
Cu (eq.ISO 8294)	less than 0,05	mg/kg

[redacted]

[redacted]

[redacted]

Ocean Nutrition Canada Ltd.
101 Research Drive
NS N2Y 4T6 Dartmouth
Canada

Certificate of analyses No. 201007004

[redacted]

Sample said to be	: See Marked
Packing	: Tinen Can
Sample quantity	: 30 ml
Sample temperature	: Ambient
Sample sealed	: Yes, lid seal
Marked	: Lotnr. : 22630
	Description : DHA3T4505 Algal Oil

Test Results:

Metal and Element analysis		
Iron (Fe) (eq.ISO 8294)	less than 0,1	mg/kg
Cu (eq.ISO 8294)	less than 0,05	mg/kg

[redacted]

[redacted]

[redacted]

Ocean Nutrition Canada Ltd.
101 Research Drive
NS N2Y 4T6 Dartmouth
Canada

Certificate of analyses No. 201007005

[redacted]

Sample said to be	: See Marked
Packing	: Tinen Can
Sample quantity	: 30 ml
Sample temperature	: Ambient
Sample sealed	: Yes, lid seal
Marked	: Lotnr. : 22740
	Description : DHA3T4505 Algal Oil

Test Results:

Metal and Element analysis

Iron (Fe) (eq.ISO 8294)	less than 0,1	mg/kg
Cu (eq.ISO 8294)	less than 0,05	mg/kg

[redacted]

[redacted]

[redacted]

Analytical Results

Desc. 1: Product Name: DHA3T4505
Desc. 2: [redacted]
Desc. 3: Lot #: 22629
Desc. 4: Qty: 3

Laboratory ID: 322103427
Condition Rec'd: NORMAL
Temp Rec'd (°C): 20

Analyte	Result	Units	Method Reference
Aerobic Colony Count USP	<10	CFU/g	USP32,NF27,2009,61
Coliforms	<3	MPN/g	MFHPB-19
E. coli USP	Negative	-	USP32,NF27,2009,62
Yeasts and Molds USP			USP32,NF27,2009,61
Yeast	<10	CFU/g	
Mold	<10	CFU/g	

Desc. 1: Product Name: DHA3T4505
Desc. 2: [redacted]
Desc. 3: Lot #: 22630
Desc. 4: Qty: 3

Laboratory ID: 322103437
Condition Rec'd: NORMAL
Temp Rec'd (°C): 20

Analyte	Result	Units	Method Reference
Aerobic Colony Count USP	<10	CFU/g	USP32,NF27,2009,61
Coliforms	<3	MPN/g	MFHPB-19
E. coli USP	Negative	-	USP32,NF27,2009,62
Yeasts and Molds USP			USP32,NF27,2009,61
Yeast	<10	CFU/g	
Mold	<10	CFU/g	

Desc. 1: Product Name: DHA3T4505
Desc. 2: [redacted]
Desc. 3: Lot #: 22740
Desc. 4: Qty: 3

Laboratory ID: 322103449
Condition Rec'd: NORMAL
Temp Rec'd (°C): 20

Analyte	Result	Units	Method Reference
Aerobic Colony Count USP	<10	CFU/g	USP32,NF27,2009,61
Coliforms	<3	MPN/g	MFHPB-19
E. coli USP	Negative	-	USP32,NF27,2009,62
Yeasts and Molds USP			USP32,NF27,2009,61
Yeast	<10	CFU/g	
Mold	<10	CFU/g	

[redacted]

[redacted]

Analytical Results

[redacted]

[redacted]

[redacted]

Analytical Results

Desc. 1:	Product Name: DHA3T4505	**Laboratory ID:**	327268529
Desc. 2:	[redacted]	**Condition Rec'd:**	NORMAL
Desc. 3:	Lot #: 22629	**Temp Rec'd (°C):**	20
Desc. 4:	Qty: 125mL		

Analyte	Result	Units	Method Reference
Salmonella	Negative	/25g	MFHPB 20
Staphylococcus aureus	<10	CFU/g	MFHPB 21

[redacted]

Desc. 1:	Product Name: DHA3T4505	**Laboratory ID:**	327268536
Desc. 2:	[redacted]	**Condition Rec'd:**	NORMAL
Desc. 3:	Lot #: 22630	**Temp Rec'd (°C):**	20
Desc. 4:	Qty: 125mL		

Analyte	Result	Units	Method Reference
Salmonella	Negative	/25g	MFHPB 20
Staphylococcus aureus	<10	CFU/g	MFHPB 21

[redacted]

Desc. 1:	Product Name: DHA3T4505	**Laboratory ID:**	327268543
Desc. 2:	[redacted]	**Condition Rec'd:**	NORMAL
Desc. 3:	Lot #: 22740	**Temp Rec'd (°C):**	20
Desc. 4:	Qty: 125mL		

Analyte	Result	Units	Method Reference
Salmonella	Negative	/25g	MFHPB 20
Staphylococcus aureus	<10	CFU/g	MFHPB 21

[redacted]

[redacted]

[redacted]

ANALYTICAL REPORT

Sample Description: DHA3T4505 Lot #22629

PO#: Q001001

Analysis Results:

Analyte	Result	Units
Residual Solvents GC/MS		
Isopropanol	<1.0	ppm

[redacted]

Results reported on as received basis unless otherwise specified. This report applies to the analysis done on the sample submitted for testing and is not necessarily indicative of the quality or condition of any other sample of an apparently identical or similar nature. As a mutual protection to clients, the public and this laboratory, all reports are submitted as the confidential property for the use of the client to whom it is addressed, and authorization for publication of statements, conclusions or extracts from or regarding our reports is reserved pending our written authorization

[redacted]

[redacted]

ANALYTICAL REPORT

Sample Description: DHA3T4505 Lot #22630

PO#: Q001001

Analysis Results:

Analyte	Result	Units
Residual Solvents GC/MS		
Isopropanol	<1.0	ppm

[redacted]

Results reported on as received basis unless otherwise specified. This report applies to the analysis done on the sample submitted for testing and is not necessarily indicative of the quality or condition of any other sample of an apparently identical or similar nature. As a mutual protection to clients, the public and this laboratory, all reports are submitted as the confidential property for the use of the client to whom it is addressed, and authorization for publication of statements, conclusions or extracts from or regarding our reports is reserved pending our written authorization.

[redacted]

[redacted]

ANALYTICAL REPORT

Sample Description: DHA3T4505 Lot #22740

PO#: Q001001

Analysis Results:

Analyte	Result	Units
Residual Solvents GC/MS		
Isopropanol	<1.0	ppm

[redacted]

Results reported on as received basis unless otherwise specified. This report applies to the analysis done on the sample submitted for testing and is not necessarily indicative of the quality or condition of any other sample of an apparently identical or similar nature. As a mutual protection to clients, the public and this laboratory, all reports are submitted as the confidential property for the use of the client to whom it is addressed, and authorization for publication of statements, conclusions or extracts from or regarding our reports is reserved pending our written authorization

[redacted]

[redacted]

[redacted]

[redacted]

ANALYTICAL REPORT

Sample Description: Oil sample Lot 22629

PO#: PO13941

Analysis Results:

Analyte	Result	Units
Moisture & Volatiles (Vacuum Oven)	<0.01	%

[redacted]

[redacted]

[redacted]

ANALYTICAL REPORT

Sample Description: Oil sample Lot 22630

PO#: PO1394 I

Analysis Results:

Analyte	Result	Units
Moisture & Volatiles (Vacuum Oven)	<0.01	%

[redacted]

[redacted]

[redacted]

ANALYTICAL REPORT

Sample Description: Oil sample Lot 22740

PO#: PO13941

Analysis Results:

