

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent of: Kirk E. Apt et al.  
U.S. Patent No.: 8,207,363 B2 Attorney Docket No.: 55973-0002IP1  
Issue Date: June 26, 2012  
Appl. Serial No.: 12/407,687  
Filing Date: March 19, 2009  
Title: THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND  
METHODS OF MAKING AND USES THEREOF

**DECLARATION OF DR. JONATHAN CURTIS**

I currently hold the opinions set expressed in this declaration. But my analysis may continue, and I may acquire additional information and/or attain supplemental insights that may result in added observations.

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I further declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code.

Dated: 17<sup>th</sup> November 2023

By:   
Jonathan M. Curtis, Ph.D.

## TABLE OF CONTENTS

|             |                                                                                                                            |            |
|-------------|----------------------------------------------------------------------------------------------------------------------------|------------|
| <b>I.</b>   | <b>EXPERIENCE AND QUALIFICATIONS.....</b>                                                                                  | <b>3</b>   |
| <b>A.</b>   | <b>Materials Considered.....</b>                                                                                           | <b>5</b>   |
| <b>B.</b>   | <b>Overview of Conclusions Formed .....</b>                                                                                | <b>10</b>  |
| <b>C.</b>   | <b>Interpretation of the '363 Patent Claims .....</b>                                                                      | <b>11</b>  |
| <b>D.</b>   | <b>Legal Standards.....</b>                                                                                                | <b>11</b>  |
|             | 1. Terminology.....                                                                                                        | 11         |
|             | 2. Legal Standards for Anticipation .....                                                                                  | 12         |
|             | 3. Legal Standards for Obviousness.....                                                                                    | 13         |
| <b>E.</b>   | <b>Background Knowledge One of Skill in the Art Would Have Had<br/>Prior to the Priority Date of the '363 Patent .....</b> | <b>18</b>  |
|             | 1. Composition of Microbial Oils .....                                                                                     | 18         |
|             | 2. Extraction of Microbial Oils .....                                                                                      | 24         |
| <b>II.</b>  | <b>SUMMARY OF THE '363 PATENT .....</b>                                                                                    | <b>29</b>  |
| <b>A.</b>   | <b>Brief Description.....</b>                                                                                              | <b>29</b>  |
| <b>B.</b>   | <b>Summary of the Prosecution History of the '363 Patent.....</b>                                                          | <b>31</b>  |
| <b>C.</b>   | <b>The Effective Priority Date of the Claims of the '363 Patent .....</b>                                                  | <b>33</b>  |
| <b>D.</b>   | <b>Level of Ordinary Skill in the Art.....</b>                                                                             | <b>33</b>  |
| <b>III.</b> | <b>THE CHALLENGED CLAIMS ARE UNPATENTABLE.....</b>                                                                         | <b>34</b>  |
| <b>A.</b>   | <b>GROUND 1: Claims 1-3 is anticipated by Radianingtyas .....</b>                                                          | <b>34</b>  |
|             | 1. Overview of Radianingtyas.....                                                                                          | 34         |
|             | 2. Analysis.....                                                                                                           | 37         |
| <b>B.</b>   | <b>GROUND 2: Claims 1-15 are obvious over Radianingtyas in view<br/>of Kralovec .....</b>                                  | <b>49</b>  |
|             | 1. Overview of Radianingtyas.....                                                                                          | 49         |
|             | 2. Overview of Kralovec.....                                                                                               | 49         |
|             | 3. Combination of Radianingtyas and Kralovec .....                                                                         | 52         |
|             | 4. Analysis.....                                                                                                           | 61         |
| <b>C.</b>   | <b>GROUND 3: Claims 1-15 are obvious over Bijl in view of Barclay<br/>.....</b>                                            | <b>92</b>  |
|             | 1. Overview of Bijl.....                                                                                                   | 92         |
|             | 2. Overview of Barclay .....                                                                                               | 93         |
|             | 3. Combination of Bijl and Barclay .....                                                                                   | 97         |
|             | 4. Analysis.....                                                                                                           | 103        |
| <b>IV.</b>  | <b>CONCLUSION.....</b>                                                                                                     | <b>123</b> |

I, Jonathan Curtis, declare as follows:

## **I. EXPERIENCE AND QUALIFICATIONS**

1. I joined the Faculty of Agricultural and Environmental Sciences at the University of Alberta in Edmonton, Alberta, Canada, in 2007, where I am currently a full professor in the Department of Agricultural, Food and Nutritional Science. I have continued my association with this department as Professor Emeritus since my retirement in July 2023. The research group which I direct within this department is known as the Lipid Chemistry Group.

2. As Professor, my teaching duties within the Nutrition and Food Science program have included responsibility for a large undergraduate course in Food Chemistry and a joint 4<sup>th</sup> year undergraduate/graduate course in Analytical Chemistry. Also, as part of my university activities, I have directed a research group which has included graduate and undergraduate students, technicians, senior research associates and postdoctoral research fellows. My research activities have been focused to a considerable extent on lipid chemistry, the conversion of fats and oils to biobased chemicals and materials, lipid analysis, and the development of novel analytical methods for lipids relevant to medical sciences, the fat and oil industries, agriculture, food science and nutrition.

3. I earned a Bachelor of Science degree in Chemistry in 1981 from the University of Southampton in the U.K. and a Master of Science in Analytical

Chemistry in 1983 from the University of Guelph in Ontario, Canada. I earned a Ph.D. in Analytical Chemistry in 1987 from Swansea University in Wales. My doctoral thesis was entitled “Novel Methods of Structure Determination by Mass Spectrometry.” From 1987 to 1988, I was a postdoctoral research associate in the School of Chemical Sciences at the University of Illinois, where I worked on the analysis of marine natural products.

4. Between 1992 and 1999 I was a Research Officer with the Canadian National Research Council, Institute for Marine Biosciences, in Halifax, Nova Scotia. My focus there was on the analytical chemistry of marine lipids and natural products, and the NRC certified reference material program.

5. Between 1999 and 2007 I worked in an industrial R&D position at Ocean Nutrition Canada Ltd. (now part of DSM) where my title was Director of Research: Analytical and Functional Food Technologies. There I directed analytical chemistry and food science groups involved in the processing of fish oils and omega-3 concentrates, oil quality measurements, and developing microencapsulated oil as a food ingredient and food grade oils with desirable sensory qualities. During that time, I also interacted with the group developing microalgal oils for omega-3 production.

6. I have co-authored or authored more than 180 published peer-reviewed articles in scientific journals and 7 book chapters. I have been co-inventor on 6 issued patents plus several provisional patents.

7. My curriculum vitae is attached as exhibit MARA-1004. In the past three years, I have not testified at deposition or trial.

8. I have no financial interest in the parties or in the outcome of this proceeding. I am being compensated for my work as an expert on an hourly basis. My compensation is not dependent on the outcome of these proceedings or the content of my opinions.

#### **A. Materials Considered**

9. I have been retained on behalf of Mara Renewables Corporation to offer technical opinions relating to U.S. Patent No. 8,207,363 (“the ’363 patent”) and prior art references relating to its subject matter. I have reviewed the ’363 patent, and relevant excerpts of the prosecution history of the ’363 patent. In addition to any materials cited in the present declaration, I have also reviewed the following references:

- MARA-1002: Prosecution History of the ’363 patent (“the Prosecution History”)
- MARA-1007: WO 2008/129358 to Radianingtyas et al.  
 (“Radianingtyas”)

- MARA-1008: WO 97/37032 to Bijl et al. (“Bijl”)
- MARA-1009: Peter D. Nichols et al., *New Australian Single Cell and Crop Plant Sources of Health-Enhancing Long-Chain Omega-3 Oils*, Australasian Biotechnology 13(5):26–28 (Oct./Nov. 2003) (“Nichols-I”)
- MARA-1010: *Single Cell Oils*, Z. Cohen and C. Ratledge (Eds.), AOCS Press, Champaign, Illinois, USA (2005)
- MARA-1015: U.S. Pat. App. Pub. No. 2004/0161831 to Komazawa et al. (“Komazawa”)
- MARA-1019: U.S. Patent No. 5,130,242 to Barclay (“Barclay”)
- MARA-1020: Peter D. Nichols et al., *Omega-3 oils down under—An update*, Inform 15(6):383–385 (June 2004) (“Nichols-II”)
- MARA-1022: *Long-Chain Omega-3 Specialty Oils*, H. Breivik (Ed.), The Oily Press, Bridgewater, UK (2007)
- MARA-1023: *Fennema’s Food Chemistry*, S. Damodaran and K.L. Parkin (Eds.), CRC Press, Boca Raton, Florida, USA (5th ed. 2017)
- MARA-1024: *Food Lipids*, C.C. Akoh and D.B. Min (Eds.), Marcel Dekker, New York, New York, USA (2nd ed. 2002)
- MARA-1025: Adam M. Burja et al., *Isolation and characterization of polyunsaturated fatty acid producing Thraustochytrium species:*

- screening of strains and optimization of omega-3 production*, Applied Microbiology and Biotechnology 72:1161–1169 (2006)
- MARA-1027: Edwin N. Frankel, *Lipid Oxidation*, The Oily Press, Cambridge, UK (2nd ed. 2005)
  - MARA-1030: WO 2009/040676 to Kralovec et al. (“Kralovec”)
  - MARA-1032: Thomas Eric Lewis, *Characterisation and application of Australian thraustochytrids*, Doctoral Thesis, University of Tasmania (June 2001)
  - MARA-1033: Seshagiri Raghukumar, *Thraustochytrid Marine Protists: Production of PUFAs and Other Emerging Technologies*, Marine Biotechnology 10:631–640 (2008)
  - MARA-1034: U.S. Pat. App. Pub. No. 2003/0161864 to Tanaka et al. (“Tanaka”)
  - MARA-1035: W.M.N. Ratnayake et al., *Preparation of Omega-3 PUFA Concentrates from Fish Oils via Urea Complexation*, Fat Science Technology 90(10):381–386 (1988)
  - MARA-1036: Fereidoon Shahidi and Priyatharini Ambigaipalan, *Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits*, Annual Review of Food Science and Technology 9:345–381 (2018)
  - MARA-1038: Owen P. Ward and Ajay Singh, *Omega-3/6 Fatty Acids:*

*Alternative Sources of Production*, *Process Biochemistry* 40:3627–3652 (2005)

- MARA-1039: Anthony P. Bimbo, *Marine Oils*, AOCS Lipid Library, <https://lipidlibrary.aocs.org/edible-oil-processing/marine-oils> (last accessed August 21, 2023)
- MARA-1040: King Wai Fan and Feng Chen, *Chapter 11. Production of High-Value Products by Marine Microalgae Thraustochytrids*, in *Bioprocessing for Value-Added Products from Renewable Resources: New Technologies and Applications* 293-323, S.-T. Yang (Ed.), Elsevier B.V., Amsterdam, The Netherlands (2007) (“Fan”)
- MARA-1041: Eneko Ganuza Taberna, *Heterotrophic cultivation of microalgae as a source of docosahexaenoic acid for aquaculture*, Doctoral Thesis, University of Las Palmas de Gran Canaria (2008) (“Taberna”)
- MARA-1042: Kim Jye Lee-Chang et al., *Docosahexaenoic Acid Is Naturally Concentrated at the sn-2 Position in Triacylglycerols of the Australian Thraustochytrid Aurantiochytrium sp. Strain TC 20*, *Marine Drugs* 19:382 (2021)

- MARA-1045: U.S. Provisional App. No. 60/821,084<sup>1</sup>
- MARA-1046: J.J. Myher et al., *Stereospecific Analysis of Triacylglycerols Rich in Long-Chain Polyunsaturated Fatty Acids*, *Lipids* 31(2):207–215 (1996)

10. I have also reviewed various supporting references and other documentation as further noted in my opinions below.

11. Counsel has informed me that I should consider these materials through the lens of one of ordinary skill in the art related to the '363 patent as of March 2009. I have done so. The '363 patent claims priority to one non-provisional application filed on March 19, 2009 (“the Critical Date”). Counsel has informed me that the Critical Date represents the earliest possible priority date to which the challenged claims of the '363 patent are entitled, and I have therefore used that Critical Date in my analysis below.

12. In writing this Declaration, I have considered the following: U.S. Patent No. 8,207,363 (MARA-1001), my own knowledge and experience, including my work experience in the field of high omega-3 oils, my experience in teaching food chemistry, oil processing, oleochemistry, and experience in working with others involved in this field. In addition, I have analyzed the foregoing

---

<sup>1</sup> Priority document for MARA-1007.

publications and materials, in addition to any other materials I may cite in my declaration.

13. My opinions, as explained below, are based on my education, experience, and expertise in the fields relating to the '363 patent. Unless otherwise stated, my testimony below refers to the knowledge of one of ordinary skill in the art as of the Critical Date, or before. Any figures that appear within this document have been prepared with the assistance of Counsel and reflect my understanding of the '363 patent and the prior art discussed below.

#### **B. Overview of Conclusions Formed**

14. This expert Declaration explains the conclusions that I have formed based on my analysis. To summarize those conclusions:

- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion, claims 1-3 of the '363 patent are anticipated by Radianingtyas;
- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion, claims 1-15 of the '363 patent are obvious in light of Radianingtyas and Kralovec; and
- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion, claims 1-15 of

the '363 patent are obvious in light of Bijl and Barclay.

### **C. Interpretation of the '363 Patent Claims**

15. I understand that, for purposes of my analysis in this *inter partes* review proceeding, the terms appearing in the patent claims should be interpreted “in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent.” 37 C.F.R. § 42.100(b).

16. For purposes of this proceeding only, all terms should be given their plain meaning. I reserve the right to opine on the construction of any other claim terms, should Patent Owner propose any other words that it contends require construction.

### **D. Legal Standards**

#### **1. Terminology**

17. I have been informed by Counsel and understand that the best indicator of claim meaning is its usage in the context of the patent specification as understood by one of ordinary skill. I further understand that the words of the claims should be given their plain meaning unless that meaning is inconsistent with the patent specification or the patent’s history of examination before the Patent Office. Counsel has also informed me, and I understand that, the words of the claims should be interpreted as they would have been interpreted by one of

ordinary skill at the time of the invention was made (not today). Because I do not know at what date the invention as claimed was made, I have used the earliest possible priority date of the '363 patent as the point in time for claim interpretation purposes (the Critical Date).

## **2. Legal Standards for Anticipation**

18. I have been informed by Counsel and understand that documents and materials that qualify as prior art can render a patent claim unpatentable as anticipated. I am informed by Counsel and understand that all prior art references are to be looked at from the viewpoint of a person of ordinary skill in the art.

19. I am informed by Counsel and understand that a challenged claim is unpatentable as “anticipated” under 35 U.S.C. § 102 if it is determined that all the limitations of the claim are described in a single prior art reference. I am informed by Counsel and understand that, to anticipate a claim, a prior art reference must disclose, either expressly or inherently, each and every limitation of that claim and enable one of ordinary skill in the art to make and use the invention. I further understand from counsel that an overlap in a range can demonstrate anticipation.

20. I have been informed by Counsel and understand that in an *inter partes* review, “the petitioner shall have the burden of proving a proposition of unpatentability,” including a proposition of anticipation, “by a preponderance of the evidence.” 35 U.S.C. § 316(e).

### **3. Legal Standards for Obviousness**

21. I have been informed by Counsel and understand that documents and materials that qualify as prior art can render a patent claim unpatentable as obvious. I am informed by Counsel and understand that all prior art references are to be looked at from the viewpoint of a person of ordinary skill in the art at the time of the invention, and that this viewpoint prevents one from using his or her own insight or hindsight in deciding whether a claim is obvious.

22. I have been informed by Counsel and understand that a claim is unpatentable for obviousness under 35 U.S.C. § 103 “if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.” I am informed by Counsel and understand that obviousness may be based upon a combination of references. I am informed by Counsel and understand that the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results. However, I am informed by Counsel and understand that a patent claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.

23. I am informed by Counsel and understand that when a patented invention is a combination of known elements, a court must determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue by considering the teachings of prior art references, the effects of demands known to people working in the field or present in the marketplace, and the background knowledge possessed by a person having ordinary skill in the art.

24. I am informed by Counsel and understand that a patent claim composed of several limitations is not proved obvious merely by demonstrating that each of its limitations was independently known in the prior art. I am informed by counsel and understand that identifying a reason those elements would be combined can be important because inventions in many instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known. I am informed by Counsel and understand that it is improper to use hindsight in an obviousness analysis, and that a patent's claims should not be used as a "roadmap."

25. I am informed by Counsel and understand that an obviousness inquiry requires consideration of the following factors: (1) the scope and content of the prior art; (2) the differences between the claims and the prior art; (3) the level of ordinary skill in the pertinent art; and (4) any objective indicia of non-obviousness,

such as commercial success, long-felt but unresolved need, failure of others, industry recognition, copying, and unexpected results. I understand that the foregoing factors are sometimes referred to as the “Graham factors.”

26. I have been informed by Counsel and understand that an obviousness evaluation can be based on a combination of multiple prior art references. I understand that the prior art references themselves may provide a suggestion, motivation, or reason to combine, but that the nexus linking two or more prior art references is sometimes simple common sense. I have been informed by Counsel and understand that obviousness analysis recognizes that market demand, rather than scientific literature, often drives innovation, and that a motivation to combine references may be supplied by the direction of the marketplace.

27. I have been informed by Counsel and understand that if a technique has been used to improve one device, and a person of ordinary skill at the time of invention would have recognized that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.

28. I have been informed by Counsel and understand that practical and common sense considerations should guide a proper obviousness analysis, because familiar items may have obvious uses beyond their primary purposes. I have been informed by Counsel and understand that a person of ordinary skill looking to

overcome a problem will often be able to fit together the teachings of multiple prior art references. I have been informed by Counsel and understand that obviousness analysis therefore takes into account the inferences and creative steps that a person of ordinary skill would have employed at the time of invention.

29. I have been informed by Counsel and understand that a proper obviousness analysis focuses on what was known or obvious to a person of ordinary skill at the time of invention, not just the patentee. Accordingly, I understand that any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.

30. I have been informed by Counsel and understand that a claim can be obvious in light of a single reference, without the need to combine references, if the elements of the claim that are not found explicitly or inherently in the reference can be supplied by the common sense of one of skill in the art.

31. I have been informed by Counsel and understand that secondary indicia of non-obviousness may include (1) a long felt but unmet need in the prior art that was satisfied by the invention of the patent; (2) commercial success of processes covered by the patent; (3) unexpected results achieved by the invention; (4) praise of the invention by others skilled in the art; (5) taking of licenses under the patent by others; (6) deliberate copying of the invention; (7) failure of others to

find a solution to the long felt need; and (8) skepticism by experts. I understand that evidence of secondary indicia of non-obviousness, if available, should be considered as part of the obviousness analysis.

32. I have been informed by Counsel and understand that there must be a relationship between any such secondary considerations and the invention, and that contemporaneous and independent invention by others is a secondary consideration supporting an obviousness determination.

33. In sum, my understanding is that prior art teachings are properly combined where one of ordinary skill, having the understanding and knowledge reflected in the prior art and motivated by the general problem facing the inventor, would have been led to make the combination of elements recited in the claims. Under this analysis, the prior art references themselves, or any need or problem known in the field of endeavor at the time of the invention, can provide a reason for combining the elements of multiple prior art references in the claimed manner.

34. I have been informed by Counsel and understand that in an *inter partes* review, “the petitioner shall have the burden of proving a proposition of unpatentability,” including a proposition of obviousness, “by a preponderance of the evidence.” 35 U.S.C. § 316.

**E. Background Knowledge One of Skill in the Art Would Have Had Prior to the Priority Date of the '363 Patent**

35. Below, I give an overview of microbial oils, including their composition, nomenclature and health benefits. I then discuss how microorganisms are sourced and processed, and fatty acids extracted and refined, to result in lipid compositions for creation of microbial oils.

**1. Composition of Microbial Oils**

36. It has long been recognised that the regular consumption of the polyunsaturated fat found in marine organisms is associated with a range of health benefits and disease risk reduction. Early studies pointed to its importance in cardiovascular diseases, and more recently wide-ranging benefits in infant and maternal health, inflammatory diseases, cognitive disorders and in many other areas have been described. MARA-1022, 11-18.

37. The two most important components of marine lipids that are associated with these benefits, are the n-3 fatty acids eicosapentaenoic acid (EPA) and docosohexaenoic acid (DHA). The structures of these compounds are shown in Figure 1. They are described as being omega-3 or n-3 fatty acids, which refers to the position of the first double bond from the terminal methyl group with all of the remaining double bonds in the cis configuration and separated by a methylene group as shown. In contrast, n-6 fatty acids such as n-6 docosapentaenoic acid (DPA) has the first double bond at the 6<sup>th</sup> carbon atom when counting from the

terminal methyl group (Figure 1). Arachidonic acid (ARA, a biologically important molecule in the membrane phospholipids of mammals, for example) is also an n-6 fatty acid like n-6 DPA, but has only four double bonds and is 20 carbon atoms long like EPA. Heptadecanoic acid (HDA) is a saturated fatty acid (having no double bonds) and is 17 carbon atoms long.

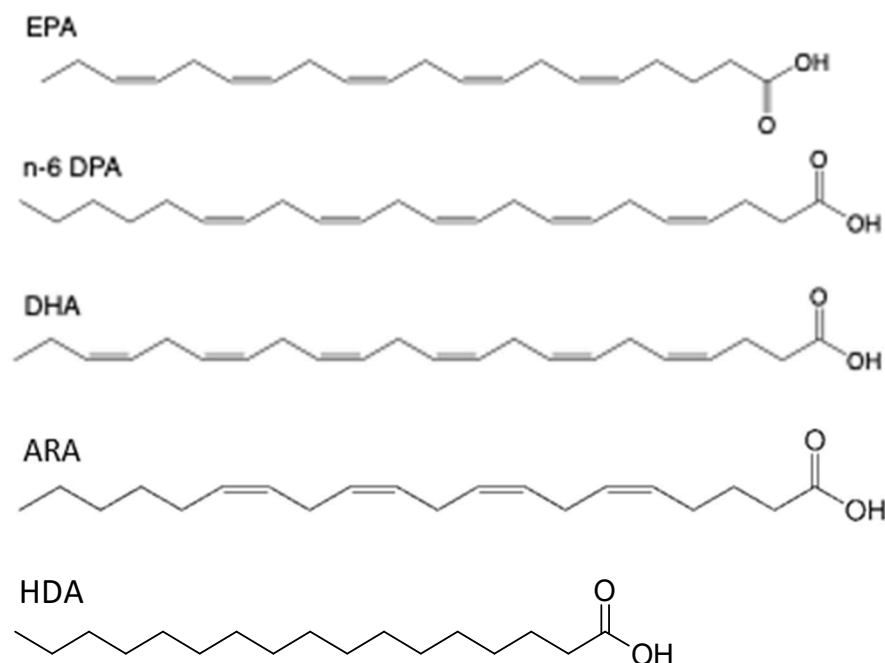


**Figure 1** EPA, DPA n-6, DHA, ARA, HDA structures

38. The vast majority of fatty acids that are found in nature in fats and oils are esterified to glycerol, predominantly as triacylglycerols (also referred to as “triglycerides”, especially in earlier literature), meaning three ester groups, through which the fatty acids are linked, are added to the glycerol molecule (Figure 2). MARA-1023, 175-176. However fatty acids may also be esterified to glycerol as

mono- or di-acylglycerols or as phospholipids, an example of which (phosphatidyl choline, PC) is given in Figure 2. MARA-1023, 176-177. A phospholipid is a class of lipids where the molecule has a hydrophilic (water-loving) “head” containing a phosphate group attached at the *sn*-3 position and two hydrophobic (water-hating) “tails” derived from fatty acids at the *sn*-1 and *sn*-2 positions (Figure 2). PC is the main component of lecithin obtained from soybean oil, and is also abundant in egg yolk lipids. Fatty acids can also be found esterified to other molecules such as cholesterol, sphingosine, or to fatty alcohols in wax esters. Edible oils and fats generally contain mainly triacylglycerols with only minor amounts of these other esters and other lipids present.

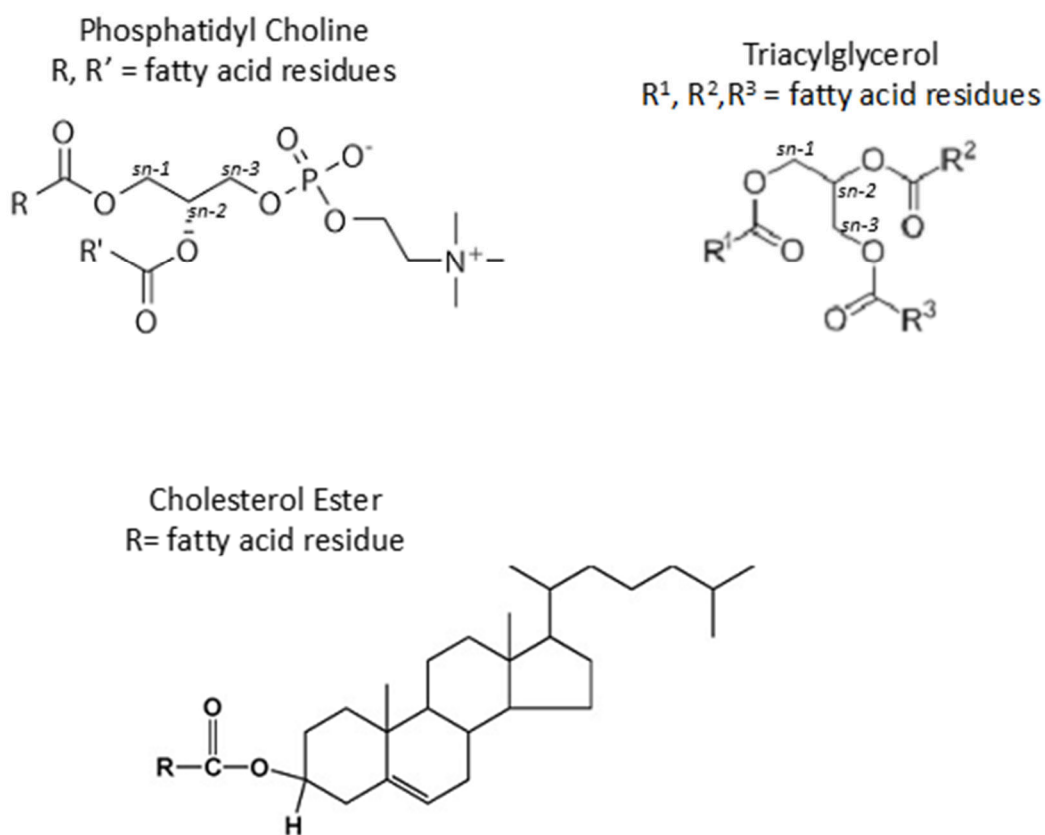


**Figure 2** TAG (triacylglycerides), PC, cholesterol ester structures.

39. There are many different fatty acids that can be found in triacylglycerols (also known as TAG, triacylglycerides, or triglycerides). The fatty acid composition can be expressed as a percentage of the “total fatty acid” (TFA, the sum of all measured fatty acid abundances), or as a weight fraction (mg/g) percentage which relates the measured weight of each fatty acid to that of the oil sample.

40. Often, when a lipid is extracted from an organism, the organism is first mechanically disrupted such as through heating, crushing, milling, drying,

etc., then solvents are used to ensure that all of the lipid is extracted. MARA-1024, 224 & Table 1, 224-245. Depending on the process used, the lipid extracted may include triacylglycerols, sterols and sterol esters, phospholipids, and other compounds soluble in that solvent. This could be described as a crude lipid extract or total lipid extract.

41. It is widely believed that most people do not have enough EPA and DHA in their diets for optimal health. MARA-1022, 14-18. As a result, a sizeable industry has been built around producing fish oils, such as from sardines, anchovies, tuna etc., and which are rich sources of these fatty acids. However, it is not sustainable to harvest increasing amounts of fish, and it is well known that fish themselves are not the primary producers of these valuable fatty acids, but rather concentrate them from the plankton they ingest. Therefore, considerable investments have been made into isolating and growing marine microbes which are the primary producers of the n-3 PUFA (polyunsaturated fatty acids) that is required for human nutrition, and can also be utilised in aquaculture.

42. Burja et al. noted that “There are two groups of marine microbes that have been found to produce these oils, namely, the marine diatoms, *Cryptheconidiaceae*, and the marine protists, *Thraustochytriidae*.” MARA-1025, 1161. They further described how whilst *thraustochytrids* show particular promise for industrial production of especially DHA, “there exists a great deal of variability

in biomass, lipid, and DHA yields within strains, mostly dependent upon fermentation conditions.” MARA-1025, 1162. For instance, in one of the thraustochytrid strains isolated in this paper, a DHA content of 35% of total fatty acids was achieved. MARA-1025, 1166. It was noted that this was similar to commercial strains at the time but lower than reported literature values of up to 50-53% of TFA. MARA-1025, 1166. In addition, they noted that the n-6 PUFA arachidonic acid was produced (as was n-6 DPA at 3-7% w/w) along with variable amounts of EPA. MARA-1025, 1166. Also illustrated was that the DHA content could be manipulated through changes to the composition of the growth medium such that the DHA content changed over the range of 16.23-31.55% as a percentage of the TFA. MARA-1025, 1166-1167 & Table 1.

43. A 2007 review chapter by Wynn and Ratledge established that by 2006 two commercial microbial oils were available, both with ~40% DHA w/w of TFA which, following extensive safety studies, had been incorporated into at least 6 infant formulas. MARA-1022, 58-67 & Table 2. Thus, refined microbial oils are entirely suitable for human nutrition.

44. It may be desirable to produce an oil which is highly concentrated (e.g., >90%) in n-3 fatty acids and especially DHA, for pharmaceutical or nutraceutical purposes. This can be achieved by refining a crude microbial oil, using many well known processes such as molecular distillation. For this purpose,

the crude microbial oil should have a low abundance of fatty acids that are less desirable for an n-3 fatty acid concentrate and have similar structures. This would include other polyunsaturated fatty acids of similar chain lengths, such as the n-6 fatty acids ARA or DPA, which would lower the n-3 fatty acid content whilst being the most difficult to remove in industrial processes due to their similar physical properties. It may therefore be preferable to optimize microbial oil production for maximum DHA content whilst controlling the final concentrations of ARA, DPA, and certain other fatty acids.

45. Also, whilst mammals generally have the ability to convert DPA ultimately to DHA, no such conversion is possible for ARA, which is a precursor to certain pro-inflammatory eicosanoids. MARA-1010, 73 (“AA is also a biosynthetic precursor to several classes of biologically active metabolites, such as eicosanoids.”).

## **2. Extraction of Microbial Oils**

46. In general, the steps required to produce edible oils include:
- a. Recovery of the crude lipid (fat or oil) by extraction, crushing or rendering an organism, depending on whether it is a plant, animal, or microbial source. This stage allows the crude lipid to be separated from the remaining biomass; the latter may have other uses such as the defatted meal recovered from oilseeds. The crude

lipid generally contains undesirable components such as residual solvent (often hexanes, if used in the extraction), phosphatides (gums), pigments, free fatty acids, and flavour and aroma compounds. Hence it is usual to remove these undesirables through refining to produce high quality edible oils.

- b. Various stages of refining are applied to the crude lipid. Common processes include degumming, a water wash process to remove phospholipids; caustic refining, to remove free fatty acids; bleaching, using adsorbents such as clays or carbon to remove pigments, lipid oxidation products, and other contaminants; and deodorization, using steam distillation at high temperatures and low pressures to remove residual flavours, aromas, and volatile compounds.
- c. Optionally, various types of conversions can also be applied to the crude or refined oil, such as fractional crystallization, hydrogenation, interesterification etc. which convert the oil to a form most suitable for a specific application. MARA-1024, 223-273.

47. For edible applications, refined oils must have acceptable organoleptic properties, be essentially free of residual solvent, have acceptably low levels of

processing or environmental contaminants, have very low levels of lipid oxidation products, and must have suitable stability. Thus, commercially produced edible oils must go through considerable chemical and physical testing to ensure that they meet these and other required specifications. There are several types of testing:

- a. A test based on the use of chromatography, which separates one component from another. Examples include determining the amount of an individual fatty acid (such as DHA or n-6 DPA) that is present in the entire lipid, which is commonly performed by gas chromatography after conversion to fatty acid methyl esters. Other types of chromatography could also be used to separate triacylglycerols (triglyceride) from other classes of lipids, such as polar lipids (e.g. phosphatidyl choline, lecithin) or free fatty acids.
- b. A test based on the accurate determination of the presence of an element in the lipid. There are many well-known techniques for elemental analyses including types of atomic spectroscopy; these could accurately measure not only the level of phosphorus but also might be employed to measure levels of undesirable heavy metals.
- c. Standardized tests based on a reaction, which indicate the level of a class of compound but do not specifically measure an individual compound. Two examples of these types of measurements are

i. Peroxide value (PV). In this test, lipid hydroperoxides that are present in the oil react with potassium iodide in the presence of acetic acid to liberate iodine; the amount of iodine liberated is measured. The PV test is a measure of oxidation applicable to oils that are only oxidised to low extent, which should be the case for freshly refined oils. Since lipid hydroperoxides are the precursors to secondary oxidation products that include flavour and aroma compounds, PV is useful indication of oil quality. However, as stated by Frankel “knowledge of the history of the oil samples is important to better interpret the significance of peroxide value measurements.” MARA-1027, 105. Thus, a PV measurement alone can be misleading, for example since peroxide levels can fall following high levels of oxidation. In addition, the PV of an oil can be lowered through the use of refining techniques like those described above; but it may also increase during storage (especially for PUFA) at a rate that depends on the presence of oxygen, antioxidants, prooxidants, temperature and other factors.

- ii. Para-Anisidine value (pAV or AV). The AV indicates the level of carbonyl compounds that are present in oils as secondary lipid oxidation products, *i.e.* that were formed by decomposition of lipid hydroperoxides, the primary oxidation products. This chemical test measures the degree of reaction of any saturated and unsaturated carbonyl compounds with the p-anisidine reagent. The AV “provides useful information on non-volatile carbonyl compounds formed in oils during processing” and hence is a widely used indication of oil quality. MARA-1027, 107. However, there is no direct correlation between AV and taste/smell since, for example, it is not sensitive to some of the low molecular weight aldehydes known to have extremely high aroma impact on the oil. The AV is also highly affected by oil refining processes (bleaching, steam deodorization) so oils with higher AV after extraction processes can have low AV after refining.
- iii. In summary, both AV and PV are widely used specifications for edible oils and can give an indication of oil quality at a particular point in time, and depending on how the oil has

been processed and stored. PUFA containing oils are particularly susceptible to oxidation and hence to changes in AV and PV. Hence AV and PV are not used to specify the composition of oils or lipid, but rather they are a transient indication of oil quality.

## **II. SUMMARY OF THE '363 PATENT**

### **A. Brief Description**

48. My review of the '363 patent indicates that it describes strains of thraustochytrids (a marine microorganism) and methods and compositions for culturing, extracting, and using fatty acid oils derived from those thraustochytrids. MARA-1001 at Abstract.

49. In particular, I note that the '363 patent identifies thraustochytrids on deposit under ATCC Accession Numbers PTA-9695, PTA-9696, PTA-9697, and/or PTA-9698. MARA-1001, 3:4-6. The '363 patent also describes “mutant, variant or recombinant” strains related to those ATCC-deposited microorganisms. MARA-1001, 5:54-6:10.

50. Microorganisms of the purported invention are described as containing varying fatty acids, including a triglyceride fraction (TG or TAG fraction) and a total fatty acid (TFA) fraction, in which the TG and/or TFA fractions has varying amounts of docosapentaenoic acid n-6 (DPA n-6),

eicosapentaenoic acid (EPA), heptadecanoic acid (HDA), arachidonic acid (ARA), and/or docosahexaenoic acid (DHA). MARA-1001, 6:23-31, 11:13-13:23, 1:34-48, 2:53-61, 3:42-62, 4:4-5. Mutant/variant strains produce differing amounts of these same fatty acids. MARA-1001, 5:66-6:5.

51. My review of the '363 patent indicates a description of culturing methods, in which thraustochytrids are cultured in varying conditions (such as dissolved oxygen, pH, glucose concentrations, and/or temperature ranges) and then their oils are extracted. MARA-1001, 4:21-39, 7:20-43, 10:1-11 (referencing MARA-1019); MARA-1019. Those conditions are known in the art and encompass "conventional medium for growth of thraustochytrids." MARA-1001, 7:20-31, 6:34-50 (referencing MARA-1019); MARA-1019.

52. I note that the '363 patent states that microbial oils of the invention extend to "any oil derived from a microorganism," which includes "a crude oil," a "refined oil that is obtained by treating a crude microbial oil," a "diluted microbial oil obtained by diluting a crude or refined microbial oil," and "an enriched oil" obtained "by treating a crude or refined microbial oil with further methods of purification to increase the concentration of a fatty acid (such as DHA) in the oil." MARA-1001, 10:13-23. The '363 patent states that these oils may then be applied in compositions, such as in "food products, pharmaceutical compositions, cosmetics, and industrial compositions." MARA-1001, 14:62-64.

53. The '363 patent includes 15 claims, of which claims 1, 3 and 4 are independent.

**B. Summary of the Prosecution History of the '363 Patent**

54. My review of the '363 patent indicates that it issued on June 26, 2012 from U.S. Patent Application No. 12/407,687 (“the '687 application”), filed on March 19, 2009. *See* MARA-1002.<sup>2</sup>

55. My review of the '363 patent’s prosecution history indicates little back-and-forth between the Patent Examiner and the Applicant.

56. I understand that the Examiner rejected nearly all of the claims following two restriction requirements over Elswyk, U.S. Pat. App. Pub. No. 2003/0060509 alone or in combination with Corley et al., U.S. Pat. No. 6,399,803. In that rejection, the Examiner relied on Elswyk for describing that “[t]he oil, as a highly purified algal oil, comprises at least 70% triglycerides by weight. *See* Abstract and paragraphs 6, 7, 9, 43, 72, 84 and 85.” MARA-1002, 143.

57. I understand that in response to that rejection, Applicant argued that Elswyk provides a highly unsaturated fatty acid (HUFA) in at least one of five forms: “highly purified algal oil comprising 70% or more of the desired HUFA,

---

<sup>2</sup> For this reference, page numbers are provided based on the PDF having a total of 337 pages.

triglyceride oil combined with phospholipid, phospholipid, protein and phospholipid combination, or dried marine microalgae”, in which each underlined phrase indicates one of the five forms. MARA-1002, 103 (emphasis in original). The Applicant also argued that the first form is “an oil comprising 70% or more of the desired HUFA, which does not require the desired HUFAs to be present in the triglyceride fraction,” that the second form is “a triglyceride oil combined with phospholipid, which does not require the combination to have at least 70% triglycerides,” and that “Elswyk's disclosure regarding a highly purified algal oil comprising 70% or more of the desired HUFA cannot be construed as being equivalent to an algal oil comprising at least 70% triglycerides by weight.” MARA-1002, 103-104.