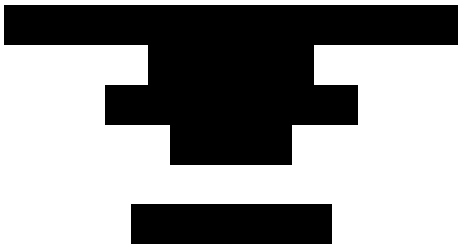
Analyte	Result	Units
Moisture & Volatiles (Vacuum Oven)	<0.01	%

[redacted]

[redacted]

Annex 3

Annex 3 – Taxonomic Report by [redacted]



Taxonomic Report, prepared for Ocean Nutrition Canada

Brief History of Taxonomic Classification:

The Labyrinthulomycota is a taxonomic phylum consisting of two families (Olive, 1975). One family (the Thraustochytriaceae) consists of several genera commonly referred to as "thraustochytrids". The traditional genera within the thraustochytrids are distinguishable based on the presence of certain morphological features, except for the genus *Thraustochytrium* (Sparrow, 1936), which serves as a "catch-all" for the group and contains members that do not show the distinguishing character(s) of any other genus. The other traditional genera include:

(1) *Japanochytrium* (Kobayashi and Ookubo, 1953), whose species are distinguishable by the presence of a swelling (the supsoral apophysis) just below the sporulating structure,

(2) *Schizochytrium* (sensu stricto) (Goldstein and Belsky, 1964), whose members are characterized by the sporangia undergoing vegetative mitosis (successive bipartitioning) before the formation of spores,

(3) *Ulkenia* (Gaertner, 1977), whose members are identified by the presence of an amoeboid protoplasm being released from the sporangium prior to cleavage into zoospores,

(4) *Althornia* (Jones and Alerman, 1971), whose members lack two of the sub-cellular structures exclusive to the Labyrinthulomycota (bothrosomes and an ectoplasmic net),

(5) *Diplophrys* (Barker, 1868), which also lacks bothrosomes, and

(6) *Aplanochytrium* ((Bahnweg and Sparrow, 1972) emend. Leander and Porter, 2000), members of which display a unique gliding motility.

By the 1990's, molecular phylogenetics began to indicate that the traditional morphological characteristics used to distinguish these genera within the thraustochytrids did not match the evolutionary relationships within the group. By this point, it had also become clear that some of the morphological characteristics used to define species and genera varied depending on age of culture and nutrient conditions (Booth and Miller 1968, Wethered and Jennings 1985, Taoka and Yousuke 2008). Thus, the taxonomic state of the thraustochytrids remains fairly turbulent.

Of particular importance to this discussion is Yokoyama and Honda's 2007 taxonomic rearrangement of *Schizochytrium*, which resulted in the erection of two new genera (*Oblongichytrium* and *Aurantiochytrium*) and an amended description of the genus *Schizochytrium*. These three genera can be distinguished with fatty acid and pigment profiles, in addition to sequence and morphological data.

(2b) *Schizochytrium* (emend. Yokoyama and Honda, 2007) members possess only beta-carotene as a carotenoid pigment and 20% arachidonic acid. Colonies are large because of

successive bipartitioning.

(7) *Oblongichytrium* (Yokoyama and Honda, 2007) members possess canthaxanthin, beta-carotene, abundant n-3 docosapentaenoic acid, and little n-6 docosapentanoic acid. Colonies are also large due to successive bipartitioning.

(8) *Aurantiochytrium* (Yokoyama and Honda, 2007) members possess astaxanthin, phoenicoxanthin, canthaxanthin, and beta-carotene as well as arachidonic acid and docosahexaenoic acid. Colonies are smaller, but sporangia still undergo successive bipartitioning.

In 2007, Yokoyama, Salleh, and Honda also amended the description for *Ulkenia* and described three new genera (*Botryochytrium*, *Parietichytrium*, and *Scyiodochytrium*). This rearrangement of *Ulkenia* also bears importance to this discussion. These new generic descriptions also include pigment and fatty acid evaluation.

(3b) *Ulkenia* (emend. Yokoyama, Salleh, Honda, 2007) species possess astaxanthin, phenicoxanthin, echinenon, and beta-carotene. The sporangia "creep around" like amoeba during certain stages.

(9) *Botryochytrium* species possess canthaxanthin, echinoenon, beta-carotene, and n-6 docosapentaenoic acid. The sporangial cell wall disappears, and a naked star-shaped protoplasm remains.

(10) *Parietichytrium* species posess beta-carotene and docosatetraenoic acid. Members of this genus display a division pattern resulting in a star-shaped sporangium with an intact cell wall.

(11) *Sicyoidochytrium* species possess canthaxanthin, echinenon, and beta-carotene. The naked sporangial protoplast divides by "pinching and pulling" to form zoospores.

Evaluation of ONC T-18:

As per my instructions, this strain was grown on solid agar media (KMV or equivalent) by ONC to evaluate gross morphological characters. Figure 1 shows ONC T18 displaying successive bipartitioning.