58. In a final Office Action, I understand that the Examiner reiterated the prior rejection. An interview was conducted with Applicant’s representatives, the Examiner, and the Supervisor, in which the Supervisor agreed with Applicant’s interpretation of Elswyk that “the 70% minimum content (for the algal oil composition) refers to only the first item in the list of five, not to all five” and in which the rejection was to be withdrawn. MARA-1002, 67.

59. I also note that Applicant acknowledged the proposed interpretation of Elswyk in the Interview. MARA-1002, 59.

60. It is my understanding that, following Applicant's confirmation of this interpretation of Elswyk and upon cancelling non-elected claims, the Examiner allowed the pending claims. MARA-1002, 20.

**C. The Effective Priority Date of the Claims of the '363 Patent**

61. My review of the '363 patent indicates that it issued from U.S. Application No. 12/407,687, filed on March 19, 2009.

62. Claims 1, 3, and 4 of the '363 patent are independent claims. Claims 2 and 5-15 depend directly or indirectly from claims 1, 3, and 4. Accordingly, claims 2 and 5-15 cannot enjoy an effective filing date earlier than that of claims 1, 3, and 4 from which they depend (*i.e.*, no earlier than March 19, 2009).

**D. Level of Ordinary Skill in the Art**

63. A person having ordinary skill in the art relating to the subject matter of the '363 patent as of March 19, 2009 (a "PHOSITA") would have had a Master of Science degree in an academic discipline emphasizing chemistry, chemical engineering, or biochemistry, in combination with training or at least two to three years of related work experience with lipid chemistry technologies, including but not limited to lipid extraction technologies. Alternatively, the person could have also had a Doctorate degree in a relevant academic discipline with one to two years of related work experience in the same discipline.

64. A PHOSITA would have been familiar with microbial oils. I have summarized some of the knowledge that a PHOSITA would have above. In particular, a PHOSITA would have understood the benefits of polyunsaturated fats (PUFAs), microbial sources for obtaining them, and methods for culturing and extracting oils from those microbial sources.

### **III. THE CHALLENGED CLAIMS ARE UNPATENTABLE**

65. As I summarize below, it is my opinion that the prior art discloses each of the elements of the Challenged Claims, and claims 1-15 of the '363 patent are unpatentable.

#### **A. GROUND 1: Claims 1-3 is anticipated by Radianingtyas**

##### **1. Overview of Radianingtyas**

66. It is my opinion that Radianingtyas, like the '363 patent, is directed to methods of culturing oil-producing microbes and extracting microbial oils rich in omega-3 and omega-6 fatty acids. MARA-1007, 1:27-2:2; MARA-1001, 1:16-21, 1:49-2:3, 2:18-37, 9:66-14. I note that the method of Radianingtyas includes collecting cell biomass, breaking or disrupting that biomass, and then extracting the biomass with solvents such as “chloroform, hexane, methanol, ethanol or via supercritical fluid extraction means.” MARA-1007, 32:10-14.

67. It appears to me that Radianingtyas's resultant oils comprise unsaturated fatty acids with approximately 96-100 wt.% triglycerides in the neutral

lipid fraction, where the percentage of neutral lipids “can be at least 95% by weight of total lipids.” MARA-1007, 32:20-21, 33:1-2.

68. Radianingtyas also discloses that constituent fatty acids include DHA, DPA, as well as low levels of heptadecanoic acid (HDA or 17:0, also known as margaric acid). MARA-1007, 11:4-9, 99:22-24, 99:29-32, 100:23-25, Figure on 9/16 (PDF page 123), Table on 11/16 (PDF page 125). As Radianingtyas discloses, naturally-occurring microorganisms produce microbial oils with “high concentrations of omega-3 fatty acids...and low concentrations of omega-6 fatty acids.” MARA-1007, 12:22-25, 13:7-10, 17:19-20, 40:2-4.

69. I note that Radianingtyas describes that microorganisms “can also produce antioxidants,” and the oils and antioxidants can be produced “both individually and cumulatively.” MARA-1007, 11:10-21, 18:4-20:4, 37:24-39:24, 89:32-90:25.

70. In my opinion, a PHOSITA would know that different yields of DHA could be obtained. Radianingtyas explains that “the composition of the lipid produced by the oil producing microbe can be manipulated based on the growing medium composition the oil producing microbe multipl[i]es in”, such as “[b]y changing both the ratios and types of carbon to nitrogen sources, the chemical composition of salt used and by the addition of certain chemical compounds which impact upon fatty acid production.” MARA-1007, 27:13-18. Radianingtyas also

explains that fatty acid production antagonists can be employed, “[f]or example, the ratio of omega n-3 and/or n-6 oils to omega n-9 oils can be increased by sumplementing [sic] the medium of an oil producing microbe with a fatty acid production antagonist.” MARA-1007, 27:22-28. More specifically, Radianingtyas teaches that increasing agitation, temperature, or dissolved oxygen during culturing have the effect of decreasing DHA content, while adding acetate, trace elements, metal, and vitamins to the production medium increases DHA content. MARA-1007, 28:14-20, 30:15-17.

71. I note that Radianingtyas contains disclosures of specific strains of thraustochytrids (i.e., a marine microorganism), one of which is labeled ONC-T18. *See* MARA-1007, 16:1-20:23, 26:29-27:3, 81:5-92:2. Radianingtyas discloses that “the fatty acid component which ONC-T18 produces under heterotrophic fermentation is almost entirely triglyceride (*at least 95%*) in nature.”<sup>3</sup> MARA-1007, 89:24-26. Further, Radianingtyas discloses that microorganisms such as ONC-T18 can produce “a lipid composition comprising from about 25% to about 40% of an omega-3 fatty acid, such as n-3 DHA, (for example, at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55% or 60% by weight)...and from about 4% to

---

<sup>3</sup> All emphasis added herein unless otherwise noted.

about 12% of an omega-6 fatty acid, such as n-6 DPA, (for example, at least 4%, 5%, 6%, 7%, 8%, 9%, or 10% by weight).” MARA-1007, 26:29-34.

## **2. Analysis**

72. I discuss below why, in my opinion, Radianingtyas anticipates claim 1 of the ’363 patent.

### Claim 1

#### ***[1pre] A microbial oil comprising***

73. In my opinion, Radianingtyas discloses a microbial oil.

74. I note that Radianingtyas describes “compositions and methods related to oil producing microbes that can produce unsaturated fatty acids which can be purified and used.” MARA-1007, Abstract, 12:1-2 (“Also disclosed are processes for the production of microbial derived (or single celled) oils.”), 40:29-31 (“Disclosed are methods of preparing a lipid composition, the method comprising: culturing a[n] oil producing microbe[]comprising one or more microorganisms from the family Thraustochytriaceae, and isolating the lipid composition”), 1:27-31 (“Disclosed are compositions and methods related oil producing microbes, which when cultured produce quantities of unsaturated fatty acids, such as omega 3 (n-3) and/or omega 6 (n-6) oils, such as DHA, EPA and DPA, capable of being purified and used....”), 1:22-26 (“Disclosed are compositions and methods related to a eukaryote of the order Thraustochytriales

and family Thraustochytriaceae which when cultured produce quantities of unsaturated fatty acids, such as omega 3 (n-3) and/or omega 6 (n-6) oils, such as DHA, EPA and DPA, capable of being purified and used as all such compositions are used”), 40:29-31.

75. I also note that Radianingtyas discloses that “[c]ellular lipids containing the (n-3) DHA, EPA and (n-6) DPA PUFAs can...be extracted from the cellular biomass...to produce a sample of concentrated lipid material.” MARA-1007, 42:10-14, 32:10-14, 42:10-43:10.

76. In my opinion, Radianingtyas’s disclosure of “concentrated lipid material” and “lipid compositions” from microbial sources is indicative that Radianingtyas discloses microbial oils.

***[1a] a triglyceride fraction of at least 70% by weight,***

77. In my opinion, Radianingtyas also discloses a triglyceride fraction of at least 70% by weight. I understand “triglyceride fraction” to simply refer to the percentage of the microbial oil that is in triglyceride form.

78. I note that Radianingtyas identifies “oil producing microbes of the order Thraustochytriales...having the ability to produce lipids” containing “omega-3 fatty acids” and “omega-6 fatty acids.” MARA-1007, 11:4-9. Radianingtyas’s compositions include triglycerides representing from “96 to about 100%” of the

neutral lipids, which “can be at least 95% by weight of total lipids.” MARA-1007, 32:17-33:5, 89:24-26.

79. In particular, I note that Radianingtyas discloses the strain ONC-T18, of which “[a]nalysis of results shows that the fatty acid component which ONC-T18 produces under heterotrophic fermentation *is almost entirely triglyceride (at least 95%)* in nature.” MARA-1007, 89:24-26.

80. In my opinion, then, Radianingtyas discloses microbial oil compositions that are almost entirely triglycerides, meaning that they have a triglyceride fraction of at least 70% by weight.

*[1b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight,*

81. In my opinion, Radianingtyas discloses examples in which the triglyceride fraction is at least 50% by weight DHA. I understand this to mean that at least 50% of the fatty acids in triglyceride form are DHA.

82. I note that Radianingtyas discloses that microorganisms “such as ONC-T18...can produce a lipid composition comprising from about 25% to about 40% of an omega-3 fatty acid, such as n-3 DHA, (for example, at least...40%, 45%, 50%, 55% or 60% by weight).”<sup>4</sup> MARA-1007, 26:29-34.

---

<sup>4</sup> I note that this paragraph identifies “a lipid composition comprising from about

83. I note that Radianingtyas discloses the strain ONC-T18, of which “[a]nalysis of results shows that the fatty acid component which ONC-T18 produces under heterotrophic fermentation *is almost entirely triglyceride (at least 95%)* in nature.” MARA-1007, 89:24-26; *see also*, MARA-1007, 32:17-33:5. In my opinion, because the majority of Radianingtyas’s microbial oils are made up of triglycerides (e.g., “at least 95%” in the lipid composition), a PHOSITA would have understood that the majority of PUFAs (e.g., at least 60% DHA in the lipid composition) in Radianingtyas must be present in triglyceride form and that such an oil would meet [1b]. MARA-1007, 89:24-26; *see also*, MARA-1007, 32:17-33:5. Consistent with this understanding, Komazawa discloses a *Thraustochytrium* strain that contains DHA components ranging from 62% to 84% in triglyceride form. *See, e.g.*, MARA-1015, [0116] (stating that “[t]he DHA component in the

---

25% to about 40% of an omega-3 fatty acid, such as n-3 DHA.” MARA-1007, 26:29-27:3. Immediately thereafter, a parenthetical states “for example, at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% by weight[.]” MARA-1007, 26:33-34. Thus, this disclosure encompasses 60% by weight DHA concentration. In my opinion, the DHA disclosure, based on the parenthetical disclosure, should be read as “from about 15% to about 60% of an omega-3 fatty acid, such as n-3 DHA.”

triglycerides produced shows characteristic tendency. Namely,...DHA component becomes a high content of 80% or more, when culturing was carried out at pH8.5"); *see*, MARA-1015, Fig. 3 (stating "DHA content in triglyceride" in the header and disclosing about 80% DHA in triglyceride form at pH 8.5 and about 58% DHA in triglyceride form at pH 4.5).

84. Moreover, in my opinion, in an oil containing 60% DHA (as contemplated by Radianingtyas), even if the fatty acid portion of the remaining 5% non-triglyceride portion *was entirely DHA* (which is unlikely as the non-triglyceride portion includes various components that do not contain any fatty acids, such as sterols, carotenoids, alkanes, etc., and phospholipids and sterol esters that contain lower amounts of fatty acids, which are unlikely to be specifically DHA given the abundance of all fatty acids) and with the understanding that this is an extreme example, the oil would still contain *at least* 55% DHA in triglyceride form and would still meet [1b].

85. High DHA content in thraustochytrid oils was contemplated and described by Radianingtyas, in which various strains of thraustochytrids were shown to contain more than 50% DHA by weight. *See, e.g.*, MARA-1007, 82 & Table 3 (showing the following strains having more than 50% DHA when taking the DHA amount (in mg/g) and dividing by the total lipid amount (in mg/g): ONC-T02 (60%), ONC-T07 (53%), ONC-T09 (58%), ONC-T10 (54%), ONC-T12

(60%), ONC-T19 (52%), ONC-T25 (59%), ONC-T46 (52%), ONC-T52 (64%), ONC-T67 (55%), and ONC-T68 (55%)); MARA-1007, 32:2-3, 3:25-28, Tables 7-8, Fig. 10 on 10/16 (PDF page 124) (showing various total lipid, TFA, or lipid content as being in units of “mg/g”).

86. I note that Radianingtyas also states that extracted oils can be further purified to increase DHA content. *See* MARA-1007, 8:2-4 (“For example, if a disclosed eukaryotic microorganism, for example, produces 35% DHA, this could be further ‘purified’ such that the final lipid composition was greater than 90% DHA.”).

87. Based on these disclosures in Radianingtyas, in my opinion, a PHOSITA would have understood that the selection of ONC-T18 may have been related to other beneficial properties of this strain (e.g., increased amount of total lipid production) and that high DHA-producing thraustochytrid strains were readily available even under non-optimized screening conditions.

88. In my opinion then, Radianingtyas teaches a triglyceride fraction that is at least 50 wt.% DHA.

***[1c] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from 0.5% by weight to 6% by weight,***

89. In my opinion, Radianingtyas discloses a microbial oil that comprises docosapentaenoic acid n-6 content of the triglyceride fraction in an amount from 0.5% by weight to 6% by weight.

90. I note that Radianingtyas discloses that microorganisms “such as ONC-T18....can produce a lipid composition comprising...from about 4% to about 12% of an omega-6 fatty acid, such as n-6 DPA, (for example, **at least 4%**, 5%, 6%, 7%, 8%, 9%, or **10% by weight**).” MARA-1007, 26:29-27:3. I also note that Radianingtyas discloses the strain ONC-T18, of which “[a]nalysis of results shows that the fatty acid component which ONC-T18 produces under heterotrophic fermentation **is almost entirely triglyceride (at least 95%)** in nature.” See, e.g., MARA-1007, 89:24-26; see also, e.g., MARA-1007, 32:17-33:5. In my opinion, a PHOSITA would have understood that because the majority of Radianingtyas’s microbial oils are made up of triglycerides (e.g., “at least 95%” in the lipid composition), the majority of the PUFAs (e.g., at least 4% DPA in the lipid composition) in Radianingtyas must be present in the triglyceride form, and that such an oil would meet [1c]. MARA-1007, 89:24-26; see also, e.g., MARA-1007, 32:17-33:5.

91. In my opinion, even if the fatty acid portion of the remaining 5% non-triglyceride portion **was entirely DPA** (which is unlikely as the non-triglyceride portion includes various components that do not contain any fatty acids, such as sterols, carotenoids, alkanes, etc., and phospholipids and sterol esters that contain lower amounts of fatty acids, which are unlikely to be specifically DPA given the abundance of all fatty acids) and with the understanding that this is an extreme

example, an oil containing at least 10% DPA (as contemplated by Radianingtyas) would still contain *at least* 5% DPA in triglyceride form (i.e., at least 10% DPA – remaining 5% non-triglyceride portion as DPA = at least 5% DPA in triglyceride form) and would still meet [1c]. Furthermore, Radianingtyas discloses oils having lower DPA content (e.g., lower than 10%, such as at least 4%, 5%, 6%, 7%, 8%, or 9%). In my opinion, a PHOSITA would have understood that at least one of these lower DPA oils would contain 0.5% to 6% DPA in triglyceride form for a high triglyceride oil.

92. I note that Radianingtyas discloses that microorganisms “such as ONC-T18....can produce a lipid composition comprising...from about 4% to about 12% of an omega-6 fatty acid.” MARA-1007, 26:29-27:3. Specifically with regard to experimental conditions provided in Fig. 11 with ONC-T18, Radianingtyas shows that DPA n-6 (C22:5w6) is present in an amount between 3.88% to 9.43% of the total fatty acid content, in which 9 of 13 experimental conditions provide between 0.5% to 6% DPA n-6 of the total fatty acid content. MARA-1007, Fig. 11 on 12/16 (PDF page 126).

93. I understand from counsel that an overlap in a range can demonstrate anticipation. Based on that understanding, it is my opinion that Radianingtyas’ disclosure of a 3.88-9.43% quantity of DPA n-6 anticipates that portion of the ’363 patent, claim 1.

94. In my opinion then, Radianingtyas teaches a docosapentaenoic acid n-6 content of the triglyceride fraction that is from 0.5% by weight to 6% by weight.

***[1d] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

95. In my opinion, Radianingtyas discloses a microbial oil that comprises 5% by weight or less of heptadecanoic acid.

96. I note that Radianingtyas discloses exemplary oils that all consistently contain low amounts of heptadecanoic acid (i.e., HDA, referred to as “17:0” or “margaric acid”). See MARA-1007, 34:4-37:14 (showing margaric acid ranging from 0.17% to 2.69% as percentage of total fatty acid); see also MARA-1007, Fig. 11 on 11/16 (PDF page 125) (showing HDA (C17:0) present in amounts between 0.31% to 2.29% of the total fatty acid content for “Control”).

97. As mentioned above, I understand from counsel that an overlap in a range can demonstrate anticipation. Based on that understanding, it is my opinion that Radianingtyas’ disclosure of a 0.17% to 2.69% or 0.31% to 2.29% quantity of HDA anticipates this claimed element of the ’363 patent, claim 1.

98. Furthermore, I understand from counsel that Element [1d] is an optional negative limitation, for which prior art disclosure is not necessary to meet the claim element. In other words, an oil containing no HDA meets this limitation. Radianingtyas discloses a microbial oil in which HDA is not disclosed at all, and is either absent or below the limit of detection. See, e.g., MARA-1007, 32:20-25

(“typical composition of fatty acids for ONC-T18, in the neutral lipids” summing to 100% without disclosing HDA).

99. In my opinion then, Radianingtyas teaches wherein the oil comprises 5% by weight or less of heptadecanoic acid.

100. Because Radianingtyas discloses all elements of claim 1, it is my opinion that Radianingtyas anticipates claim 1.

#### Claim 2

***[2] The microbial oil of claim 1, further comprising an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.***

101. In my opinion, Radianingtyas discloses the microbial oil of claim 1 that further includes an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.

102. I note that Radianingtyas discloses exemplary oils that contain 1.5% by weight or less of ARA. In Example 13, titled “Isolation and characterization of [PUFA] producing[sic] *Thraustochytrium* sp. ONC-T18; screening in order to identify strain and optimization of production therein,” Radianingtyas discloses 68 isolates (ONC-T01 to ONC-T68 strains) and states that “[i]n addition to the omega-3 oils produced, approximately 80% of all isolates synthesized the omega-6 PUFAs, *arachidonic acid (AA)* or docosapentaenoic acids (DPA), *at*

*concentrations varying between 1 and 18% and 3 and 7% w/w, respectively.”*

MARA-1007, 96:1-3, 99:29-32.<sup>5</sup>

103. Specifically with regard to experimental conditions provided in Fig. 11 with ONC-T18, Radianingtyas shows that this strain can produce oils that all consistently contain low amounts of ARA (or C20:4w6). *See* MARA-1007, Fig.