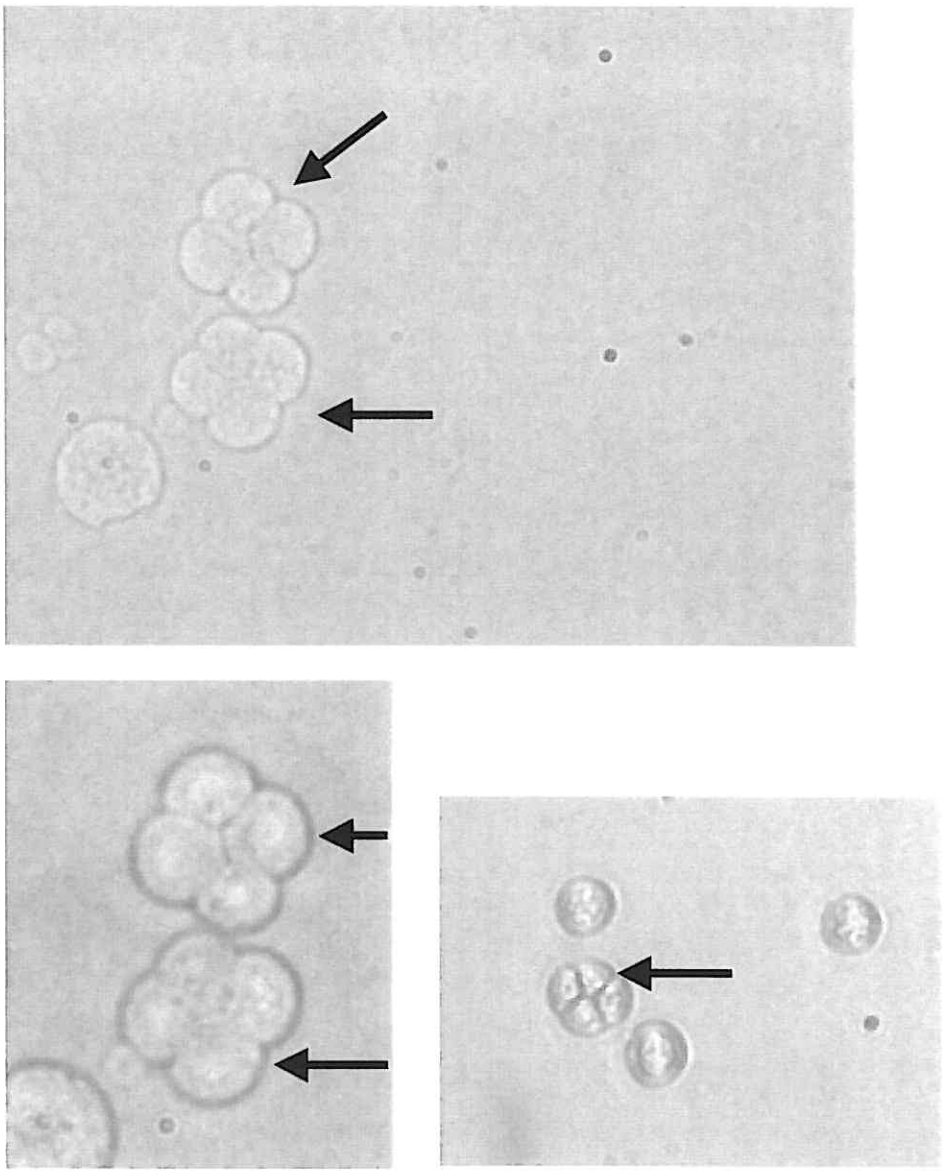
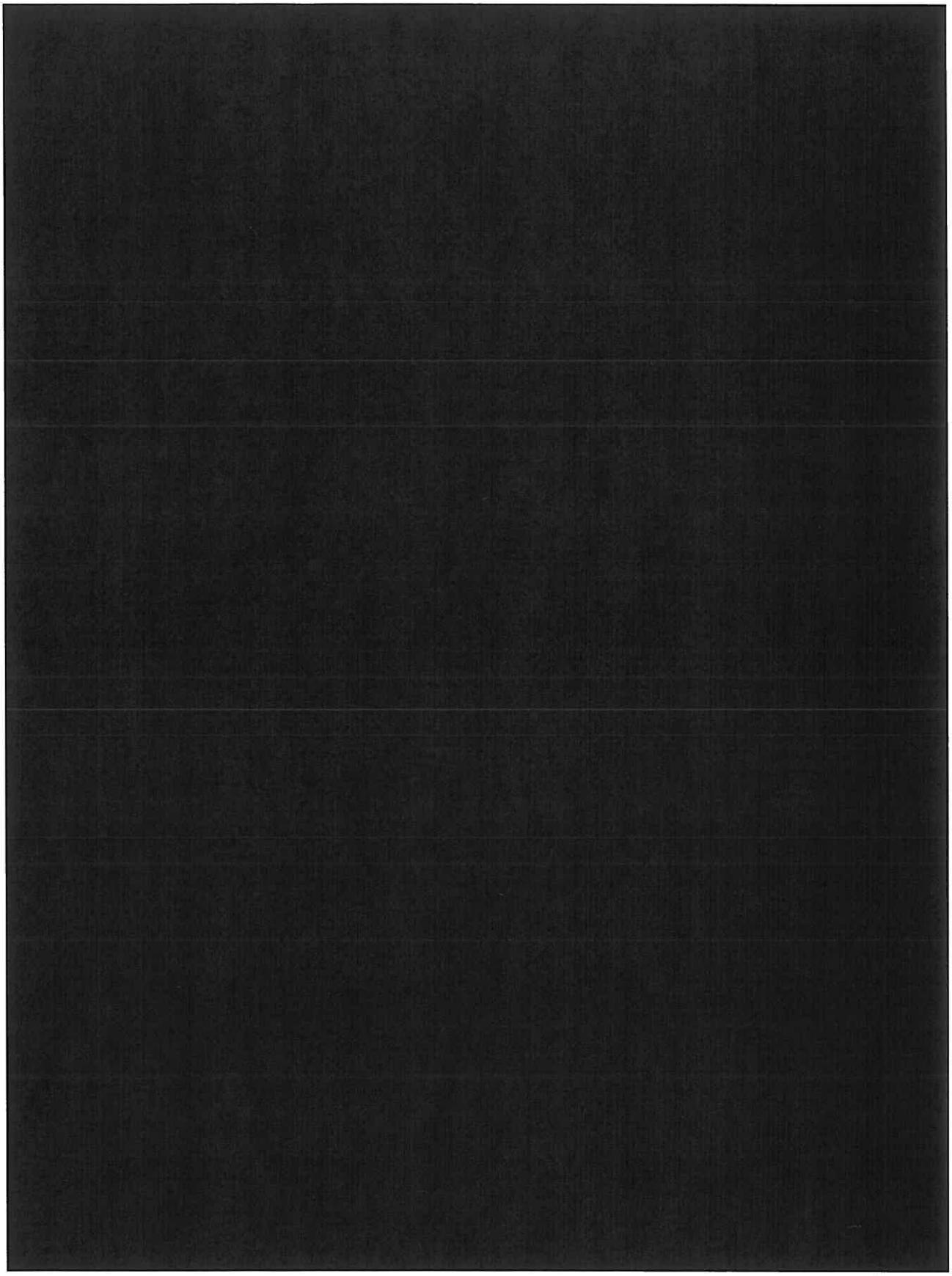


Figure 1. ONC T-18 on solid agar, showing successive bipartitioning (arrows).

Based on several images indicating successive bipartitioning in this strain, I began to suspect that this strain was a close relative to (or a member of) the *Schizochytrium, Oblongichytrium,* or *Aurantiochytrium* genera. Video footage provided by ONC suggests the presence of naked protoplasts, suggesting possible affiliation with *Ulkenia*. Personal communication via [redacted] (ONC) verified that no amoeboid cells were observed. In addition, no star-shaped protoplasts or pinching and pulling during zoospore formation were obvious. Thus, allegiance with *Ulkenia*, *Sicyoidochytrium, Parietichytrium,* or *Botryochytriu* was not strongly supported. However, because the strain does appear to have a naked protoplast stage, further investigation was necessary. [redacted]



[redacted]

I was supplied with three ssurDNA sequences by ONC for taxonomic evaluation. After alignment with other thraustochytrid sequences from Genbank, a bootstrap Maximum Liklihood phylogenetic tree (figure 4) was generated in GARLI (Zwickl, 2006). The selected sequences represent taxa from all genera within the family, and includes representation of the Labyrinthulaceae family. Figure 4 indicates that ONC T18 is closely related to *Schizochytrium*, *Thraustochytrium*, and an *Aurantiochytrium* strain.