---

<sup>5</sup> While Radianingtyas (MARA-1007) refers to Fig. 11 as describing PUFA content in isolates, this is likely a clerical error and should actually refer to Fig. 11 of the US priority document (MARA-1045) for Radianingtyas. Of note, Fig. 11 of MARA-1007 and MARA-1045 are different. In fact, Fig. 11 of MARA-1007 relates to “the gas chromatography results of *culturing ONC-T18* with examples of fatty acid production antagonists.” MARA-1007, 3:15-16. However, Fig. 11 of MARA-1045 relates to “*a grouping of isolates* collected based on their C20 and C22 PUFA profiles. Results were compared to two reference strains: ATCC 20891 and MYA-1381.” MARA-1045, 2:21-22. Indeed, Fig. 11 of MARA-1045 is similar to Fig. 2 in MARA-1025, which shows “Grouping of isolates collected based on their C20 and C22 PUFA profiles. Key:  Arachidonic acid;  Eicosapentaenoic acid;  Docosapentaenoic acid, n-6; and  Docosahexaenoic acid. Results were compared to two reference strains: ATCC 20891 and MYA-1381.” MARA-1045, Fig. 11; MARA-1025, 1163 & Fig. 2.

11 on 11/16 (PDF page 125) (showing ARA (C20:4w6) present in amounts between 0.13% to 0.9% of the total fatty acid content or between 0.13% to 0.38% of the total fatty acid content for “Control”), 34:4-37:14 (stating ARA ranging from 0.13% to 0.90% as percentage of total fatty acid).

104. Consistent with my earlier analysis for claim 1, claim 2 also recites an optional negative limitation, in which an oil containing no ARA meets this limitation. Radianingtyas discloses a microbial oil in which ARA is not disclosed at all, and is either absent or below the limit of detection. *See, e.g.*, MARA-1007, 32:20-25 (“typical composition of fatty acids for ONC-T18, in the neutral lipids” summing to 100% without disclosing ARA).

### Claim 3

#### ***[3pre] A microbial oil comprising***

105. *See* Section III(A)(2)[1pre], *supra*.

#### ***[3a] a triglyceride fraction of at least 70% by weight,***

106. *See* Section III(A)(2)[1a], *supra*.

#### ***[3b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight,***

107. *See* Section III(A)(2)[1b], *supra*.

#### ***[3c.1] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight,***

108. *See* Section III(A)(2)[1c], *supra*.

***[3c.2] wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1,***

109. Radianingtyas discloses a microbial oil that includes a ratio of docosahexaenoic acid to docosapentaenoic acid n-6 that is greater than 6:1.

110. As discussed with respect to Elements [1b] and [1c] in Section III(A)(2)[1b]-[1c], *supra*, Radianingtyas discloses a microbial oil having at least 55% DHA in triglyceride form and at least 5% DPA in triglyceride form. *See* Section III(A)(2)[1b]-[1c], *supra*. Taking such an oil, the resultant ratio of DHA to DPA is greater than 6:1 (i.e., a ratio of 11:1 for 55% DHA:5% DPA).

***[3d] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

111. *See* Section III(A)(2)(1d), *supra*.

**B. GROUND 2: Claims 1-15 are obvious over Radianingtyas in view of Kralovec**

**1. Overview of Radianingtyas**

112. I previously described Radianingtyas above in Section III(A)(1).

**2. Overview of Kralovec**

113. I note that Kralovec relates to methods for modifying oil concentrations by concentrating polyunsaturated fatty acids. MARA-1030, 1:9-11. Kralovec recognizes that omega-3 fatty acids like DHA have “well established” beneficial effects, such as reduction of “cardiovascular and heart disease risk” and “prevention and/or treatment of inflammation, neurogenerative diseases, and

cognitive development.” MARA-1030, 1:16-2:3. Kralovec also acknowledges that omega-3 fatty acids can be found in microbial and algal oils, which “typically provide the PUFA in a triglyceride form where other undesired, fatty acids...are present along side a desired PUFA in the triglyceride molecule.” MARA-1030, 2:4-8. Kralovec is thus directed to a modification method to produce “an oil having higher levels of a PUFA...than the starting oil composition.” MARA-1030, 5:24-28. Kralovec also acknowledges that “[s]everal methods of producing PUFA concentrates from oils, such as marine, microbial, and/or algal oils, are known, for example, selective lipase hydrolysis, PUFA complexation using urea...and a physical removal of unwanted components by fractionation...Fractionation involving molecular distillation is usually conducted on ethyl esters prepared from the starting triglycerides since they are more volatile than corresponding triglycerides”, thus showing that the Kralovec method is only one example that a PHOSITA would consider for further concentrating PUFA, including DHA, from oils including microbial and algal oils. MARA-1030, 2:9-16.

114. I also note that Kralovec indicates its method can be used with “any suitable oil composition that comprises a glyceride,” including microbial and algal oils. MARA-1030, 6:28-7:2. Kralovec suggests that a “specific composition of a treated oil” can be “adjusted and/or tailored to have a target ratio of various

polyunsaturated fatty acids,” which can allow for “high concentrations of DHA.” MARA-1030, 6:9-13.

115. My review indicates that Kralovec’s method employs three steps: hydrolysis, separation, and esterification. Hydrolysis utilizes enzymatic lipase to contact a starting oil, which induces hydrolysis of the oil. MARA-1030, 9:8-10 (stating that “[t]he hydrolysis step comprises the use of a lipase enzyme in liquid form to hydrolyze at least a portion of the glyceride and provide a hydrolyzed glyceride fraction and a free saturated fatty acid fraction.”). This process creates hydrolyzed glycerides and free saturated fatty acids. MARA-1030, 9:8-10. Using modifications such as atmospheric conditions, mixing/agitation, or elevated temperature, a user induces a “desired amount of hydrolysis.” MARA-1030, 11:15-28.

116. Then, the “hydrolyzed glyceride composition...can then be separated...to separate at least a portion of the free saturated fatty acid from the glyceride.” MARA-1030, 12:13-16.

117. After, lipase is utilized to attach “desirable polyunsaturated fatty acid components, for example, EPA and/or DHA” to the hydrolyzed glyceride compositions. MARA-1030, 13:27-30. This method provides compositions that Kralovec describes as containing “triglycerides with higher DHA...contents than the starting triglycerides.” MARA-1030, 35:20-32.

### 3. Combination of Radianingtyas and Kralovec

#### a) DHA as the preferred PUFA

118. I noted above that Radianingtyas teaches methods of culturing oil-producing microbes and extracting microbial oils rich in omega-3 and omega-6 fatty acids. MARA-1007, 1:27-2:2; MARA-1001 1:27-32; 1:63-2:18; 2:33-52; 10:16-14:24. Importantly, Radianingtyas discloses that these oils, which contain unsaturated fatty acids including DHA, are “capable of being purified as all such compositions are used.” MARA-1007, 1:25-26, 1:29-30.

119. More specifically, I noted that Radianingtyas discloses examples directed to “[o]ptimized production of [DHA] by strain ONC-T18” and “[o]ptimal time for harvesting of ONC-T18 for maximal DHA production.” MARA-1007, 87:6-89:10.

120. Based on those disclosures, it is my opinion that it would have been obvious for a PHOSITA to have sought microbial oils having high levels of DHA as the preferred PUFA based on its teachings as well as those of other references available before the Critical Date. *See, e.g.*, MARA-1009, 27 (stating that a certain microbial strain is “particularly attractive” because it “contains very high levels of DHA (61%)”).

121. First, it is my opinion that a PHOSITA would have been motivated to increase DHA in Radianingtyas’s extracted microbial oil because there were many

recognized health benefits associated with DHA. For example, Radianingtyas teaches that “[t]here is overwhelming scientific evidence that (n-3) highly unsaturated fatty acids such as docosahexaenoic acid (DHA) have a positive effect on cardio-circulatory diseases, chronic inflammations and brain disorders....”

MARA-1007, 1:6-8.

122. Second, it is my opinion that a PHOSITA would have been motivated to increase DHA content in Radianingtyas’s microbial oil because Kralovec teaches that DHA is an omega-3 fatty acid, known to be “vital to everyday life and function.” MARA-1030, 1:13-14. For example, “[o]mega-3 fatty acids not only exhibit proven effects on lowering serum triglyceride levels, but they have strong connection to diabetes.” MARA-1030, 18:8-12. Omega-3 fatty acids were also “known for other cardioprotective benefits,” such as “reduc[ing] cardiovascular and heart disease risk.” MARA-1030, 1:13-2:3. With regard to DHA, Kralovec teaches that DHA was associated with “a strong insulin permeability enhancement effect” and was “viewed as a potential absorption enhancer for intestinal delivery of insulin.” MARA-1030, 18:10-19:2. For example, DHA was understood to “prevent[] certain biochemical processes that originate from insulin deficiency” and “significantly increase fasting insulin levels.” MARA-1030, 19:2-6.

123. Third, and additionally, it is my opinion that a PHOSITA would have been motivated to focus on increasing DHA in Radianingtyas’s oil because DHA

would have been an obvious choice selected from a small and finite number of preferred omega-3 fatty acids. For example, Radianingtyas discloses DHA as one of the two desirable n-3 PUFAs: docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3). MARA-1007, 1:6-20. Based on these limited desirable choices, a PHOSITA would have expected to be successful in pursuing increased DHA in a microbial oil as contemplated by Radianingtyas (and others). MARA-1007, 1:6-31, 26:29-34, 27:4-12; *see, e.g.*, MARA-1038, 3639 (stating that “[s]tudies with thraustochytrids have established these marine protists as preeminent industrial strains for production of DHA. Initial research, at relatively, low cell densities, (5-20 g/L) established the capacities of *Thraustochytrium* species to accumulate greater than 50% of their lipids as DHA and to produce > 1 g DHA/L of culture, with productivities of about 0.2 g/(L day).”).

124. Moreover, it is my opinion that a PHOSITA would have expected success in obtaining extracted oils with high levels of DHA given that it was already known that certain strains of microbes contained high levels of DHA, including various strains of thraustochytrids in Radianingtyas that contained more than 50% DHA by weight. MARA-1007, 82 & Table 3 (showing at least eleven (11) of sixty-eight (68) strains having more than 50% DHA (ONC-T02, -T07, -T09, -T10, -T12, -T19, -T25, -T46, -T52, -T67, -T68) and three strains having at

least 60% DHA (ONC-T02, -T12, -T52)); *see also, e.g.*, MARA-1015, [0116], Fig. 3 (disclosing a *Thraustochytrium* strain (LFF1 strain) with a DHA content of about 80% in triglyceride form); *see also, e.g.*, MARA-1009, 27 (stating that “[s]train O is particularly attractive as it contains very high levels of DHA (61%)”); *see also, e.g.*, MARA-1040, 300-301 (stating that “[a]mong the microalgal groups, thraustochytrids are one of the most promising sources of DHA” and that “DHA may represent as much as 50% of the total fatty acids in thraustochytrids”) and MARA-1040, 305 (further stating that “*Thraustochytrium* spp., in general, contained a larger proportion of DHA, ranging from 38.5%–59.5% of the total fatty acids”); *see also, e.g.*, MARA-1019, Table 3 (showing that, excluding “PRIOR STRAINS” (a term employed by Barclay), more than twenty (20) of 108 strains have more than 50% DHA and 0.5% to 6% DPA (n-6) of total fatty acids, of which three (3) strains have more than 70% DHA).

*b) Increased Omega-3 Fatty Acid (DHA) Concentration in Microbial Oils*

125. I also note that Radianingtyas teaches suitable starting oils that have not been treated, such as microbial oils from organisms that “can be used in a process for preparing a lipid or fat containing DHA, EPA and DPA.” MARA-1007, 16:31-17:3. As discussed above, Kralovec provides methods for obtaining compositions containing “triglycerides with higher DHA...contents than the starting triglycerides.” MARA-1030, 35:20-32. It would have been obvious for a

PHOSITA to combine the teachings of Radianingtyas, directed to microbial oils having high levels of triglycerides (e.g., “at least 95%” in the lipid composition) and DHA (at least 60% by weight in the lipid composition), with Kralovec, directed to methods for modifying an oil composition, to increase the concentration of DHA in the microbial oil. MARA-1007, 26:31-34, 32:17-21, 33:1-2, 89:24-26; MARA-1030, 1:9-11.

126. Specifically, it is my opinion that a PHOSITA would have found it obvious to implement Kralovec’s oil modification techniques (or similar) to Radianingtyas’s extracted microbial oil to increase the amount of DHA for at least the following reasons.

127. First, it is my opinion that a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to Radianingtyas’s extracted microbial oil to increase its DHA content because “purifying and concentrating PUFA’s [sic] in a triglyceride form is generally desired.” MARA-1030, 2:7-8. It would have been well-known that concentrating PUFA is desirable to reduce the concentration of unwanted components (e.g., saturated fatty acids) in a microbial oil so as to deliver high levels of active PUFA in lower amounts of oil, which is preferable for supplements and food applications. *See, e.g.*, MARA-1030, 2:4-8. Furthermore, decreasing the amount of unwanted components in a microbial oil would be desirable because “[p]olyunsaturated fatty acids, such as, for example

omega-3 fatty acids, are often derived from marine oils, microbial, and/or algal oils,” which “typically provide the PUFA in a triglyceride form where other undesired, fatty acids...are present along side a desired PUFA in the triglyceride molecule.” MARA-1030, 2:4-8.

128. In fact, a higher-purity, DHA-containing oil would be preferable because it results in a consumer needing to take fewer supplements (i.e. capsules) and reduces the “calories ingested from unwanted fatty acids.” MARA-1022, 113; MARA-1035, 381 (“Concentrates keep the intake of calories and other possibly physiologically active fatty acids as low as possible.”).

129. Second, it is my opinion that a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to Radianingtyas’s extracted microbial oil to increase its DHA content because the produced oils include “‘designer’ glycerides” and “often non-naturally occurring, ratios of polyunsaturated fatty acid ester” that provide “triglycerides that have better color and lower levels of trans-isomers” as well as “better sensory properties.” MARA-1030, 35:21-36:2. Kralovec describes that “[s]uch oils can be incorporated into food products, nutraceuticals, pharmaceutical formulations, and microcapsules.” MARA-1030, 35:21-36:2.

130. Third, I believe that a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to Radianingtyas’s extracted microbial oil

because such combinations reflect nothing more than the use of a known technology (e.g., enzymatic modification) to improve similar oils (e.g., extracted microbial oils) according to known methods (e.g., oil refinement methods) to yield predictable results (e.g., obtain oil with an increased concentration of a desired PUFA, e.g., DHA).

131. Fourth, in my opinion, a PHOSITA would also have been motivated to apply Kralovec's oil modification techniques (or similar) to Radianingtyas's microbial oils because concentrating high DHA oils from thraustochytrids can simplify processing, as compared to complex oils. A complex oil can include, e.g., "fish oil with up to 20 fatty acids (representing a continuous range of saturated, mono-, di-, tri-, and polyenoic fatty acids, and as such, a series of overlapping crystallization temperatures)." MARA-1019, 14:30-33. Instead, a PHOSITA would have sought microbial oils having high levels of the desired PUFA (such as DHA) that are more amenable to further processing to concentrate the PUFA of interest. *See, e.g.,* MARA-1019, 14:18-33 (stating that "[t]he advantage of this process is that it is not necessary to go through the urea complex process or other expensive extraction methods...to remove saturated and mono-unsaturated fatty acids prior to cold crystallization" and that this advantage begins "by starting the purification process with *an oil consisting of a simple fatty acid profile such as that produced by Thraustochytrids* (3 or 4 saturated or monounsaturated fatty

acids with 3 or 4 HUFAs, two groups of fatty acids widely separated in terms of their crystallization temperatures) rather than a complex oil such as fish oil with up to 20 fatty acids (representing a continuous range of saturated, mono-, di-, tri-, and polyenoic fatty acids, and as such, a series of overlapping crystallization temperatures)”).

132. It is my opinion that a PHOSITA would have expected success in applying Kralovec’s techniques to increase the concentration of DHA in the microbial oil because Radianingtyas teaches that “[t]he omega-3 and omega-6 PUFAs may be further concentrated by hydrolyzing the lipids and concentrating the highly unsaturated fraction by employing traditional methods.” MARA-1007, 42:14-16, 42:19-20 (“The extracted oils can be refined by well-known processes routinely employed to refine vegetable oils (e.g. by chemical or physical refining)”).

133. To me, a PHOSITA would have naturally considered “traditional” or “well-known” methods for modifying an oil such as Radianingtyas’s oil, and would have looked to methods including hydrolysis, such as in Kralovec’s methods or similar, for use in making DHA concentrates, for example. Thus, a PHOSITA would have been motivated to refine Radianingtyas’s oils in accordance with Radianingtyas’s teachings by looking to Kralovec.

134. Moreover, it is my opinion that a PHOSITA would have expected success in using Kralovec's method techniques for increasing the concentration of the preferred PUFA (i.e., DHA) particularly in the context of an extracted microbial oil given that Radianingtyas and Kralovec teach that their oils are microbial in origin. MARA-1007, 1:22-31; MARA-1030, 2:28-29, 7:18-21. Both Radianingtyas and Kralovec acknowledge their oils can be derived from *Thraustochytrium* or *Schizochytrium* species. MARA-1007, 11:4-5; MARA-1030, 7:20. Both acknowledge their oils contain omega-3 PUFAs, including DHA. MARA-1007, 1:22-31; MARA-1030, 19:7-12.

135. Furthermore, I note that both Radianingtyas and Kralovec acknowledge similar uses for their oils, such as human nutritional uses. Radianingtyas, for example, teaches that its resultant oil is "used as a nutritional supplements[sic] for humans, as a food additive, or as pharmaceutical applications." MARA-1007, 43:6-8. Kralovec too discloses that its oils "can be incorporated into food products, nutraceuticals, pharmaceutical formulations, and microcapsules." MARA-1030, 35:31-36:2.

136. Therefore, it is my opinion that a PHOSITA would have known to combine the teachings of Radianingtyas with a method such as that given by Kralovec to increase the final DHA content, and would have expected success in

doing so. I detail my opinion on how this combination renders claims 1-15 of the '363 patent obvious below.

#### **4. Analysis**

##### Claim 1

##### ***[1pre] A microbial oil comprising***

137. It is my opinion that Radianingtyas and Kralovec disclose a microbial oil. As I described above in Section III(A)(2)[1pre], it is my opinion that Radianingtyas discloses a microbial oil.

138. Kralovec also discloses a microbial oil, as it describes that its methods can be broadly used with “any suitable oil composition that comprises a glyceride,” including microbial and algal oils. MARA-1030, 6:28-7:2.

139. Radianingtyas’s disclosures are consistent with a PHOSITA’s knowledge of microbial oils, which were well known prior to the Critical Date. Other literature, such as Barclay, describes an extracted microbial oil. *See* MARA-1019, 1:23-28. It describes an “oil consisting of a simple fatty acid profile such as that produced by Thraustochytrids,” as well as oils produced from *Schizochytrium*, also a microbe. MARA-1019, 14:23-47. Contemporaneous literature describes that Barclay’s work employed “technology for DHA production from thraustochytrids,” which included “microbial production of single cell oil.” MARA-1033, 632. And at the same time, thraustochytrids were known as a source

of “oils with a suite of PUFA profiles that can have specific uses,” including “*Schizochytrium* oil, known as DHA Gold, [which was] marketed by Omega Tech Inc to supplement animal feeds.” MARA-1033, 631, 635.

***[1a] a triglyceride fraction of at least 70% by weight,***

140. It is my opinion that Radianingtyas and Kralovec also disclose a triglyceride fraction of at least 70% by weight. As I described above in Section III(A)(2)[1a], Radianingtyas discloses high-triglyceride-content microbial oils.

141. Similarly, it is my opinion that Kralovec discloses oils that “have a high TG (triglyceride) content,” for example, “the oil can comprise greater than or equal to about 60%, 65%, **70%, 75%, or 80% TG** by weight of the total composition.” MARA-1030, 37:22-24; 36 (Table 5 disclosing “TG content” of “60-80%” “after the disclosed hydrolysis and esterification”); *see also* MARA-1030, 40-41 & Table 8.1 (disclosing “TG” of “98.5%” or “99.0%” for oil samples after “enzymatic re-esterification”); *see also* MARA-1030, 43, Table 9.2 (disclosing “TG” of “91.2%” for the “TG product”); *see also* MARA-1030, 46, Table 10.2 (disclosing “TG” of “96.1%” or “99.0%” for oil samples after “enzymatic re-esterification”).

142. It is also my opinion triglyceride-rich oils were well known to a PHOSITA prior to the Critical Date. Consistent with the teachings in Radianingtyas and Kralovec, other references also disclosed triglyceride-rich oils.

MARA-1008, 2:30-33 (stating that “[a]ccording to a first aspect of the present invention there is provided a microbial oil, comprising at least one polyunsaturated fatty acid (PUFA), which has a triglyceride content of greater than 90%.”); MARA-1034, [0037]-[0038] (disclosing that “[t]ypically, the content of neutral lipids in the lipids of the present invention is very high (more than 90[%] of total lipids)” and that “[t]he neutral lipids from the lipids of the present invention contain at least 85% triacylglycerols, preferably at least 90% triacylglycerols”); MARA-1040, 301 (stating that “Fig. 4. summarizes the distribution of the major lipid classes in thraustochytrids and indicates that the major lipid class in thraustochytrids is neutral lipids, making up from 44.7–95.0% of total lipids” and that “[t]he proportions of different lipid classes in *T. aureum* ATCC 34304 varied when grown in different media, indicating the profound influence of media composition on lipid composition (Fig. 4 [showing ATCC 34304 as having about 65% or about 90% neutral lipids of the total lipids]))” (citations omitted); MARA-1040 at 302 (stating that “[i]n the neutral lipid fractions, triacylglycerols were the major component followed by diacylglycerols and monoacylglycerols (Table 1). Triacylglycerols account for 86.2% of the neutral lipids in *T. aureum* ATCC 34304, but in *Schizochytrium* sp. SR21, it can constitute 98.0% of the neutral lipids. Diacylglycerols and monoacylglycerols, however, were reported to be

minor components (< 10.0%) of the neutral lipids of thraustochytrids.”) (citations omitted).

143. It is thus my opinion that the combination of Radianingtyas and Kralovec discloses or suggests a triglyceride fraction of at least 70% by weight.

***[1b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight,***

144. It is my opinion that Radianingtyas and Kralovec disclose a triglyceride fraction that comprises at least 50% by weight DHA. As I described above in Section III(A)(2)[1b], Radianingtyas discloses that the ONC-T18 strain can produce a lipid composition comprising an omega-3 fatty acid, such as n-3 DHA in an amount of “at least...40%, 45%, 50%, 55% or 60% by weight” and that other strains of thraustochytrids contained more than 50% DHA by weight. MARA-1007, 26:29-34, 82 & Table 3 (showing at least eleven strains having more than 50% DHA (ONC-T02, -T07, -T09, -T10, -T12, -T19, -T25, -T46, -T52, -T67, -T68) and three strains having at least 60% DHA (ONC-T02, -T12, -T52)).

145. Additionally, a PHOSITA would have understood and/or found obvious to apply Kralovec’s (or similar) methods to the Radianingtyas microbial oil to produce a DHA-enriched microbial oil containing at least 50 wt.% DHA, or higher, such as at least 60 wt.% DHA in triglyceride form.

146. It is thus my opinion that the combination of Radianingtyas and Kralovec discloses or suggests that the docosaheptaenoic acid content of the triglyceride fraction is at least 50% by weight.

***[1c] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from 0.5% by weight to 6% by weight,***

147. It is my opinion that Radianingtyas and Kralovec disclose and/or render obvious a microbial oil that comprises the docosapentaenoic acid n-6 content of the triglyceride fraction that is from 0.5% by weight to 6% by weight. As I described above in Section III(A)(2)[1c], Radianingtyas discloses that microorganism ONC-T18 can produce a lipid composition comprising an omega-6 fatty acid, such as n-6 DPA in an amount of “at least 4%, 5%, 6%, 7%, 8%, 9%, or 10% by weight.” MARA-1007, 26:29-27:3. Additionally, a PHOSITA would have understood and/or found obvious to apply Kralovec’s (or similar) methods to the Radianingtyas microbial oil to produce an enriched microbial oil containing a desired amount of DPA in triglyceride form. That in turn discloses or renders obvious [1c].

***[1d] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

148. It is my opinion that Radianingtyas and Kralovec disclose an oil that comprises 5% by weight or less of heptadecanoic acid. As I described above in Section III(A)(2)[1d], Radianingtyas discloses examples in which exemplary oils all consistently contain low amounts of heptadecanoic acid (i.e., HDA, referred to

as “17:0” or “margaric acid”). *See* MARA-1007, 34:4-37:14 (showing margaric acid ranging from 0.17% to 2.69% as percentage of total fatty acid).

149. It would have been obvious to a PHOSITA that Radianingtyas’s oil has 5% by weight or less of HDA (margaric acid) because Radianingtyas discloses production of a wide range of oils with consistently low amounts of HDA. *See* MARA-1007, 34:4-37:14. In some embodiments, HDA is not detectable at all. *See, e.g.*, MARA-1007, 32:20-25. As described in more detail in Section III(A)(2)[1d], *supra*, “5% by weight or less” includes compositions with 0% HDA.

150. Additionally, I note that Kralovec describes specific saturated fatty acids and esters that can be removed by hydrolysis, and one such specific compound includes “margaric acid (C17),” which is a synonym for HDA and 17:0. MARA-1030, 9:1-3. Additionally, Kralovec teaches that in an embodiment using Kralovec’s method for increasing DHA, HDA is present at 0.1% in the product after re-esterification. MARA-1030, 42-43 & Table 9.1.

151. I also note that a PHOSITA would have understood and/or found obvious that it was desirable to have any non-DHA component (of which HDA is just one example) present at only low levels in a primarily DHA-containing oil. As motivation, a PHOSITA would recognize that high-DHA, low-HDA concentrations would be preferable to DHA from fish oil because of “the low content of the desired fatty acids” in fish oils. MARA-1034, [0005]. A PHOSITA

would have therefore wanted to achieve a high-purity, DHA-containing oil free of contaminants or other PUFAs for more efficient supplementation of human and animal food products. Indeed, a higher-purity, DHA-containing oil would be preferable because it results in a consumer needing to take fewer supplements (i.e. capsules) and reduces the “calories ingested from unwanted fatty acids.” MARA-1022, 113; MARA-1035, 381 (“Concentrates keep the intake of calories and other possibly physiologically active fatty acids as low as possible.”).

152. Furthermore, HDA (or 17:0) is an odd-chain fatty acid (OCFA). As explained by Taberna, “the physiological implications of OCFA nutrition for humans are relatively unknown,” and “15:0 and 17:0 accumulation can decrease the percentage of other fatty acids including the valuable DHA.” MARA-1041, 82-84. Also, Radianingtyas discloses production of a wide range of oils with consistently low amounts of HDA. *See* MARA-1007, 34:4-37:14. Thus, a PHOSITA would have sought out microbial oils that would contain or maintain low amounts of HDA.

153. Therefore, it is my opinion that Radianingtyas and Kralovec disclose a microbial oil that renders obvious claim 1 of the '363 patent.

Claim 2

**[2] The microbial oil of claim 1, further comprising an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.**

154. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious an oil further comprising an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.

155. In my opinion, Radianingtyas discloses [2]. *See* Section III(A)(2)[2], *supra*. Radianingtyas discloses production of a wide range of oils with consistently low amounts of ARA. *See* MARA-1007, 34:4-37:14. In some embodiments, ARA is not detectable at all. *See, e.g.*, MARA-1007, 32:20-25. As described in more detail in Section III(A)(2)[1d]-[2], *supra*, “1.5% by weight or less” includes compositions with 0% ARA.

156. Moreover, in my opinion, a PHOSITA would have understood and/or found obvious that it was desirable to have any omega-6 fatty acid (of which ARA is just one example) present at only low levels in a primarily DHA (an omega-3 fatty acid)-containing oil. As motivation, a PHOSITA would recognize that high-DHA, low-omega-6 (of which low ARA is only an example) concentrations would be preferable for *economical production* of high purity, DHA-containing oils. *See, e.g.*, MARA-1019, 6:44-50 (stating that “economically desirable characteristics for the production of omega-3 highly unsaturated fatty acids” can include “high content of omega-3 highly unsaturated fatty acids” and “preferably

low content of saturated and omega-6 highly unsaturated fatty acids”). In turn, high purity, DHA-containing oils can simplify processing, including those that can be used to form concentrates of such DHA-containing oils. *See, e.g.*, MARA-1019, 14:18-33, stating that a recited processing “advantage is enabled by starting the purification process with ***an oil consisting of a simple fatty acid profile such as that produced by Thraustochytrids*** (3 or 4 saturated or monounsaturated fatty acids with 3 or 4 HUFAs, two groups of fatty acids widely separated in terms of their crystallization temperatures) rather than a complex oil such as fish oil with up to 20 fatty acids (representing a continuous range of saturated, mono-, di-, tri-, and polyenoic fatty acids, and as such, a series of overlapping crystallization temperatures).”

157. A PHOSITA would have therefore wanted to achieve a high-purity, DHA-containing oil free of contaminants or other PUFAs for more efficient and economic processing. Thus, a PHOSITA would have sought out microbial oils containing low amounts of ARA, such as in Radianingtyas or Barclay, for example. MARA-1007, 99: 29-32 (stating that “[i]n addition to the omega-3 oils produced, approximately 80% of all isolates synthesized the omega-6 PUFAs, ***arachidonic acid (AA)*** or docosapentaenoic acids (DPA), ***at concentrations varying between 1 and 18%*** and 3 and 7% w/w, respectively.”); MARA-1019, 19:46-49 (stating that “[s]trains can also be isolated which have less than 1% (as %

of total fatty acids) of the ***undesirable C20:4w6*** [ARA] and C22:5w6 highly unsaturated fatty acids”); MARA-1019, 10:40-45 (stating that “[t]he unicellular strains of heterotrophic microorganisms isolated by the screening procedure outlined above, tend to have high concentrations of three omega-3 highly unsaturated fatty acids: C20:5w3, C22:5w3 and C22:6w3 and ***very low concentration of C20:4w6*** [ARA].”).

### Claim 3

#### ***[3pre] A microbial oil comprising***

158. See Section III(B)(4)[1pre], *supra*.

#### ***[3a] a triglyceride fraction of at least 70% by weight,***

159. See Section III(B)(4)[1a], *supra*.

#### ***[3b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight,***

160. See Section III(B)(4)[1b], *supra*.

#### ***[3c.1] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight,***

161. See Section III(B)(4)[1c], *supra*.

#### ***[3c.2] wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1,***

162. I note that Radianingtyas discloses a microbial oil in which the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1.

163. As discussed with respect to Elements [1b] and [1c] in Section III(B)(4)[1b]-[1c], *supra*, Radianingtyas discloses a microbial oil having at least

55% DHA in triglyceride form and at least 5% DPA in triglyceride form. *See* Section III(B)(4)[1b]-[1c], *supra*. Taking such an oil, the resultant ratio of DHA to DPA is greater than 6:1 (i.e., a ratio of 11:1 for 55% DHA:5% DPA).

***[3d] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

164. *See* Section III(B)(4)[1d], *supra*.

#### Claim 4

***[4pre] A microbial oil comprising***

165. *See* Section III(B)(4)[1pre], *supra*.

***[4a] a triglyceride fraction of at least 70% by weight,***

166. *See* Section III(B)(4)[1a], *supra*.

***[4b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight,***

167. Radianingtyas-Kralovec discloses and/or renders obvious an oil wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight.

168. I note that Radianingtyas describes various strains of thraustochytrids that contain more than 60% DHA by weight. *See, e.g.*, MARA-1007, 82 & Table 3 (showing the following strains having at least 60% DHA when taking the DHA amount (in mg/g) and dividing by the total lipid amount: ONC-T02 (60%), ONC-T12 (60%), and ONC-T52 (64%)).

169. I also note that Radianingtyas also discloses that microorganisms “such as ONC-T18....can produce a lipid composition comprising from about 25% to about 40% of an omega-3 fatty acid, such as n-3 DHA, (*for example, at least...40%, 45%, 50%, 55% or 60% by weight*).” MARA-1007, 26:29-34. In particular, I note that Radianingtyas discloses the strain ONC-T18, of which “[a]nalysis of results shows that the fatty acid component which ONC-T18 produces under heterotrophic fermentation *is almost entirely triglyceride (at least 95%)* in nature.” MARA-1007, 89:24-26; *see also*, MARA-1007, 32:17-33:5. A PHOSITA would have understood that because the majority of Radianingtyas’s microbial oils are made up of triglycerides (e.g., “at least 95%” in the lipid composition), the majority of the PUFAs in Radianingtyas must be present in triglyceride form, including the DHA, and that such an oil would meet [4b]. MARA-1007, 89:24-26; *see also*, MARA-1007, 32:17-33:5.

170. In my opinion, in the unlikely circumstance that the fatty acid portion of the remaining 5% non-triglyceride portion *was entirely DHA* (which is unlikely as the non-triglyceride portion includes various components that do not contain any fatty acids, such as sterols, carotenoids, alkanes, etc., and phospholipids and sterol esters that contain lower amounts of fatty acids, which are unlikely to be specifically DHA given the abundance of all fatty acids) and with the

understanding that this is an extreme example, the oil would still contain *at least* 55% DHA (i.e., nearly 60% DHA) in triglyceride form and would still meet [4b].

171. Additionally, compositions having a DHA content of the triglyceride fraction that is at least 60% by weight were also well known to a PHOSITA based on the prior art that was available before the Critical Date. It was known prior to the Critical Date that there were microorganisms of a *Thraustochytrium* strain that had “a high ability of producing docosahexaenoic acid” and were producing oil with at least 60% by weight DHA. MARA-1015, Abstract; *see also* MARA-1015, [0016], [0116]; *see also* MARA-1015, Fig. 3 (disclosing a *Thraustochytrium* strain (LFF1 strain) with a DHA content of about 80% in triglyceride form); *see also* MARA-1019, Table 3 (showing that, excluding “PRIOR STRAINS” (a term employed by Barclay), twenty (20) of 108 strains have more than 60% DHA and 0.5% to 6% DPA (n-6) of total fatty acids, of which three (3) strains have more than 70% DHA); *see also* MARA-1009, 27; *see also* MARA-1032, 27-31 & Table 2.5. It was also known how to increase the yield of desired fatty acids, including DHA. For example, it was known that decreasing agitation or dissolved oxygen during culturing would result in increases of DHA. MARA-1007, 28:14-20, 27:13-21.

172. To the extent the DHA content in the triglyceride fraction in Radianingtyas’s microbial oil is determined to be less than 60%, Radianingtyas

teaches that “if a disclosed eukaryotic microorganism, for example, produces 35% DHA, this could be further ‘purified’ such that the final lipid composition was greater than 90% DHA.” MARA-1007, 8:2-4.

173. Additionally, a PHOSITA would also readily know how to increase the DHA content in triglyceride form using methods disclosed in references like Kralovec. As discussed above (Section III(B)(3), *supra*), in the combination, a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques (or similar) to Radianingtyas’s extracted microbial oil to increase its DHA content. In doing so, Kralovec discloses its methods can be used to produce “concentrated oils with triglycerides containing higher proportions of polyunsaturated fatty acid esters than the starting glycerides,” such as “oils that comprise greater than or equal to about...80% DHA by weight of the total composition.” MARA-1030, 35:20-27, 36:12-15. Such oils would “also have a high TG (triglyceride) content” and the method would “produce triglycerides of fatty acids.” MARA-1030, 35:27-30, 37:22. The end result would be “an oil having higher levels of a PUFA...than the starting oil composition.” MARA-1030, 5:24-32.

174. A PHOSITA would have therefore understood and/or found obvious to apply Kralovec’s (or similar) methods to Radianingtyas’s microbial oil to

produce a DHA-enriched microbial oil containing at least 60 wt.% DHA in triglyceride form.

175. In my opinion, a PHOSITA would understand that having higher levels of DHA in triglyceride form was desirable because it would mean a more homogeneous oil, desirable for use in pharmaceutical preparations. See MARA-1039, 20 (identifying free fatty acids and phospholipids as impurities that would be removed in “[p]roduction of pharmaceutical- and food-grade omega-3 marine oils”) (citing A.P. Bimbo, *Processing of Marine Oils*, Long Chain Omega-3 Specialty Oils 77-109 (2007)). Additionally, the presence of phospholipids, as opposed to triglycerides, was known to make oils less stable, more cloudy, and more difficult to process efficiently. See MARA-1039, 17 (identifying free fatty acids and phospholipids as impurities) (citing A.P. Bimbo, *Guidelines for Characterizing Food-Grade Fish Oil*, Inform 9:473-483 (1998)). Moreover, when enrichment of DHA is performed via conversion to the ethyl ester form followed by distillation (a commercially important process), the resulting ethyl ester-form DHA concentrates can be considered as an “unnatural form.” In contrast, providing high levels of DHA in the triglyceride form more closely resembles the “natural” form in which lipids are predominantly ingested. See MARA-1039, 19 (“Conversion of concentrated fatty acids or ethyl esters back to the triglyceride form...is considered more natural.”) (citing A.P. Bimbo, *Processing of Marine*

*Oils*, Long Chain Omega-3 Specialty Oils 77-109 (2007)). Hence, the triglyceride form of DHA would be expected to have greater consumer acceptability, along with the possibility of greater bioavailability according to some literature. *See* MARA-1036, 352-354, Table 2.

***[4c] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

176. *See* Section III(B)(4)[1d], *supra*.

#### Claim 5

***[5] The microbial oil of any one of claims 1 to 4, wherein at least 20% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at two positions in the triglyceride selected from any two of the sn-1, sn-2, and sn-3 positions.***

177. Radianingtyas-Kralovec discloses and/or renders obvious the microbial oil of any one of claims 1 to 4, wherein at least 20% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at two positions in the triglyceride selected from any two of the sn-1, sn-2, and sn-3 positions.

178. Each of claims 1-4 recites at least 40-60% DHA in triglyceride form, and this recited DHA percentage provides a presence of at least 20% of that DHA at any two of the sn-1, sn-2, and sn-3 positions. A PHOSITA would have readily understood that an oil having at least 40% DHA would possess at least 20% DHA at any two of the sn-1, sn-2, and sn-3 positions.

179. A triglyceride (or triacylglycerol) includes three fatty acids that are linked, by way of ester groups, to the glycerol molecule. In turn, the glycerol

molecule includes three carbon atoms providing sn-1, sn-2, and sn-3 positions, in which one fatty acid is attached to one of the sn-1, sn-2, and sn-3 positions.

Depending on the fatty acid being employed, various structures can be provided.

Assuming that D is DHA and X is another fatty acid that is not DHA, all possible triglyceride structures are as follows:

|             |                                                    |
|-------------|----------------------------------------------------|
| DDD         | (having three DHA at each of sn-1, sn-2, and sn-3) |
| DDX DXD XDD | (having two DHA at two of sn-1, sn-2, and sn-3)    |
| DXX XDX XXD | (having one DHA at one of sn-1, sn-2, and sn-3)    |
| XXX         | (having three X at each of sn-1, sn-2, and sn-3)   |

180. Based on the eight possible triglyceride structures (shown above), the probability of DHA being at a certain position can be determined for a certain DHA content in triglyceride form.