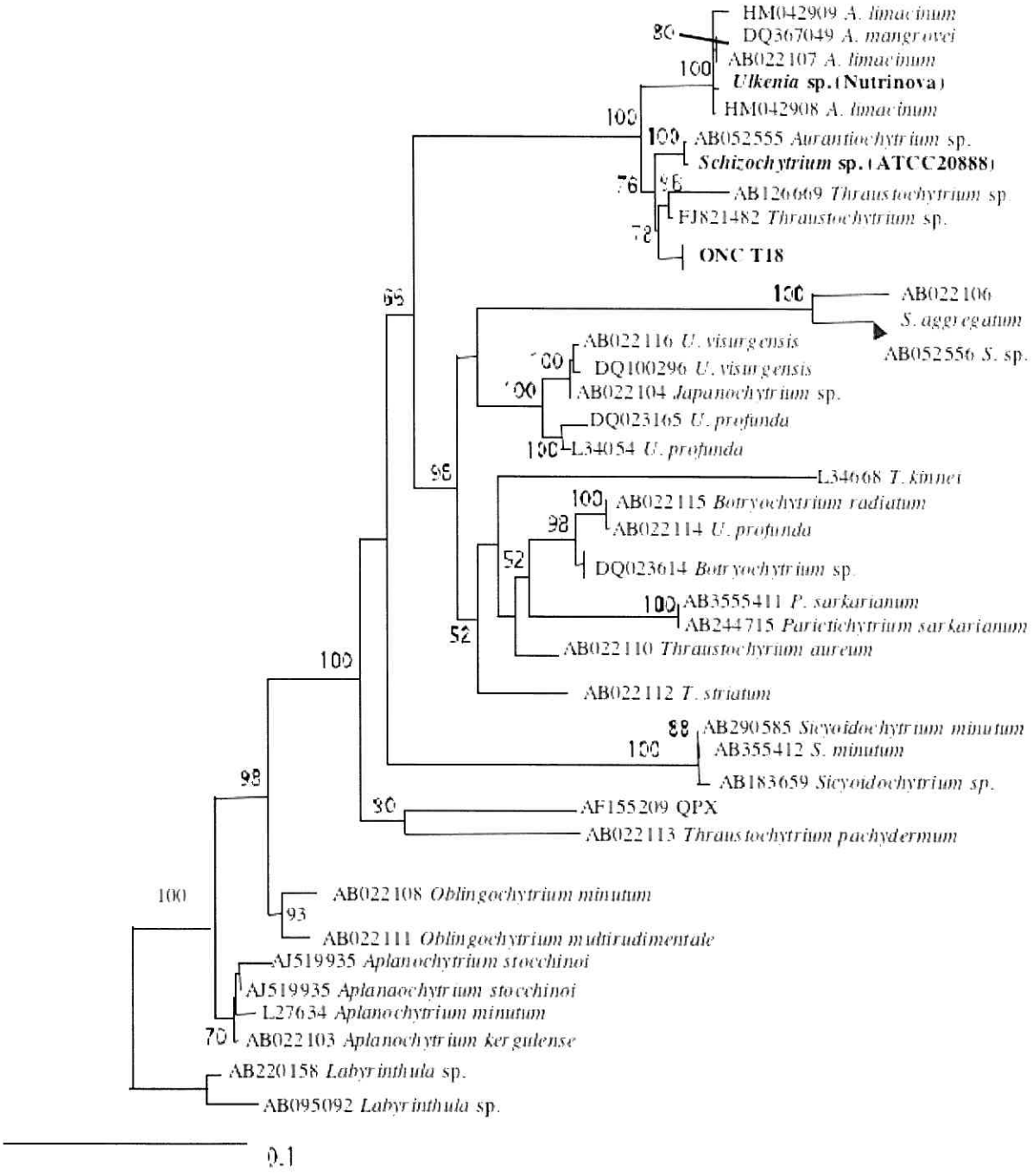


Figure 4. ML tree showing relationships among select thraustochytrid sequences. Genbank accession numbers are shown with current taxonomic names for each associated strain.

Morphological evaluation of video provided of growth of the "*Ulkenia* sp. (Nutrinova)" clearly

indicate that this strain is very similar to *Aurantiochytrium limacinum*. Phylogenetic analysis supports the inclusion of this strain within the *A. limacinum* group. To be sure that ONC T18 is not part of the closely related *Aurantiochytrium* lineage, a second phylogeny (figure 5) was generated including all published sequences of *Aurantiochtrium* strains (a total of 16 sequences). This analysis shows *Aurantiochytrium* is a monophyletic group, with the exception of the Nutrinova *Ulkenia* strain, and that ONC T18 does not fall within this lineage.

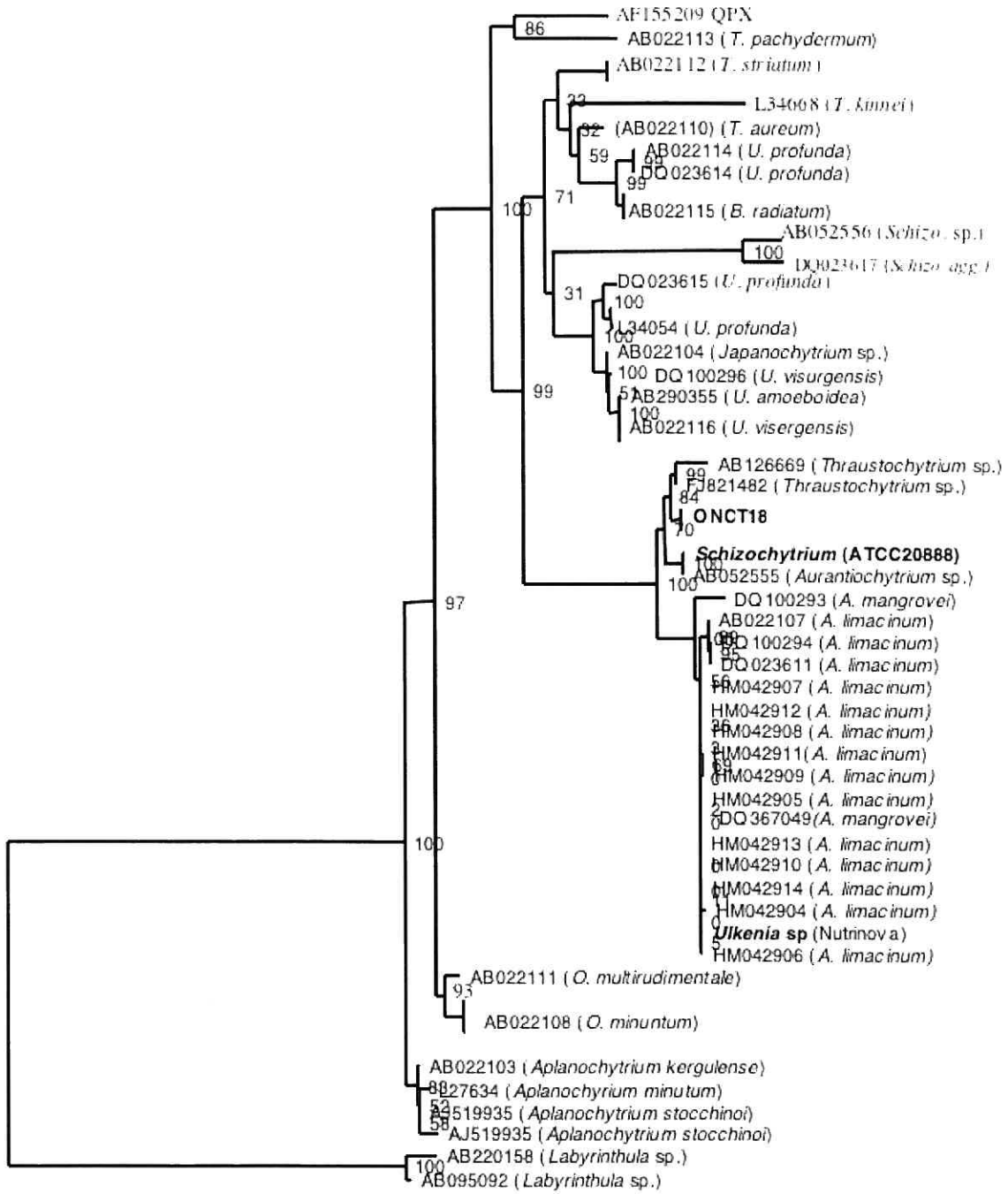


Figure 5. ML tree showing expanded relationships of *Aurantiochytrium* sp.

The two sequences ONC T18 and *Schizochytrium* sp (Martek) are closely related to one another. These strains belong to a moderately well supported larger group including two *Thraustochytrium* strains and one *Aurantiochtrium* strain.