181. Claim 5 depends from claims 1-4, of which claims 1, 3, and 4 recite DHA content of the triglyceride (TG) fraction that is at least 50%, 40%, or 60%, respectively. Taking the upper limit for DHA content in claim 1, the DHA content in triglyceride (TG) form is 50% by weight, thus the X content in TG form is 50% (i.e.,  $100\% - 50\% \text{ DHA} = 50\% \text{ X}$ ). Assuming a random distribution of fatty acid at each of the sn-1, sn-2, and sn-3 positions with no preference for a certain position, the assumed abundance of it being D at any position is 50%, and the assumed

abundance of it being X at any position is 50%. The probability for each of the eight possible TG structures can be determined as follows:

**Table 1A. DHA in TG form is 50% by weight**

| TG Structure | Calculations for Probability      | Calculated Probability | Calculated Abundance |
|--------------|-----------------------------------|------------------------|----------------------|
| DDD          | $(0.5)^3$                         | 0.125                  | 12.5% **             |
| DDX DXD XDD  | $(0.5)^2 \times (0.5)^1 \times 3$ | 0.375                  | 37.5% *              |
| DXX XDX XXD  | $(0.5)^1 \times (0.5)^2 \times 3$ | 0.375                  | 37.5%                |
| XXX          | $(0.5)^3$                         | 0.125                  | 12.5%                |
|              |                                   | 1.000                  | 100%                 |

182. Therefore, if DHA is at least 50% of the TG, then *at least 37.5%* of the TG must contain 2 DHA molecules at any two of the sn-1, sn-2, and sn-3 positions (marked with \* in **Table 1A**), assuming a random distribution of fatty acid substitution onto glycerol with no preference for a certain position. However, claim 5 recites “at least 20%” of the TG contain DHA at any two of the sn-1, sn-2, and sn-3 positions, which is less than the calculated abundance of *at least 37.5%*. Thus, a PHOSITA would have readily understood that an oil having at least 50% DHA would possess at least 20% DHA at any two of the sn-1, sn-2, and sn-3 positions.

183. Similarly, probability calculations can be assessed for other DHA content recited in other claims. Taking the upper limit for DHA content in claim 3, the DHA content in TG form is 40% by weight, thus the X content in TG form is 60% (i.e., 100% - 40% DHA = 60% X). Assuming a random distribution of fatty

acid at each of the sn-1, sn-2, and sn-3 positions with no preference for a certain position, the assumed abundance of it being D at any position is 40%, and the assumed abundance of it being X at any position is 60%. The probability for each of the eight possible TG structures can be determined as follows:

**Table 1B. DHA in TG form is 40% by weight**

| TG Structure | Calculations for Probability      | Calculated Probability | Calculated Abundance |
|--------------|-----------------------------------|------------------------|----------------------|
| DDD          | $(0.4)^3$                         | 0.064                  | 6.4% **              |
| DDX DXD XDD  | $(0.4)^2 \times (0.6)^1 \times 3$ | 0.288                  | 28.8% *              |
| DXX XDX XXD  | $(0.4)^1 \times (0.6)^2 \times 3$ | 0.432                  | 43.2%                |
| XXX          | $(0.6)^3$                         | 0.216                  | 21.6%                |
|              |                                   | 1.000                  | 100%                 |

184. Therefore, if DHA is at least 40% of the TG, then ***at least 28.8%*** of the TG must contain 2 DHA molecules at any two of the sn-1, sn-2, and sn-3 positions (marked with \* in **Table 1B**), assuming a random distribution of fatty acid substitution onto glycerol with no preference for a certain position. However, claim 5 recites “at least 20%” of the TG contain DHA at any two of the sn-1, sn-2, and sn-3 positions, which is less than the calculated abundance of ***at least 28.8%***. Thus, a PHOSITA would have readily understood that an oil having at least 40% DHA would possess at least 20% DHA at any two of the sn-1, sn-2, and sn-3 positions.

185. Taking the upper limit for DHA content in claim 4, the DHA content in TG form is 60% by weight, thus the X content in TG form is 40% (i.e., 100% -

60% DHA = 40% X). Assuming a random distribution of fatty acid at each of the sn-1, sn-2, and sn-3 positions with no preference for a certain position, the assumed abundance of it being D at any position is 60%, and the assumed abundance of it being X at any position is 40%. The probability for each of the eight possible TG structures can be determined as follows:

**Table 1C. DHA in TG form is 60% by weight**

| TG Structure | Calculations for Probability      | Calculated Probability | Calculated Abundance |
|--------------|-----------------------------------|------------------------|----------------------|
| DDD          | $(0.6)^3$                         | 0.216                  | 21.6% **             |
| DDX DXD XDD  | $(0.6)^2 \times (0.4)^1 \times 3$ | 0.432                  | 43.2% *              |
| DXX XDX XXD  | $(0.6)^1 \times (0.4)^2 \times 3$ | 0.288                  | 28.8%                |
| XXX          | $(0.4)^3$                         | 0.064                  | 6.4%                 |
|              |                                   | 1.000                  | 100%                 |

186. Therefore, if DHA is at least 60% of the TG, then ***at least 43.2%*** of the TG must contain 2 DHA molecules at any two of the sn-1, sn-2, and sn-3 positions (marked with \* in **Table 1C**), assuming a random distribution of fatty acid substitution onto glycerol with no preference for a certain position. However, claim 5 recites “at least 20%” of the TG contain DHA at any two of the sn-1, sn-2, and sn-3 positions, which is less than the calculated abundance of ***at least 43.2%***. Thus, a PHOSITA would have readily understood that an oil having at least 40% DHA would possess at least 20% DHA at any two of the sn-1, sn-2, and sn-3 positions (assuming a random distribution of fatty acids on TG).

187. For the calculations above, a random distribution was assumed, in which the fatty acid has no preference for a certain position on the glycerol. This calculation can be revised to provide enrichment or greater abundance of a fatty acid for a particular position. For example, the calculation can be revised to reflect a greater abundance of DHA on sn-2 for the case where more of the DHA is located on sn-2. MARA-1042 describes regiospecific distribution from algal oils, in which the ratio of DHA on sn-2 to DHA on sn-1 or sn-3 (i.e., a ratio of DHA (sn-2) : DHA (sn-1 or sn-3)) for the three algal oils can be determined to be 4.3:1, 2.3:1, and 1.9:1. MARA-1042, Table 1 (using data from columns a and b for *Aurantiochytrium* sp. strain TC 20 oil, Neuromins Algal DHA oil, and Solgar Algal DHA oil). Taking the average ratio of 2.8:1 as an example, then an oil having the minimum overall DHA of 40% in TFA would have 70% DHA abundance on sn-2 and 25% DHA abundance on sn-1 and on sn-3 (i.e., since  $70/25=2.8$ ). Hence, with greater DHA abundance on sn-2, the amount of TG containing 2 DHA molecules on any two of the sn-1, sn-2, and sn-3 positions can be determined to be 28.1% (i.e.,  $(DDX+DXD+XDD) = \{(0.25) \times (0.70) \times (0.75)\} + \{(0.25) \times (0.30) \times (0.25)\} + \{(0.75) \times (0.70) \times (0.25)\} = \mathbf{28.1\%}$ ; compare **28.1%** for greater abundance of DHA at sn-2 with a ratio of 2.8:1 and with 2 DHA per TG in a 40% DHA oil *with 28.8%* for a random distribution of DHA and with 2 DHA per TG in a 40% DHA oil (as indicated by \* in **Table 1B**)). Hence, even assuming

a non-random substitution of DHA to the extent indicated, and with the minimum DHA abundance of 40% (e.g., as in claim 3), more than 20% of the TG must contain at least 2 DHA. Also note that as the overall DHA percentage rises (e.g., as in claims 1 and 4), so does this probability.

188. Taking another example, MARA-1046 describes regiospecific distribution for microalgal (microbial) oil from *Chrypthecodinium cohnii* with a DHA content of 43.5%, which in turn was determined to have an averaged positional distribution of DHA as follows: sn-1=23.58%; sn-2=64.62%; sn-3=42.34%. MARA-1046, Tables 1 & 6 (for *C. cohnii* oil that is labelled as “D”). From this distribution, the amount of TG containing 2 DHA molecules on any two of the sn-1, sn-2, and sn-3 positions can be calculated as follows:

$$(DDX+DXD+XDD) = \{(0.236) \times (0.646) \times (0.577)\} + \{(0.236) \times (0.354) \times (0.423)\} + \{(0.764) \times (0.646) \times (0.423)\} = 33.2\%.$$

Thus, taking an example of a microbial oil from *C. cohnii* having at least 40% DHA in TG form and having a measured regiospecific distribution of DHA for each of the sn-1, sn-2, and sn-3 positions, the oil can be characterized as having about 33.2% of TG containing 2 DHA molecules on any two of the sn-1, sn-2, and sn-3 positions. A PHOSITA would have understood and/or found obvious that such an oil, even having a greater abundance of DHA on certain positions, provides at least 20% triglycerides that contain 2 DHA molecules on any two of the sn-1, sn-2, and sn-3 positions.

189. Taken together, a PHOSITA would have understood and/or found obvious that an overall percentage of at least 40% DHA will always result in at least 20% triglycerides that contain 2 DHA molecules on any two of the sn-1, sn-2, and sn-3 positions (assuming a random distribution of fatty acids on these positions). Furthermore, a PHOSITA would have understood and/or found obvious that the impact of any positional specificity for DHA (e.g., as in a non-random substitution of DHA on the sn-1, sn-2, and sn-3 positions in TG) has limited impact on the probability of TG including at least 2 DHA. A PHOSITA would have also understood that as the overall DHA percentage rises (e.g., from 40% DHA as in claim 3 to 50% DHA or 60% DHA as in claims 1 and 4), so does this probability (i.e., of at least 20% DHA at two of the recited sn positions). Thus, claim 5 is redundant in view of the recited DHA content in claims 1, 3, and 4.

#### Claim 6

***[6] The microbial oil of any one of claims 1 to 4, wherein at least 5% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at all three of the sn-1, sn-2, and sn-3 positions in the triglyceride.***

190. Radianingtyas-Kralovec discloses and/or renders obvious the microbial oil of any one of claims 1 to 4, wherein at least 5% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at all three of the sn-1, sn-2, and sn-3 positions in the triglyceride.

191. Each of claims 1-4 recites at least 40-60% DHA in triglyceride form, and this recited DHA percentage provides a presence of at least 5% of that DHA at

all three of the sn-1, sn-2, and sn-3 positions. A PHOSITA would have readily understood that an oil having at least 40% DHA would possess at least 5% DHA at all three of the sn-1, sn-2, and sn-3 positions.

192. The probability of DHA being at a certain position can be determined for a certain DHA content in triglyceride form. As discussed above, ***at least 6.4%*** of the TG must contain 3 DHA molecules at each of the sn-1, sn-2, and sn-3 positions (marked with \*\* in **Tables 1A-1C** above), assuming a random distribution of fatty acid substitution onto glycerol with no preference for a certain position. However, claim 6 recites “at least 5%” of the TG contain DHA at all three of the sn-1, sn-2, and sn-3 positions, which is less than the calculated abundance of ***at least 6.4%***. Thus, a PHOSITA would have readily understood that an oil having at least 40% DHA would possess at least 5% DHA at all three of the sn-1, sn-2, and sn-3 positions (assuming a random distribution of fatty acids on TG).

193. For the calculations above in **Tables 1A-1C**, a random distribution was assumed, in which the fatty acid has no preference for a certain position on the glycerol. This calculation can be revised to provide enrichment or greater abundance of a fatty acid for a particular position. Taking the average ratio of 2.8:1 as an example provided in MARA-1042 and discussed in Section III(B)(5), *supra*, then an oil having the minimum overall DHA of 40% in TFA would have

70% DHA abundance on sn-2 and 25% DHA abundance on sn-1 and on sn-3 (i.e., since  $70/25=2.8$ ). Hence, with greater DHA abundance on sn-2, the amount of TG containing 2 DHA molecules on any two of the sn-1, sn-2, and sn-3 positions can be determined to be 4.4% (i.e.,  $DDD = (0.25) \times (0.70) \times (0.25) = 4.4\%$ ; compare 4.4% for greater abundance of DHA at sn-2 with a ratio of 2.8:1 and with 3 DHA per TG in a 40% DHA oil *with 6.4%* for a random distribution of DHA and with 3 DHA per TG in a 40% DHA oil (as indicated by \*\* in **Table 1B**)). In fact, the maximum DHA (sn-2): DHA(sn-1 or sn-3) ratio is 2.2:1 in order to have an abundance of DDD (in TG form) to be more than 5% at the minimum DHA concentration of 40% (e.g., as in claim 3); this would be a distribution of sn1=sn3=29% and sn2=63% and would have an abundance of DDD (in TG form) to be 5.3%. Hence, although not explicitly stated in the '363 patent, claim 3 is equivalent to a limitation (when the minimum DHA content of 40% is present) to having this DHA (sn-2) : DHA (sn-1 or sn-3) ratio of 2.2:1 or less. Otherwise, an abundance of DDD (in TG form) that is less than 5% will be present. An oil having such a ratio is provided in MARA-1046. As discussed in Section III(B)(5), *supra*, MARA-1046 discloses a microalgal (microbial) oil from *C. cohnii* with a DHA content of 43.5%, which in turn was determined to have an averaged positional distribution of DHA as follows: sn-1=23.58%; sn-2=64.62%; sn-3=42.34%. MARA-1046, Tables 1 & 6 (for *C. cohnii* oil that is labelled as “D”).

From this distribution, the amount of TG containing 3 DHA can be calculated as follows:  $DDD = 0.236 \times 0.646 \times 0.423 = 6.4\%$ . Thus, taking an example of a microbial oil from *C. cohnii* having at least 40% DHA in TG form and having a measured regiospecific distribution of DHA for each of the sn-1, sn-2, and sn-3 positions, the oil can be characterized as having about 6.4% of TG containing 3 DHA molecules. A PHOSITA would have understood and/or found obvious that such an oil, even having a greater abundance of DHA on certain positions, provides at least 5% triglycerides that contain 3 DHA molecules.

194. A PHOSITA would have also understood and/or found obvious that as the overall DHA percentage rises (e.g., from 40% DHA as in claim 3 to 50% DHA or 60% DHA as in claims 1 and 4), so does this probability (i.e., of at least 5% DHA at all three of the recited sn positions). For example, as DHA rises from 40% to 42% of TFA and for the ratio of DHA (sn-2) : DHA(sn-1 or sn-3) = 2.8:1, the determined distribution would be sn-1=sn-3=26% and sn-2=74%. In turn, the abundance of DDD (in TG form) is 5.0% (i.e.,  $(0.26) \times (0.74) \times (0.26) = 5.0\%$ ). Hence, a PHOSITA would have understood and/or found obvious that in all cases for DHA abundance greater than 42% with the assumed non-random distribution (i.e., ratio of DHA (sn-2) : DHA(sn-1 or sn-3) = 2.8:1), 5% or more of the TG contains DHA at all three of the sn-1, sn-2, and sn-3 positions in the triglyceride.

Thus, claim 6 is redundant in view of the recited DHA content in claims 1, 3, and 4.

Claim 7

***[7] A food product, cosmetic, or pharmaceutical composition for animals or humans, comprising the microbial oil of any one of claims 1 to 4.***

195. Radianingtyas-Kralovec discloses and/or renders obvious a food product, cosmetic, or pharmaceutical composition for animals or humans, comprising the microbial oil of any one of claims 1 to 4.

196. I note that Radianingtyas states that its “oils can be used directly as a feed or food additive” and that its lipid can be included in “[a] *foodstuff*, supplement, *pharmaceutical composition* for both *human and animal* (including marine)[.]” MARA-1007, 42:22-23, 40:20-26; *see also* MARA-1007, 12:3-7, 43:6-8, 72:4-17. Radianingtyas also states that its compounds can be formulated for topical administration and that its formulations can include “ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders.” MARA-1007, 70:23-26.

Claim 8

***[8] The food product of claim 7, wherein the food product is an infant formula.***

197. Radianingtyas-Kralovec discloses and/or renders obvious wherein the food product is an infant formula.

198. I note that Radianingtyas discloses that the “compositions can be used in food products (e.g., baby milk).” MARA-1007, 13:15-16; *see also* MARA-

1007, 40:25-26 (“disclosed is an infant formula comprising the composition”), 67:18-19, 79:12-17, claim 38.

Claim 9

***[9] The food product of claim 8, wherein the infant formula can be consumed by premature infants.***

199. Radianingtyas-Kralovec discloses and/or renders obvious wherein the infant formula can be consumed by premature infants.

200. I note that Radianingtyas discloses that the “compositions can be used in food products (e.g., baby milk).” MARA-1007, 13:15-16; *see also* MARA-1007, 40:25-26 (“disclosed is an infant formula comprising the composition”), 67:18-19, 79:12-17, claim 38. Based on this disclosure, a PHOSITA would have understood and/or found obvious that Radianingtyas’s oil-containing baby milk and infant formula for infants would also be provided to premature infants.

201. This understanding is consistent with Tanaka, which describes formula that can be suitable for infants as well as immature infants: “[t]he present invention provides a nutrient-supplementing food, a formula suitable for feeding infants, ***a formula suitable for feeding immature infants***, a baby food, a food for expectant or nursing mothers....” MARA-1034, [0016].

202. Furthermore, a PHOSITA would have understood and/or found obvious the benefits of polyunsaturated fats (PUFAs) to supplement formula for infants, including preterm infants. *See, e.g.*, MARA-1010, Preface at v, stating that

“[s]ingle cell oils (SCO) have come of age. They have become accepted biotechnological products fulfilling key roles in the supply of the major very long chain polyunsaturated fatty acids (PUFA), now known to be essential for infant nutrition and development”; MARA-1010,53 (stating that “[n]ewborns obtain most of these PUFA from breast milk, and it was thus suggested that the diet of preterm infants who are not breast-fed should be supplemented with these PUFA. Indeed, various health authorities now recommend the incorporation of both AA and DHA into baby formulae and the FDA has recently approved their combined use.”) (citations omitted).

#### Claim 10

***[10] The food product of claim 7, wherein the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.***

203. Radianingtyas-Kralovec discloses and/or renders obvious wherein the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.

204. I note that Radianingtyas discloses that the “compositions can be used in food products (e.g., baby milk).” MARA-1007, 13:15-16; *see also* MARA-1007, 72:15-17 (“the foodstuff is...milk...fruit juice, a dry beverage powder...”), 72:5-6 (“By ‘foodstuff’ is meant any article that can be consumed (e.g., eaten, ***drank, or ingested***) by a subject”); 79:11-13, 79:19-21, claim 40.

Claim 11

***[11] The food product of claim 7, wherein the food product is an additive for animal or human food.***

205. Radianingtyas-Kralovec discloses and/or renders obvious wherein the food product is an additive for animal or human food.

206. I note that Radianingtyas discloses that its compounds “are useful as...***food additives***.” MARA-1007, 11:18-21; *see also* MARA-1007, 12:3-7, 42:22-25, 43:6-8.

Claim 12

***[12] The food product of claim 7, wherein the food product is a nutritional supplement.***

207. Radianingtyas-Kralovec discloses and/or renders obvious wherein the food product is a nutritional supplement.

208. I note that Radianingtyas discloses that its compounds “are useful as...***nutraceuticals***.” MARA-1007, 11:18-21; *see also* MARA-1007, 43:6-8 (discloses oils can be “used as a ***nutritional supplements*** [sic] for humans”), 12:3-7, 59:16, 59:21-24.