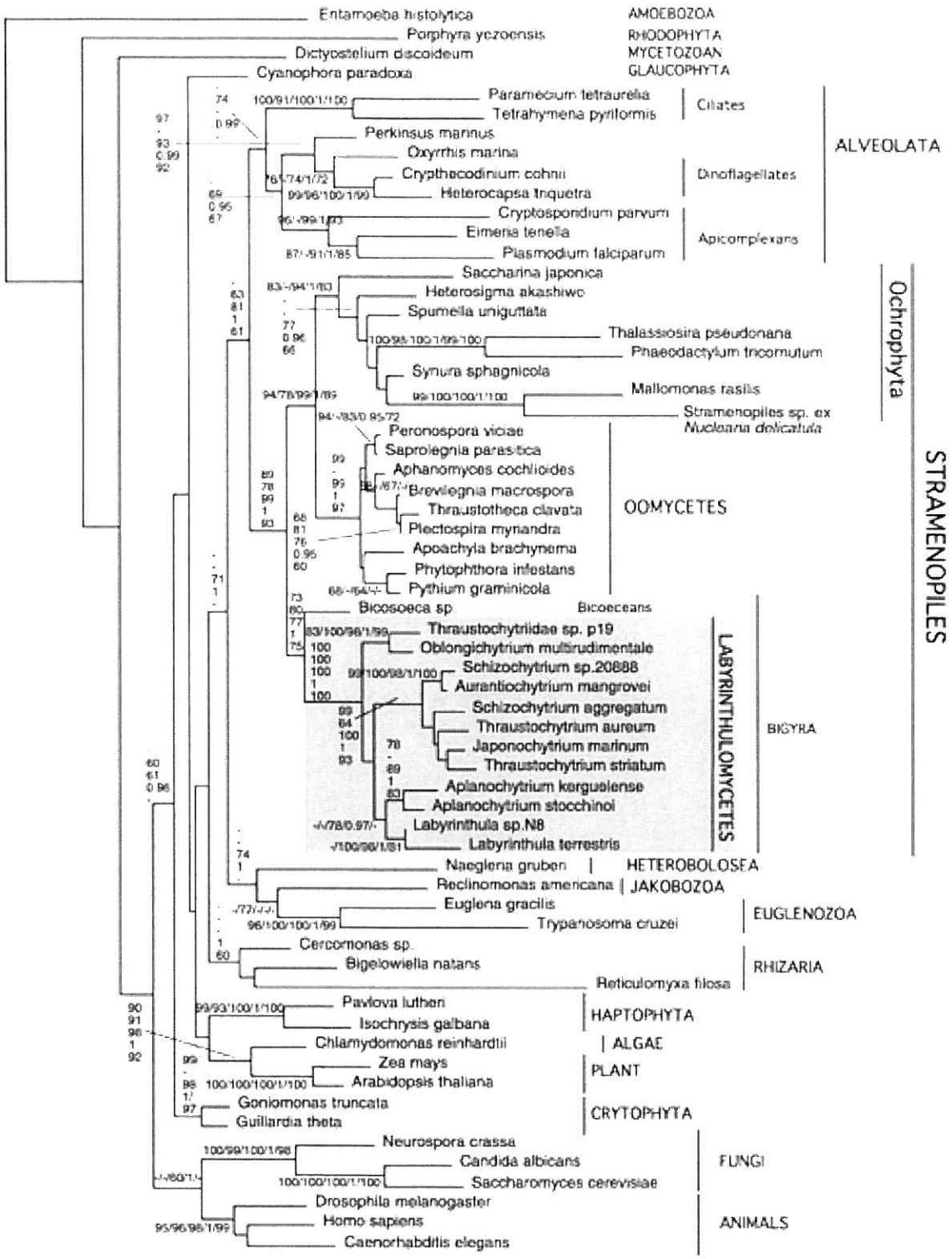


Fig. 6. A phylogeny of eukaryotes published by Tsui et al, 2009.

Figure 6 illustrates a broader context of affinity within the Labyrinthulomycota. Please note that this phylogeny was published independently and includes some (but not all) of the thraustochytrid sequences used in this report. The family Labyrinthulaceae is represented in Figure 6 by two sequences of *Labyrinthula*. These two sequences form a larger group with 2 representatives of the *Aplanochytrium* genus. The rest of the thraustochytrids (shaded in grey) form an independent lineage that comprises the lineages of the Thraustochytriaceae focused on in Fig 4 and Fig 5. These organisms form an exclusive well supported group within the eukaryotes.

Conclusion:

Given the morphological characteristic of successive bipartioning observed in ONC T18 as well as its close phylogenetic affinity with other *Schizochytrium* strains (notably ATCC20888), I would classify this strain as closely related to the genus *Schizochytrium*. Prior to 2007, this strain would have been best classified as belonging to the *Schizochytrium* genus. The 2007 amendment of *Schizochytrium* prevents inclusion of ONC T18 because of differences in pigment profiles. This strain shows phylogenetic affinity to the *Aurantiochtrium* (which was part of the *Schizochytrium* genus prior to 2007), but is nested in a separate clade. Because successive bipartitioning was observed only occasionally and under certain nutritive conditions, my guess is that this character may exist in the other close phylogenetic relatives of ONC T18 which are currently classified as members of the *Thraustochytrium* genus, but may not be readily observed under all growth conditions. There is little (if any) reason to classify this strain as closely related to *Ulkenia* sp. (including Nutrinova's strain).

Summary:

* ONC T18 shares a major defining character (the division pattern, successive bipartitioning) with *Schizochytrium* species, including the *Schizochytrium* ATCC20888.

* If I were going to publish a description of this strain, I would define it as a *Schizochytrium* according to the "old" definition (prior to 2007). Alternately, formal inclusion of ONC T18 in the *Schizochytrium* genus would require re-defining of the genus to expand pigment and fatty-acid profiles. (This process would likely better reflect the genus from a relationship standpoint, but has not formally been done.)

*Sequence data suggests that ONC T18 is closely related to, but is not a member of, the *Aurantiochytrium* lineage. In addition, ONC T18 does not have the amoeboid protoplast stage inidicitive of *A. limacinum* or *Ulkenia*.

For the purpose of producing oils rich in omega-3 fatty acids, it is in my opinion that *Schizochytrium* ATCC20888 and ONCT18 are substantially equivalent. This conclusion is based on morphological similarity between the two strains, [redacted] and their close phylogenetic affinity. Finally, members of the Thraustochytriaceae are not known to make toxins that affect any mammals, including humans.

Expertise:

I have over 10 years experience specializing in the taxonomy and phylogeny of the Labyrinthulomycota. I began working on this group as a graduate student in [redacted] and continue to do isolations and identification of these organisms at the [redacted] Please see attached full Curriculum Vitae.

I was contacted by Ocean Nutrition Canada (ONC) regarding taxonomic placement of a strain of thraustochytrid, ONC T-18. The previous report outlines my conclusions and rationale regarding the taxonomic placement of this organism.

References:

Bahnweg and Sparrow 1972. *Aplanochytrium kerguelensis* gen. nov. spec. nov., a new phycomycete from subantarctic marine waters. Arch. Mikrobiol. 81: 45-49.

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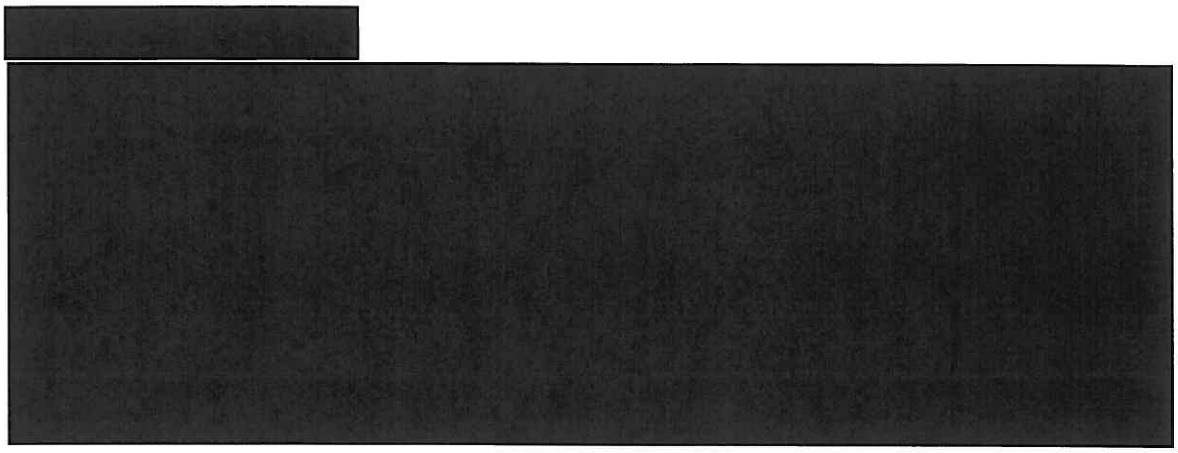
Yokoyama and Honda 2007. Taxonomic rearrangement of the genus *Schizochytrium* sensu lato based on morphology, chemotaxonomic characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for *Schizochytrium* an erection of *Aurantiochytrium* and *Oblongichytrium* gen. nov., Mycoscience 48: 199-211.

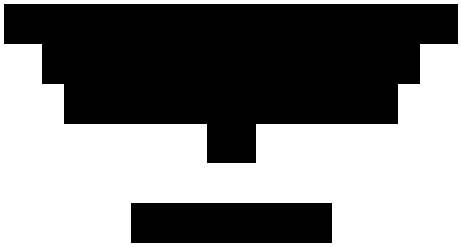
Yokoyama, Salleh, and Honda 2007. Taxonomic rearrangement of the genus *Ulkenia* sensu lato based on morphology, chemotaxonomical characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for *Ulkenia* and erection of *Botryochytrium*, *Parietichytrium*, and *Sicyoidochytrium* gen. nov., Mycoscience 48: 329-341.

Zwickl, 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.



Annex 4

Annex 4 – Taxonomic Report, [redacted]



Areas Of Expertise

I have over 40 years of experience in the field of marine microbiology, including the biology of fungi and fungoid protists, with a particular interest in Thraustochytriales. I have extensive experience analyzing and providing morphological characterization of microorganisms, particularly microorganisms of the order Thraustochytriales. In particular, I have isolated and worked extensively with *Schizochytrium* species including S. *limacinum* and also various isolates of *Ulkenia.*

Educational Background

I have a BSc (Hons) in Botany from [redacted] I received a Master's degree from the [redacted] for a research study on the Labyrinthulomycota, the taxonomic grouping which includes the thraustochytrids.

Relevant Professional Experience

I am currently the [redacted] my responsibilities and activities have included teaching undergraduates, supervising graduate students, consultancy, and conducting innovative research in the field of marine microbiology.

[redacted]

Research on thraustochytrids

[redacted]

I have studied thraustochytrids since [redacted] and have nine publications on the subject, five of these as senior author. My research has been primarily focused on environmental and biotechnological applications of these organisms, and, in particular, on the production of omega-3 polyunsaturated fatty acids (PUFAs) by the fungoid protists the Thraustochytriales. I have authored or co-authored well over 25 peer-reviewed original and review papers dealing with fungal and protistan biology (see attached *curriculum vitae*), and have been invited as a guest speaker at many conferences on these subjects. I have successfully supervised the doctoral studies of six postgraduate students (four on the subject of thraustochytrid biology) and I am reviewer/referee for several academic journals on the subject of thraustochytrid biology.

Review of taxonomic status of isolate ONC T18

I was asked by [redacted] of Ocean Nutrition Canada (ONC) to comment on the taxonomic status of an isolate known as ONC T18. I was provided with the following documentary evidence.

1.A copy of a publication by Burja *et al.*, (2006) from Applied Microbiology and Biotechnology 72:1161-1169 which described the isolation and characterisation of the organism ONC T18.

2. A taxonomic report by [redacted] prepared on behalf of ONC on the taxonomy of isolate ONC T18.

3. A copy of a paper on the taxonomic rearrangement of the genus *Schizochytrium* by Yokoyama and Honda (2007) from Mycoscience 48:199-211.

4.. A set of video recordings taken under a microscope of isolate ONC T18, an isolate from the company Nutrinova identified by them as the thraustochytrid *Ulkenia* and a thraustochytrid isolate from the company Martek identified by them as *Schizochytrium.*

5. A set of fatty acid profiles derived for the 3 above mentioned isolates.

Opinion based on the examination of the documentary evidence.

I will first deal with the photographic evidence. The video recordings of the development of the three isolates showed the following;

The recording of the isolate designated ONCT 18 was made at an insufficiently high magnification to discern clearly the cellular division giving rise to the zoospores but it was possible to state that the organism is a thraustochytrid. The still photographs of ONC T18 growing on nutrient agar were taken at a higher magnification and showed clear evidence that the vegetative cells divided by successive bipartioning to give tetrads of cells giving rise to 8 putative sporangia (arrowed Figure 1)

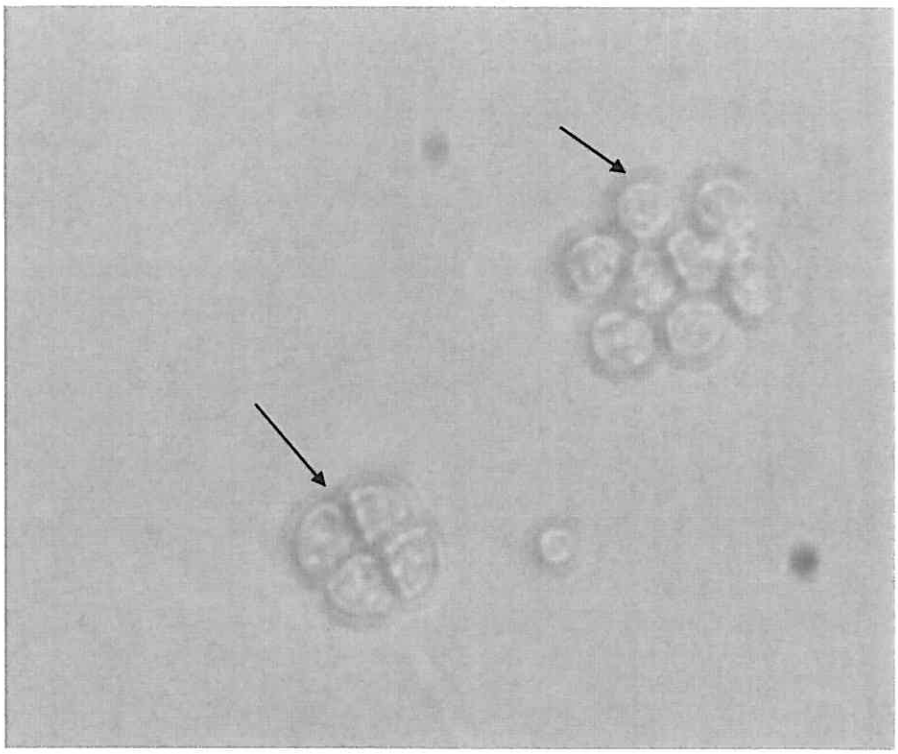


FIGURE 1 Isolate ONCT 18 growing on nutrient agar.

This feature is diagnostic of the genus *Schizochytium* as described by Goldstein and Belsky (1966). No amoeboid cells were seen in either the video or still images thus ruling out the species *S.mangrovei* and *S. limacinum,*

Examination of images of Nutrinova strain of *"Ulkenia"*and the Martek strain.

I had previously (between 2004 and 2006) examined in detail living material of both these organisms The images provided for me by ONC of these two isolates confirmed my previous identification. The Nutrinova strain is not *Ulkenia* but *Schizochytrium limacinum* Honda & Yokochii emended validly to *Aurantiochytrium* gen.nov Yokoyama et Honda. The Martek isolate is *Schizochytrium* Goldstein & Belsky emend. Yokoyama et Honda

Submission by [redacted]

This is a thorough and accurate assessment of the taxonomic status of isolate ONCT18 and I would not dispute any of her findings in any of the aspects that she has analysed and documented.