Claim 13

***[13] The food product of claim 7, wherein the food product is an animal feed.***

209. Radianingtyas-Kralovec discloses and/or renders obvious wherein the food product is an animal feed.

210. I note that Radianingtyas discloses a “*foodstuff*, supplement, pharmaceutical composition for both human *and animal* (including marine) can comprise the composition (lipid, lipid with antioxidant and antioxidant alone).” MARA-1007, 40:20-26; *see also* MARA-1007, 67:18 (“These lipids and antioxidants are targeted for use in *animal feeds*”), 12:3-7, claim 39.

Claim 14

***[14] The animal feed of claim 13, wherein the animal feed is an aquaculture feed.***

211. Radianingtyas-Kralovec discloses and/or renders obvious wherein the animal feed is an aquaculture feed.

212. I note that Radianingtyas discloses a “composition for both human *and animal (including marine)* can comprise the composition.” MARA-1007, 40:20-26; *see also* MARA-1007, 79:11-18 (“The foodstuff can also be...an *aquaculture feed*.”), claim 39.

Claim 15

***[15] The animal feed of claim 13, wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.***

213. Radianingtyas-Kralovec discloses and/or renders obvious that the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

214. I note that Radianingtyas discloses a “*foodstuff*, supplement, pharmaceutical composition for both human *and animal* (including marine) can

comprise the composition[.]” MARA-1007, 40:20-26; *see also* MARA-1007, 79:11-18 (“The foodstuff can also be a fish product, a *companion pet food*, a *livestock* or an aquaculture *feed*.”), claim 39.

**C. GROUND 3: Claims 1-15 are obvious over Bijl in view of Barclay**

**1. Overview of Bijl**

215. It appears to me that Bijl and the ’363 patent are directed to microbial oils. MARA-1001, 1:16-21; 1:49-2:3; 2:18-37; 9:65-14:3; MARA-1008, 2:30-36, 9:5-7. Bijl also discloses high quality microbial oils sourced from naturally-occurring microorganisms and processes for obtaining quality microbial oils. MARA-1008, 1:7-12, 12:22-37; 2:35-3:2.

216. My review of Bijl indicates that its disclosed microbial oils contain one or more polyunsaturated fatty acids and a triglyceride content of at least 90 wt.%. MARA-1008, 2:30-33, 5:24-29. The microbial oils of Bijl also can contain additional compounds, such as anti-oxidants, which remain from the sourced microorganisms. MARA-1008, 3:9-14. Bijl discloses oils having low diglyceride content and low monoglyceride content. MARA-1008, 5:14-19. Bijl’s microbial oils are usable in food, nutritional supplements, and cosmetics. MARA-1008, 4:13-18.

217. To me, Bijl also describes processes for obtaining microbial oils. One process involves extracting microbial oil from dried granules. MARA-1008, 6:23-

34. Another process involves culturing microorganisms in fermentation broth, pasteurising the resulting broth or biomass, and then extracting microbial oil from the biomass. MARA-1008, 7:2-11. These processes may be combinable as well. MARA-1008, 8:1-6, 28:28-29:19.

218. Bijl teaches using pasteurisation to partially inactivate one or more compound degrading substance(s), such as enzymes (e.g., lipases) that may be present in the biomass or broth. MARA-1008, Abstract, 7:12-30. The pasteurisation process removes undesirable substances that can cause degradation of parts of the compound and contributes to obtaining high quality PUFAs. MARA-1008, 7:12-30. For example, Bijl teaches that pasteurization “inactivate[s] various lipases” that “cleave fatty acids off a triglyceride backbone,” which is “disadvantageous for PUFAs where a high triglyceride content is preferred.” MARA-1008, 7:34-37.

219. Use of Bijl’s processes for obtaining microbial oils utilize less solvent for extracting microbial oils than prior methods and produce higher quality crude oils. MARA-1008, 9:5-19.

## **2. Overview of Barclay**

220. Barclay relates to a process for the “production of whole-celled or extracted microbial products with a high concentration of omega-3 highly unsaturated fatty acids [HUFAs]...using biologically pure cultures of heterotrophic

single-celled fungi microorganisms of the order Thraustochytriales,” which is the former order for thraustochytrids. MARA-1019, Abstract.

221. In particular, Barclay describes that microorganisms can be “characterized by having a high concentration of fatty acids of which a high percentage are omega-3 highly unsaturated fatty acids,” and the microorganisms themselves or extracted microbial HUFAs can be included in food or pharmaceutical applications. MARA-1019, 4:54-64, Abstract.

222. Barclay describes such organisms and “process[es] for culturing them for the production of *lipids* with high concentrations of omega-3 highly unsaturated *fatty acids* (HUFA).” MARA-1019, 1:23-28. Rather than solely using the terms “triglycerides” or “triacylglycerols,” Barclay also employs the term “lipids” and states that “[l]ipids are understood to be fats or oils including the *glyceride esters of fatty acids* along with associated phosphatides, sterols, alcohols, hydrocarbons, ketones, and related compounds.” MARA-1019, 6:6-11; *see also*, MARA-1019, 13:52-58 (describing a process in which “[t]he solvent is removed (for example by a vacuum rotary evaporator, which allows the solvent to be recovered and reused) and the lipids hydrolyzed by using any of the well-known methods for converting *triglycerides* to free fatty acids or esters of fatty acids including base hydrolysis, acid hydrolysis, or enzymatic hydrolysis”).

223. In my opinion, a PHOSITA would have understood and/or found obvious that the “lipids” of Barclay would include “triglycerides,” depending on the context.

224. I note that Barclay describes culture conditions and extraction conditions to provide high concentrations of omega-3 HUFAs in extracted microbial oils. For instance, culture conditions can include “harvesting the cells in the exponential phase of growth”; “nutrient-limited or nitrogen-limited conditions ...and harvesting the Thraustochytriales during the period of nitrogen limitation in order to increase the concentration of omega-3 HUFAs in the microorganisms”; addition of an antioxidant “in order to prevent degradation of the omega-3 HUFAs”; or application of stress “with low temperatures during culturing, maintaining a high dissolved oxygen concentration in the medium during culturing, and adding to the medium effective amounts of phosphorous and a microbial growth factor...to provide sustained growth of the microorganisms.” MARA-1019, 10:17-20; 5:29-46.

225. Barclay also notes that processes may increase both lipids (e.g., including triglycerides) and omega-3 HUFAs (e.g., DHA), in which “[i]f a product significantly *higher in lipids and omega-3 highly unsaturated fatty acids is desired*, the culture can be manipulated to become nutrient limited, preferably, nitrogen limited for a suitable time, preferably in the range from 6 to 24 hours.”

MARA-1019, 10:20-24. Extraction conditions can include a process that involves fractional crystallization, in which the process “ comprises rupturing the microorganism cells, extracting the lipid mixture from the ruptured cells with a solvent, hydrolyzing the lipid mixture, removing non-saponifiable compounds and cold-crystallizing the non-HUFAs in the lipid mixture”, in which one example of such a process can provide “[a] fatty acid mixture containing > 90% omega-3 UFA’s.” MARA-1019, 5:50-57, 33:17-66, 34:20-24.

226. Barclay also describes desirable characteristics for strains, as well as examples of strains having those characteristics. For instance, Barclay states that “desirable characteristics for the production of omega-3 highly unsaturated fatty acids” can include “high content of omega-3 highly unsaturated fatty acids” and “preferably low content of saturated and omega-6 highly unsaturated fatty acids,” among others. MARA-1019, 6:45-50.

227. In particular, Barclay states that “many strains of the invention [] produce a high proportion of total omega-3 fatty acids as C22:6w3 [DHA]” and that “[f]ifteen strains had at least 94% by weight of total omega-3 fatty acids as C22:6w3 [DHA].” MARA-1019, 22:47-54, Tables 3-4. Excluding “PRIOR STRAINS” (a term employed by Barclay in Table 3), Barclay shows more than twenty (20) of 108 strains having more than 50% DHA and 0.5% to 6% DPA (n-6)

of total fatty acids, of which three (3) strains have more than 70% DHA. MARA-1019, Table 3.

### **3. Combination of Bijl and Barclay**

228. In my opinion, a PHOSITA would have combined the teachings of Bijl, directed to processes to provide stable microbial oils having high levels of triglycerides (e.g., >90%) with a preferred PUFA (DHA), with Barclay, directed to high DHA microorganism strains and culture conditions for such strains, to increase the concentration of that PUFA in a stable microbial oil. MARA-1008, 2:30-33, 4:24-28; MARA-1019, 10:17-20; 5:29-57, 33:17-34:24.

229. Specifically, a PHOSITA would have found it obvious to implement Barclay's strains and culture conditions (or similar) to Bijl's processes for at least the following reasons.

230. First, a PHOSITA would have been motivated to apply Barclay's strains and culture conditions to Bijl's processes to provide a high DHA oil from thraustochytrids because Bijl teaches that organisms, such as thraustochytrids, can have one predominant PUFA. Specifically, Bijl states that "[f]or example, oil of the invention can comprise docosahexaenoic acid (DHA) preferably obtained from algae or fungi. Algae include dinoflagellates (e.g. those of the genus) *Cryptothecodinium*. The fungus can be of the genus *Mucorales*, e.g. *Thraustochytrium*....Typically, the oils obtained from these organisms

predominantly contain one particular polyunsaturated fatty acid. However, they can additionally contain other polyunsaturated fatty acids in lesser amounts.”

MARA-1008, 6:3-14.

231. In my opinion, this understanding is consistent with Barclay, which states that some thraustochytrid strains in Barclay “tend to have high concentrations of three omega-3 highly unsaturated fatty acids: C20:5w3 [EPA], C22:5w3 [DPA(n-3)] and C22:6w3 [DHA] and very low concentration of C20:4w6 [DPA(n-6)].” MARA-1019, 10:42-45, Abstract. Even from the three possible omega-3 HUFAs, Barclay describes DHA as being in high amounts, as compared to EPA and DPA(n-3). MARA-1019, 10:45-60 (“Ratios of C20:5w3 to C22:6w3 [EPA:DHA] can run from about 1:1 to 1:30. Ratios of C22:5w3 to C22:6w3 [DPA(n-3):DHA] can run from 1:12 to only trace amounts of C22:5w3. In the strains that lack C22:5w3 [DPA(n-3)], the C20:5w3 to C22:6w3 [EPA:DHA] ratios can run from about 1:1 to 1:10.”).

232. Second, it is my opinion that a PHOSITA would have been motivated to apply Barclay’s strains and culture conditions to Bijl’s processes to provide a high triglyceride oil, which in turn can provide DHA in triglyceride form, because Bijl states that PUFA-containing high triglyceride oils with beneficial oil quality parameters can be stable. See MARA-1008, 4:19-23 (“The oil of the present invention contains one or more polyunsaturated fatty acids and can have a high

triglyceride content. This oil has a much higher oxidative stability than the microbial polyunsaturated fatty acid-containing oils described in the art.”); *see also* MARA-1008, 5:14-24 (“Additional characteristics of the oil of the invention may include a low diglyceride content, preferably below 2%, and/or a low monoglyceride content, preferably below 0.1%. It may have a light colour, a low level of off-flavours and/or a low anisidine value (anisidine is a test for aldehydes, a product of degradation by oxidation). The anisidine value typically varies from 0.1 to 5, preferably from 0.1 to 2, more preferably from 0.1 to 1. The colour of the oil of the invention is typically yellow to light yellow.”).

233. Moreover, in my opinion a PHOSITA would have understood and/or found obvious that Barclay’s oils included triglycerides and, based on references available before the Critical Date, likely included high triglyceride content. MARA-1019, 6:6-11 (stating that “[l]ipids are understood to be fats or oils including the glyceride esters of fatty acids along with associated phosphatides, sterols, alcohols, hydrocarbons, ketones, and related compounds”); *see also* MARA-1019, 13:52-58 (describing a process in which “[t]he solvent is removed (for example by a vacuum rotary evaporator, which allows the solvent to be recovered and reused) and the lipids hydrolyzed by using any of the well-known methods for converting triglycerides to free fatty acids or esters of fatty acids including base hydrolysis, acid hydrolysis, or enzymatic hydrolysis”); *see, e.g.,*

MARA-1007, 89:24-26 (stating that “[a]nalysis of results shows that the fatty acid component which ONC-T18 produces under heterotrophic fermentation is almost entirely triglyceride (at least 95%) in nature”); *see also*, e.g., MARA-1034, [0037]-[0038] (disclosing that “[t]ypically, the content of neutral lipids in the lipids of the present invention is very high (more than 90[%] of total lipids)” and that “[t]he neutral lipids from the lipids of the present invention contain at least 85% triacylglycerols, preferably at least 90% triacylglycerols”).

234. Also, in my opinion a PHOSITA would have understood and/or found obvious that PUFAs in triglyceride form are preferred and would have been expected to have greater consumer acceptability, along with the possibility of greater bioavailability according to some literature. *See* MARA-1038, Table 15 (stating that “PUFA should be present as triglyceride if for human consumption”); *see also* MARA-1036, 352-354 & Table 2; *see also* MARA-1040, 301 (stating that “DHA in the triacylglycerol layer is the only economically feasible form for commercialization and downstream processing”).

235. In addition to maximizing the amount of a preferred PUFA (e.g., DHA), it is my opinion that it would have been well-known that increasing the amount of DHA in triglyceride form would also be economically desirable. *See* MARA-1010, 37 (stating that “[c]ost-effective production of DHA from microbes

would require the use of microbial strains that could produce large amounts of triacylglycerols”).

236. Third, in my view, a PHOSITA would have been motivated to apply Barclay’s strains and culture conditions to Bijl’s processes because Barclay’s strains possess “economically desirable characteristics for the production of omega-3 highly unsaturated fatty acids.” MARA-1019, 6:42-54. Barclay describes such strain characteristics as including “high content of omega-3 highly unsaturated fatty acids” and “preferably low content of saturated and omega-6 highly unsaturated fatty acids.” MARA-1019, 6:42-54.

237. Further in my opinion, a PHOSITA would have understood and/or found obvious that such strains in Barclay provide DHA (a preferred PUFA, *see* Section III(B)(3)(a), *supra* and/or Section III(C)(2), *supra*) and that high DHA oils from thraustochytrids can simplify processing. *See* MARA-1019, 14:18:22 (stating that “[t]he advantage of this process is that it is not necessary to go through the urea complex process or other expensive extraction methods...to remove saturated and mono-unsaturated fatty acids prior to cold crystallization. This advantage is enabled by starting the purification process with an oil consisting of a simple fatty acid profile such as that produced by Thraustochytrids (3 or 4 saturated or monounsaturated fatty acids with 3 or 4 HUFAs, two groups of fatty acids widely separated in terms of their crystallization temperatures) rather than a complex oil

such as fish oil with up to 20 fatty acids (representing a continuous range of saturated, mono-, di-, tri-, and polyenoic fatty acids, and as such, a series of overlapping crystallization temperatures)’’).

238. Fourth, in my view, a PHOSITA would have been motivated to apply Barclay’s strains and culture conditions to Bijl’s processes because such combinations reflect nothing more than the use of a known technology (e.g., known strains and identified culture conditions) to improve similar cultures (e.g., microbial cultures) according to known methods (e.g., culture refinement methods) to yield predictable results (e.g., obtain microorganisms and microbial products with an increased concentration of DHA).

239. I believe that a PHOSITA would have expected success in applying Barclay’s strains and culture modifications to increase the concentration of the preferred PUFA (i.e., DHA) in the microbial oil because Bijl contemplates that its invention can “comprise docosahexaenoic acid (DHA) preferably obtained from algae or fungi”, in which “[t]he fungus can be of the genus Mucorales, e.g. *Thraustochytrium*.” MARA-1008, 6:3-6. Thus, in my view, a PHOSITA would have looked to employ Bijl’s processes in accordance with Bijl’s teachings by looking to high DHA *thraustochytrid* strains in Barclay.

240. Moreover, it is my opinion that a PHOSITA would have expected success in using Barclay’s strains and culture conditions for increasing the

concentration of the preferred PUFA (i.e., DHA) given that Bijl and Barclay teach that their oils are microbial in origin. MARA-1008, 13:19-22, 12:22-37; MARA-1019, 4:59-61. Bijl and Barclay both acknowledge their oils are specific to containing omega-3 PUFAs, of which DHA is an example. MARA-1008, 6:3-5, 7:18-19; MARA-1019, 10:40-60, 6:31-41.

241. Furthermore, I also note that both Bijl and Barclay acknowledge similar uses for their oils, such as human nutritional or pharmaceutical uses. MARA-1008, 9:30-36, 14:23-27; MARA-1019, 13:35-40, 14:12-14, 14:48-68.

#### **4. Analysis**

##### Claim 1

##### ***[1pre] A microbial oil comprising***

242. Bijl-Barclay discloses and/or renders obvious a microbial oil.

243. I note that Bijl discloses a “pure and stable microbial oil” which comprises “at least one polyunsaturated fatty acid (PUFA), which has a triglyceride content of greater than 90%.” MARA-1008, 1:8-11, 2:30-33.

244. Bijl discloses obtaining “microbially derived” oils by “extracting, isolating or recovering the compound from the microbial biomass.” MARA-1008, 3:3-5, 7:10-11. And Bijl notes that “PUFA (oil) which has been extracted can be used” with or without further refining. MARA-1008, 9:20-22. Barclay similarly discloses microbial oils. *See* MARA-1019, 4:59-61, 10:40-60.

***[1a] a triglyceride fraction of at least 70% by weight,***

245. Bijl-Barclay discloses and/or renders obvious microbial oil comprising a triglyceride fraction of at least 70% by weight.

246. I note that Bijl discloses a microbial oil “which has a triglyceride content of greater than 90%.” MARA-1008, 2:30-33. Bijl discloses a triglyceride content that is preferably more than or equal to 93%, suitably more than or equal to 95 wt.%, and optionally more than or equal to 97 wt.%. MARA-1008, 4:24-28.

***[1b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight,***

247. Bijl-Barclay discloses and/or renders obvious wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight.

248. In my opinion, a PHOSITA would have applied the teachings of using a preferred microbial strain (e.g., “strains of the invention which produce a high proportion of total omega-3 fatty acids as C22:6w3 [DHA],” such as those in Table 3 or 4), as taught by Barclay, to Bijl’s processes to yield an improved oil composition having higher amounts of DHA. MARA-1019, 22:47-54, Tables 3-4. Indeed, Barclay shows more than twenty (20) of 108 strains having more than 50% DHA and 0.5% to 6% DPA(n-6) of total fatty acids (TFA), of which three (3) strains have more than 70% DHA. MARA-1019, Table 3.

249. A PHOSITA would have found it obvious that the DHA content of the triglyceride fraction is at least 50 wt.% in the proposed combination because more

than twenty (20) of Barclay's strains produce an oil composition with greater than 50 wt.% DHA (of TFA) and three (3) of Barclay's strains produce an oil composition with greater than 70 wt.% DHA (of TFA). MARA-1019, Table 4.

250. Consistent with Barclay's disclosure, other prior art references indicated that many microbial strains (e.g., strains in the *Thraustochytrium* family) contained DHA in amounts greater than 45 wt.% and even greater than 60 wt.%. MARA-1032, 27-31, Table 2.5 (discloses 67.2, 65.6, 67.8, 63.7, 64.3, 62.4 and other percentages of DHA); MARA-1015, [0107]-[0108], Table after [0108], [0113]-[0114], [0016], [0116], Fig. 3; MARA-1019, Tables 3-4 (showing multitude of strains with >50% DHA (C22:6w3)).