The effect of the taxonomic rearrangement by Yokoyama & Honda (2007) of the genus *Schizochytrium* on the taxonomic status of ONC T18

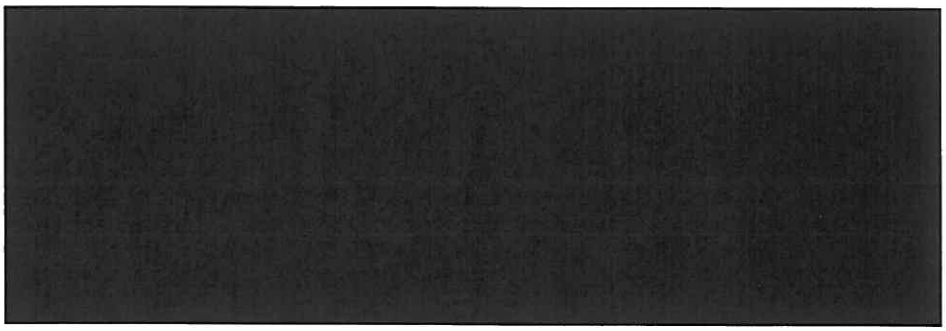
The thraustochytrid was identified in the 2006 publication of Burja et al. as *Thraustochytrium,* if it had, at that time , been correctly assigned to the genus *Schizochytrium,* the 2007 rearrangement would have placed it in the genus *Schizochytrium* sensu lato. Therefore, no change in status, similarly the Martek isolate which I examined in this study, also remains as *Schizochytrium* sensu lato.

SUMMARY

My opinions on isolate ONC T18 are:

- At the time of its description in 2006 Burja *et al.* placed too great a reliance on 18SrRNA gene sequencing without confirmation by morphological and developmental studies. This lead them to identify the isolate as *Thraustochytrium* ,whereas, it is without any doubt an isolate of *Schizochytrium.*

If isolate ONC T18 were to be used for the production of PUFA for human consumption then this should not prove problematical since *Schizochtrium* strains have been used commercially for many years in patented processes by companies such as Omegatech and Martek for the production of DHA containing lipid for human consumption.



Annex 5

Annex 5 – Screening for marine algal toxins, [redacted]

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[redacted]

NRC·CNRC

From **Discovery** to **Innovation...**

Science at work for Canada

National Research Council Canada

LC-MS screening of algal toxins

[redacted]

[redacted]

National Research Council Canada | Conseil national de recherches Canada

Canada

C. METHOD DEVELOPMENT AND QUALITY CONTROL

Precision and recovery test of extraction protocol

The recovery of the liquid/liquid extraction procedure was determined by spiking known amounts of okadaic acid into sunflower oil, which is presumably void of any marine toxins. Okadaic acid was chosen as a model lipophilic toxin Portions of ~ 4 grams of sunflower oil were spiked at three different levels and extracted with 1:1 methanol:water. Precision and recovery test results are shown in **Table 1**. Recovery was greater than 80% in all cases with precision of roughly 10%. It should be noted, that previous work in our laboratory using an identical liquid/liquid extraction procedure for the highly polar domoic acid also yielded recovery levels greater than 80%. Therefore, this extraction procedure is suitable for a wide polarity range and was chosen to extraction the range of toxins investigated in this study.

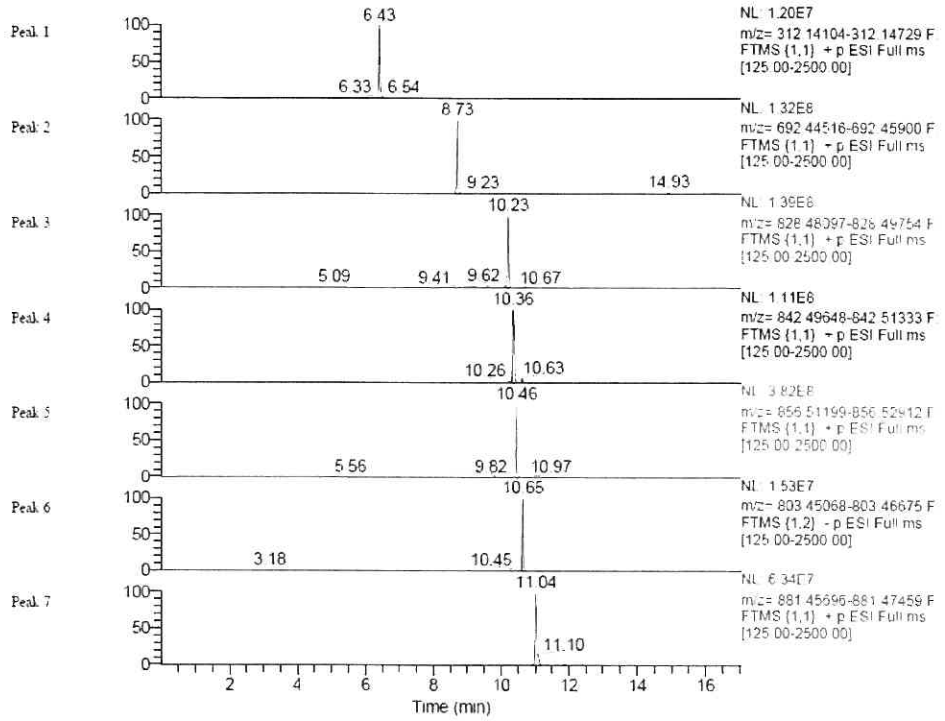
Table 1. Precision and recovery of extraction protocol

Sample	Target concentration (µg/g)	Mean recovery (%)	Standard Deviation (%)
1	0.48	83	9
2	0.24	83	9
3	0.12	84	10

LC-MS method

The high resolution mass spectrometry method was performed on a Thermo Exactive mass spectrometer with a resolving power of 100 000. To accommodate four of the analytes that required negative polarity ionization, alternating positive and negative polarity scans were acquired throughout the length of the chromatographic run. In order to maintain a sufficient number of data points across chromatographic peaks and reduce cycle times, data was acquired at a reduced resolution of 50,000 to allow for acquisitions at 2 Hz. Data was acquired in a non-targeted manner over a wide mass range, in contrast to conventional triple-quadrupole mass spectrometry methods where the analytes are specified in the acquisition method. Data is then processed extracting narrow mass windows (ie 5 ppm) centered around the masses from a specified target list. Finally, the non-targeted data acquired in this study will be archived and available for screening of additional toxins or contaminants upon request.

Shown in **Figure 1** is a typical LC-MS chromatogram for a mixture of toxin standards containing domoic acid, desmethylspirolide C, azaspiracid-1, azaspiracid-2, azaspiracid-3, okadaic acid and pectenotoxin-2. With the exception of okadaic acid that is detected in negative ion mode, all toxins were detected at 1-2 ppm mass accuracy. This method was run daily throughout this study as a quality control of instrument performance.



#	Comp. Index	Compound Name	Formula	Detected m/z	Delta (ppm)	Expected RT	Actual RT	Intensity	Adducts			Fragments		
									H+	NH4+	Na+	1	2	3
1	0	Domoic Acid	C15H21NO6	312.14365	-1.7	0.00	6.43	12021743	Y*	N	N	-	-	-
2	0	Desmethylspirolide C	C42H61NO7	692.45123	-1.2	0.00	8.73	132176160	Y*	N	N	-	-	-
3	0	Azaspiracid 3	C46H69NO12	828.48773	-1.8	0.00	10.23	139214896	Y*	N	N	-	-	-
4	0	Azaspiracid 1	C47H71NO12	842.50354	-1.6	0.00	10.36	110544712	Y*	Y	N	-	-	-
5	0	Azaspiracid 2	C48H73NO12	856.51898	-1.8	0.00	10.46	382147584	Y*	N	N	-	-	-
6	0	Okadaic Acid	C44H68O13	803.46191	4.0	0.00	10.65	15336107	Y*	N	N	-	-	-
7	0	Pectenotoxin 2	C47H70O14	881.46405	-2.0	0.00	11.04	63398068	N	Y	Y*	-	-	-

Figure 1. Typical LC-MS chromatograms for marine toxin standards generated by extracting narrow mass windows (5ppm) centered around the masses from a specified target list.

D. SCREENING RESULTS

Using the LC-MS methodology described above, none of the toxins listed in **Table 2** were detected in either the algal biomass nor the oil sample. When a chromatographic peak was observed in any of the extracted mass chromatograms, even at a retention time not corresponding to the appropriate toxin, data was inspected manually to determine the origin of the signal. For instance, **Figure 1A** displays an apparent signal in the extracted ion chromatograms for domoic acid at *m/z* 312.1447, although the retention time for the peak eluting at 5.3 min does not match the expected retention time for domoic acid. Upon inspection of the mass spectrum for this peak in **Figure 1B**, it is apparent that the signal observed at m/z 312.1447 is due to an isotope or noise generated from the peak at m/z 309.18121, and thus is not attributed to domoic acid. This manual inspection of the data was performed in all cases when a signal of any type was observed, and in all cases the signal could not be attributed to one of the target toxins.

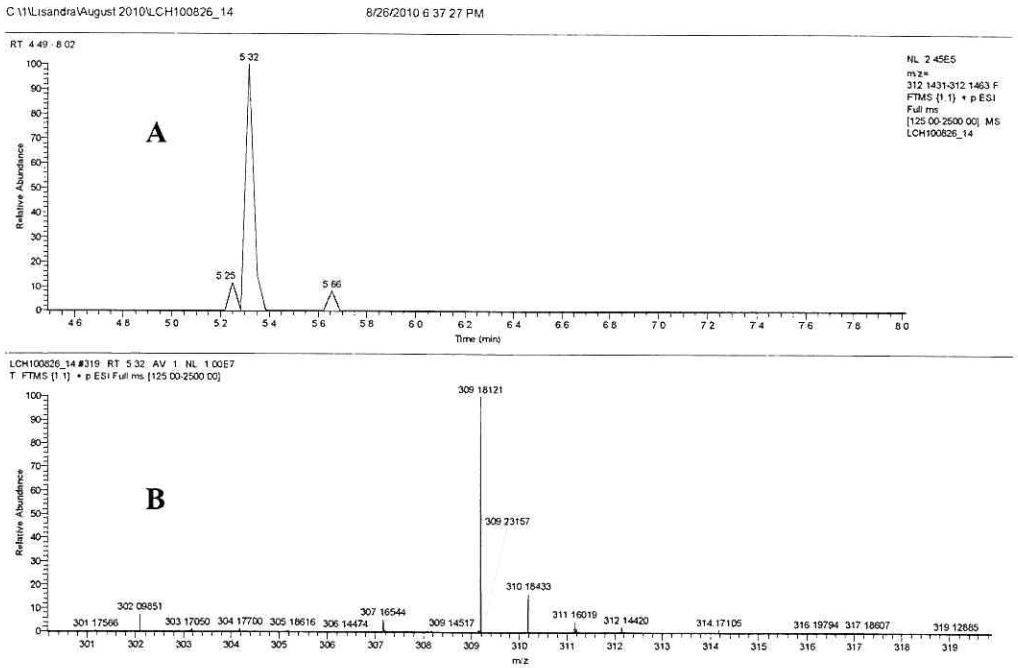


Figure 2. Extracted ion chromatogram for *m/z* 312.1447 (domoic acid) (**A**) and mass spectrum (**B**) for peak eluting at 5.3 minutes in dried biomass sample.

As no toxins were detected in either the algal biomass nor the oil sample, toxin levels were reported as less than the limits of detection (LOD) for each toxin, as listed in **Table 2**. Due to the widely varying ionization efficiencies for the toxins investigated, limits of detection vary by nearly two orders of magnitude, with those detected in negative mode (okadaic acid, Dinophysistoxin-1&2, and yessotoxin) having the largest LODs. To correct for loses and dilution during extraction, instrumental LODs were increased to account for the 80% recovery and the dilution factor of the extraction procedure. As standards are not available for the prymnesins, their LODs were estimated as the average LOD of all other toxins studied. This is a reasonable assumption given the fact that the prymnesins' structures contain a primary amine group that will enhance their ionization efficiencies in positive mode similar to the azaspiracids, while their larger structure would likely yield broader peaks that would lower sensitivity. Therefore, a moderate response factor would be anticipated for the prymnesins.

Table 2. List of toxins screened for this analysis, their monoisotopic masses and limits of detection corrected for recovery and dilution during extraction.

Toxin	**Mass-to-charge (m/z)**	**Result (< LOD in all cases)**
Domoic Acid	312.1447	< 27 ppb
Gymnodimine	508.3427	< 3.2 ppb
Desmethylspirolide C	692.4526	< 4.2 ppb
Azaspiracid-1	842.5055	< 4.1 ppb
Azaspiracid-2	856.5211	< 5.0 ppb
Azaspiracid-3	828.4898	< 4.8 ppb
Pectenotoxin-2	881.4663	< 7.9 ppb
Okadaic Acid	803.4582	< 220 ppb
Dinophysistoxin-1	817.4738	< 160 ppb
Dinophysistoxin-2	803.4582	< 120 ppb
Yessotoxin	1141.4706	< 400 ppb
Prymnesin-2	1968.8037	< 86 ppb*
Prymnesin-1	2262.8988	< 86 ppb*

* Standards not available for prymnesins, LOD based on average of all toxins.

Summary

Liquid chromatography - mass spectrometry (LC-MS) was employed to screen for multiple classes of marine toxins in both dried algal biomass and processed oil. An established extraction procedure was employed for algal biomass and an extraction protocol for oil samples was developed and tested. Recovery of the liquid/liquid extraction procedure was measured to be 80% by spiking known amounts of a typical lipophilic toxin into an oil sample void of any toxins and measuring levels by mass spectrometry. Using an in-house high-resolution LC-MS method, no toxins were detected in either the algal biomass nor the oil sample. Therefore, toxin levels were reported as less than the instrumental limits of detection (LOD) for each toxin, corrected for the recovery and the dilution factor of the extraction procedure.

A. SAMPLE INFORMATION

One algal oil sample and one dried biomass sample were received in 15 mL sample vials.

[redacted]

B. EXPERIMENTAL PROCEDURES

1. Extraction Method

Two sub-samples of algal oil (~ 4 g) and one sub-sample of dried biomass (~4 g) were extracted for marine toxins. To each sample were added three aliquots of 6 mL of methanol/water (1:1, v/v). Samples were placed in the vortex for 10 minutes and centrifuged for 15 minutes @ 3000 ppm. Supernatants were decanted and combined into 25 ml volumetric flasks with 1:1 methanol:water. An aliquot (0.5 mL) was removed from each extract solution and filtered through a centrifugal "spin-filter" (0.45 µm) prior to mass spectrometric analysis.

2. Liquid Chromatographic (LC) Conditions

HPLC column: Waters Acquity HSS T3 1.8 µm 2.1×100 mm
Mobile phase A: Water 0.1 % formic acid
Mobile phase B: Acetonitrile 0.1 % formic acid
Flow rate: 0.4 mL/min, Temperature: 40°C, Injection volume: 3 µL
Gradient elution: 0-30% B in 6 min followed by 30-100% in 4 min.

3. Mass Spectrometric (MS) Conditions

LC-MS instrumentation consisted of a Thermo Accela quaternary pump coupled to a Thermo Exactive mass spectrometer equipped with a HESI-II probe for electrospray ionization. Alternating positive and negative polarity scans were acquired and data was collected at a resolution setting of 50,000 at 2 Hz over a mass range of 100 – 2500 *m/z*.