251. Komazawa is another example that discloses a *Thraustochytrium* strain (LFF1 strain) that contains DHA components ranging from 62% to 84% in triglyceride form. MARA-1015, [0113]-[0114]; *see also*, MARA-1015, [0116] (stating that "[t]he DHA component in the triglycerides produced shows characteristic tendency. Namely,...DHA component becomes a high content of 80% or more, when culturing was carried out at pH8.5"); *see also*, MARA-1015, Fig. 3 (stating "DHA content in triglyceride" in the header and disclosing about 80% DHA in triglyceride form at pH 8.5 and about 58% DHA in triglyceride form at pH 4.5).

252. A PHOSITA would have understood and/or found obvious since the majority of Bijl's microbial oil is made up of triglycerides (e.g., > 90% in the oil), that the majority of the DHA in Bijl-Barclay must be present in the triglyceride form. In fact, Bijl discloses that DHA is a predominant PUFA in thraustochytrids and that its oils or "compound preferably comprises a triglyceride, such as one of the PUFAs previously mentioned." MARA-1008, 6:3-5, 7:18-19.

253. Moreover, a PHOSITA would have understood and/or found obvious that Barclay's oils included triglycerides and, based on references available before the Critical Date, likely included high triglyceride content. MARA-1019, 6:6-11 (stating that "[l]ipids are understood to be fats or oils including the *glyceride esters of fatty acids* along with associated phosphatides, sterols, alcohols, hydrocarbons, ketones, and related compounds"), 1:23-28 (describing organisms and "process[es] for culturing them for the production of *lipids* with high concentrations of omega-3 highly unsaturated *fatty acids* (HUFA)"), 13:52-58 (describing a process in which "[t]he solvent is removed (for example by a vacuum rotary evaporator, which allows the solvent to be recovered and reused) and the lipids hydrolyzed by using any of the well-known methods for converting *triglycerides* to free fatty acids or esters of fatty acids including base hydrolysis, acid hydrolysis, or enzymatic hydrolysis"); *see, e.g.*, MARA-1007, 89:24-26 (stating that "[a]nalysis of results shows that the fatty acid component which ONC-T18 produces under heterotrophic

fermentation is almost entirely triglyceride (at least 95%) in nature”); *see, e.g.*, MARA-1007, 32:17-33:5 (stating that “[t]he percentage of neutral lipids can be at least 95% by weight of total lipids” and that “[t]he percentage of mono-, di- and tri-glycerides in the neutral lipid fraction of ONC-T18 is 0% to about 2%, 0 to about 2% and 96 to about 100%, respectively”); *see also, e.g.*, MARA-1034, [0037]-[0038] (disclosing that “[t]ypically, the content of neutral lipids in the lipids of the present invention is very high (more than 90[%] of total lipids)” and that “[t]he neutral lipids from the lipids of the present invention contain at least 85% triacylglycerols, preferably at least 90% triacylglycerols”).

254. A PHOSITA would have therefore understood and/or found obvious to apply Barclay’s (or similar) strains or culture methods to Bijl’s processes to produce a DHA-enriched microbial oil containing at least 50 wt.% DHA in triglyceride form, or higher such as at least 60 wt.% DHA in triglyceride form.

255. For at least the reasons above, Bijl-Barclay discloses and/or renders obvious [1b].

***[1c] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from 0.5% by weight to 6% by weight,***

256. Bijl-Barclay discloses and/or renders obvious wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from 0.5% by weight to 6% by weight.

257. Bijl discloses microbial oils including PUFAs, which “predominantly (or only) contains one particular polyunsaturated fatty acid, but which may additionally contain lesser amounts of other polyunsaturated fatty acids.” MARA-1008, 5:24-29.

258. In the combination, Barclay describes characteristics for organisms having “preferably low content of saturated and *omega-6 highly unsaturated fatty acids*,” in which DPA n-6 is an example of an omega-6 HUFA. MARA-1019, 6:48-50; *see* MARA-1019, 19:46-49 (stating that “[s]trains can also be isolated which have less than 1% (as % of total fatty acids) of the undesirable C20:4w6 and C22:5w6 highly unsaturated fatty acids.”). The reason for this preference is further discussed, in which “*C22:5w6 [DPA n-6] fatty acid is considered undesirable* as a dietary fatty acid because it can retroconvert to the C20:4w6 fatty acid.” MARA-1019, 10:54-57. Using the microorganisms or culture conditions of Barclay with the process of Bijl would provide an oil with decreased DPA n-6 content.

259. A PHOSITA would have been motivated to use Barclay’s microorganisms with Bijl’s processes to provide an oil with high DHA content and low DPA n-6 content. Barclay discloses that its microorganisms can have low DPA n-6 component, i.e., “[t]he screening procedure outlined in this invention, however, facilitates the isolation of some strains that contain no (*or less than 1%*) omega-6 highly unsaturated fatty acids (C20:4w6 or C22:5w6).” MARA-1019,

10:57-60; *see* MARA-1019, Table 3 (showing that, excluding “PRIOR STRAINS” (a term employed by Barclay), twenty-five (25) of 108 strains have more than 50% DHA and 0.5% to 6% DPA (n-6) of total fatty acids, of which twenty (20) strains have more than 60% DHA and of which three (3) strains have more than 70% DHA). It would have therefore been obvious and/or understood that the extracted microbial oil of the combination produces a concentration of oils that falls within the range of 0.5% by weight to 6% by weight DPA n-6 of the triglyceride fraction.

260. A PHOSITA would have understood and/or found obvious since the majority of Bijl’s microbial oil is made up of triglycerides (e.g., > 90% in the oil), that the majority of the PUFAs (of which DPA is an example of a C22 omega-6 PUFA) in Bijl-Barclay must be present in the triglyceride form. In fact, Bijl discloses that its oils or “compound preferably comprises a triglyceride, such as one of the PUFAs previously mentioned.” MARA-1008, 7:18-19; *see also* MARA-1008, 4:5-7 (stating that “[t]he polyunsaturated fatty acid can be a C18, C20 or C22 ω-3 and C18, C20, or C22 ω-6 polyunsaturated fatty acids”), 5:30-32 stating that “[t]he polyunsaturated fatty acids that maybe present in the microbial oil of the invention are C20 ω-3 and C18, C20 and C22 ω-6 polyunsaturated fatty acids”). Based on at least the reasons provided above, Bijl-Barclay discloses and/or renders obvious Element [1c].

***[1d] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

261. Bijl-Barclay discloses and/or renders obvious “wherein the oil comprises 5% by weight or less of heptadecanoic acid.”

262. Barclay describes microbial oils having low amounts of HDA. *See* MARA-1019, Table 3 (showing that four (4) of 108 strains have more than 50% DHA, 0.5% to 6% DPA (n-6), and less than 5% of other fatty acids (Other FA) of total fatty acids, in which these strains include SBG5, 46A, 27A, and 26B). A PHOSITA would have understood and/or found obvious that a recitation of less than 5% of other fatty acids would include a composition with “5% by weight or less” of HDA.

263. In the combination, a PHOSITA would have applied the teachings of using a preferred microbial strain (e.g., “strains of the invention which produce a high proportion of total omega-3 fatty acids as C22:6w3 [DHA],” such as those in Table 3 or 4), as taught by Barclay, to Bijl’s processes to yield an improved oil composition having higher amounts of DHA. MARA-1019, 22:47-54, Tables 3-4. Upon employing Barclay’s oils having high DHA and low HDA content (e.g., such as oils from strains SBG5, 46A, 27A, and 26B in Table 3) with Bijl’s processes, a PHOSITA would have understood and/or found obvious that the HDA levels in the Barclay-Bijl oils would have been maintained.

264. Furthermore, HDA (17:0) is an odd-chain fatty acid (OCFA), and a PHOSITA would have understood and/or found obvious that “the physiological implications of OCFA nutrition for humans are relatively unknown” and that “15:0 and 17:0 accumulation can decrease the percentage of other fatty acids including the valuable DHA.” MARA-1041, 82-84. Also, Radianingtyas discloses production of a wide range of oils with consistently low amounts of HDA. *See* MARA-1007, 34:4-37:14. Thus, a PHOSITA would have sought out microbial oils that would contain or maintain low amounts of HDA.

Claim 2

***[2] The microbial oil of claim 1, further comprising an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.***

265. Bijl-Barclay discloses and/or renders obvious a microbial oil comprising an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.

266. Element [2] is an optional negative limitation, for which prior art disclosure is not necessary to meet the claim element. In other words, an oil containing no ARA meets this limitation.

267. Barclay describes oils having no ARA and oils having non-zero amounts of ARA that are less than 1.5 wt.%. Specifically, Barclay states that [t]he screening procedure outlined in this invention, however, facilitates the isolation of some strains that ***contain no (or less than 1%) omega-6 highly unsaturated fatty***

*acids (C20:4w6 [ARA] or C22:5w6 [DPA n-6]).*” MARA-1019, 10:57-60;

MARA-1019, 19:46-49 (stating that “[s]trains can also be isolated which have less than 1% (as % of total fatty acids) of the undesirable C20:4w6 and C22:5w6 highly unsaturated fatty acids”).

268. Barclay also provides microbial oils having between 0-0.9% ARA (C20:4w6). *See* MARA-1019, Table 3 (showing that four (4) strains having more than 50% DHA, less than 5% DPA (n-6), less than 1.5% ARA, and less than 5% of other fatty acids (Other FA) of total fatty acids, in which the strains include SBG5, 46A, 27A, and 26B). That in turn discloses or renders obvious [2].

269. In the Bijl-Barclay combination, a PHOSITA would have applied the teachings of using a preferred microbial strain (e.g., “strains of the invention which produce a high proportion of total omega-3 fatty acids as C22:6w3 [DHA],” such as those in Table 3 or 4), as taught by Barclay, to Bijl’s processes to yield an improved oil composition having higher amounts of DHA. MARA-1019, 22:47-54, Tables 3-4. Upon employing Barclay’s oils having high DHA and low ARA content (e.g., such as oils from strains SBG5, 46A, 27A, and 26B in Table 3) with Bijl’s processes, a PHOSITA would have understood and/or found obvious that the ARA levels in the Barclay-Bijl oils would have been maintained.

270. Additionally, a PHOSITA would have understood and/or found obvious that it was desirable to have any non-DHA component (of which ARA is

just one example) present at only low levels in a primarily DHA-containing oil. As motivation, a PHOSITA would recognize that high-DHA, low-ARA concentrations would be preferable to DHA from fish oil because of “the low content of the desired fatty acids” in fish oils. MARA-1034, [0005]. A PHOSITA would have therefore wanted to achieve a high-purity, DHA-containing oil free of contaminants or other PUFAs for more efficient supplementation of human and animal food products. Indeed, a higher-purity, DHA-containing oil would be preferable because it results in a consumer needing to take fewer supplements (i.e. capsules) and reduces the “calories ingested from unwanted fatty acids.” MARA-1022, 113; MARA-1035, 381 (“Concentrates keep the intake of calories and other possibly physiologically active fatty acids as low as possible.”). Thus, a PHOSITA would have sought out microbial oils that would contain low amounts of a non-DHA component (e.g., ARA).

271. In addition, Barclay states that one of the “economically desirable characteristics for the production of omega-3 highly unsaturated fatty acids” includes “preferably *low content of* saturated and *omega-6 highly unsaturated fatty acids*”, in which ARA (20:4 w-6) is an example of an omega-6 HUFA. MARA-1019, 6:42-50. Barclay further discloses strains having “high concentrations of three omega-3 highly unsaturated fatty acids: C20:5w3, C22:5w3

and C22:6w3 and *very low concentration of C20:4w6* [ARA]”. MARA-1019, 10:40-45.

272. A PHOSITA would have understood and/or found obvious that it was desirable to have any non-DHA component (of which ARA is just one example) present at only low levels in a primarily DHA-containing oil because higher purity oils result in more efficient processing that is economically desirable. For this reason as well, a PHOSITA would have sought out microbial oils that would contain low amounts of a non-DHA component (e.g., ARA), as taught in Bijl-Barclay.

### Claim 3

***[3pre] A microbial oil comprising***

273. See Section III(C)(4)[1pre], *supra*.

***[3a] a triglyceride fraction of at least 70% by weight,***

274. See Section III(C)(4)[1a], *supra*.

***[3b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 40% by weight,***

275. See Section III(C)(4)[1b], *supra*.

***[3c.1] wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least 0.5% by weight to 6% by weight,***

276. See Section III(C)(4)[1c], *supra*.

***[3c.2] wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than 6:1,***

277. Bijl-Barclay discloses and/or renders obvious [3c.2].

278. I note that Barclay discloses that DHA is desirable and that DPA n-6 is undesirable. Regarding DHA, Barclay shows more than twenty (20) of 108 strains in Table 3 as having more than 50% DHA and 0.5% to 6% DPA (n-6), and three (3) strains in Table 3 as having more than 70% DHA. MARA-1019, Tables 3-4. Regarding DPA n-6, Barclay states that “C22:5w6 fatty acid is considered undesirable as a dietary fatty acid because it can retroconvert to the C20:4w6 fatty acid. The screening procedure outlined in this invention, however, facilitates the isolation of some strains that *contain no (or less than 1%) omega-6 highly unsaturated fatty acids (C20:4w6 or C22:5w6 [DPA n-6])*.” MARA-1019, 10:55-60. Taking a greater than 50% DHA and 0.5% to 6% DPA (n-6) (as contemplated by Barclay), Barclay discloses a ratio of DHA:DPA n-6 that is greater than 6:1 (e.g., a ratio of 50% DHA:0.5% DPA (n-6) ~ 100:1 and a ratio of 50% DHA:6% DPA (n-6) ~ 8.3:1). This in turn discloses or renders obvious [3c.2].

***[3d] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

279. See Section III(C)(4)[1d], *supra*.

#### Claim 4

##### ***[4pre] A microbial oil comprising***

280. See Section III(C)(4)[1pre], *supra*.

##### ***[4a] a triglyceride fraction of at least 70% by weight,***

281. See Section III(C)(4)[1a], *supra*.

***[4b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight,***

282. See Section III(C)(4)[1b], *supra*.

***[4c] and wherein the oil comprises 5% by weight or less of heptadecanoic acid.***

283. See Section III(C)(4)[1d], *supra*.

Claim 5

***[5] The microbial oil of any one of claims 1 to 4, wherein at least 20% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at two positions in the triglyceride selected from any two of the sn-1, sn-2, and sn-3 positions.***

284. Bijl-Barclay discloses and/or renders obvious the microbial oil of any one of claims 1 to 4, wherein at least 20% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at two positions in the triglyceride selected from any two of the sn-1, sn-2, and sn-3 positions.

285. Each of claims 1-4 recites at least 40-60% DHA in triglyceride form, and this recited DHA percentage provides a presence of at least 20% of that DHA at any two of the sn-1, sn-2, and sn-3 positions.

286. As I explain above, a PHOSITA would have readily understood that an oil having at least 40% DHA would possess at least 20% DHA at any two of the sn-1, sn-2, and sn-3 positions. See Paragraphs 177-189 above.

Claim 6

***[6] The microbial oil of any one of claims 1 to 4, wherein at least 5% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at all three of the sn-1, sn-2, and sn-3 positions in the triglyceride.***

287. Bijl-Barclay discloses and/or renders obvious the microbial oil of any one of claims 1 to 4, wherein at least 5% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at all three of the sn-1, sn-2, and sn-3 positions in the triglyceride.

288. Each of claims 1-4 recites at least 40-60% DHA in triglyceride form, and this recited DHA percentage provides a presence of at least 5% of that DHA at all three of the sn-1, sn-2, and sn-3 positions.

289. As I explain above, a PHOSITA would have readily understood that an oil having at least 40% DHA would possess at least 5% DHA at all three of the sn-1, sn-2, and sn-3 positions. *See* Paragraphs 190-194 above.

Claim 7

***[7] A food product, cosmetic, or pharmaceutical composition for animals or humans, comprising the microbial oil of any one of claims 1 to 4.***

290. Bijl-Barclay discloses and/or renders obvious a food product, cosmetic, or pharmaceutical composition for animals or humans, comprising the microbial oil of any one of claims 1 to 4.

291. I note that Bijl describes that the oil of its invention can “be used in *foods, foods stuffs* or *food compositions* or serve as a nutritional supplement, for

*humans* as well as for *animals*” and “can be used in *cosmetics*.” MARA-1008, 4:13-16; *see also* MARA-1008, 4:17-18, 9:28-10:8, 14:23-27, claims 33-36.

292. I note that Barclay also describes that “heterotrophic organisms and a process for culturing them for the production of lipids with high concentrations of omega-3 highly unsaturated fatty acids (HUFA) suitable for human and animal consumption as *food additives* or for use in *pharmaceutical* and industrial products,” as well as microbial products to “be used as a food or *feed supplement*.” MARA-1019, 1:23-28; 17:13-15.

#### Claim 8

***[8] The food product of claim 7, wherein the food product is an infant formula.***

293. Bijl-Barclay discloses and/or renders obvious that the food product is an infant formula.

294. I note that Bijl teaches that the oil of its invention “are particularly suitable for inclusion in *infant formula*.” MARA-1008, 9:37-10:1; *see also* MARA-1008, 4:13-18, 9:28-10:8, 14:23-27, 29:20-25, claims 33-36.

#### Claim 9

***[9] The food product of claim 8, wherein the infant formula can be consumed by premature infants.***

295. Bijl-Barclay discloses and/or renders obvious that the infant formula can be consumed by premature infants.

296. I note that Bijl teaches that the oil of its invention “are particularly suitable for inclusion in *infant formula*.” MARA-1008, 9:37-10:1; *see also*

MARA-1008, 4:13-18, 9:28-10:8, 14:23-27, 29:20-25, claims 33-36. Based on this disclosure, a PHOSITA would have understood and/or found obvious that Bijl's oil-containing infant formula would also be provided to premature infants. In my opinion, this understanding is consistent with Tanaka, which describes formula that can be suitable for infants as well as immature infants: "[t]he present invention provides a nutrient-supplementing food, a formula suitable for feeding infants, *a formula suitable for feeding immature infants*, a baby food, a food for expectant or nursing mothers ...." MARA-1034, [0016].

297. Furthermore, a PHOSITA would have understood and/or found obvious the benefits of polyunsaturated fats (PUFAs) to supplement formula for infants, including preterm infants. *See, e.g.*, MARA-1010, Preface at v, stating that "[s]ingle cell oils (SCO) have come of age. They have become accepted biotechnological products fulfilling key roles in the supply of the major very long chain polyunsaturated fatty acids (PUFA), now known to be essential for *infant nutrition and development*."; MARA-1010,53 (stating that "[n]ewborns obtain most of these PUFA from breast milk, and it was thus suggested that the *diet of preterm infants who are not breast-fed should be supplemented with these PUFA*. In fact, various health authorities now recommend the incorporation of both AA and DHA into baby formulae and the FDA has recently approved their combined use") (citations omitted).

#### Claim 10

***[10] The food product of claim 7, wherein the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.***

298. Bijl-Barclay discloses and/or renders obvious that the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.

299. I note that Bijl teaches that the oil of its invention can be used in “***milk, health drinks, and bread,***” as well as ***infant formula***. MARA-1008, 9:28-10:8; *see also* MARA-1008, 4:13-18, 14:23-27, 29:20-25, claims 33-36.

#### Claim 11

***[11] The food product of claim 7, wherein the food product is an additive for animal or human food.***

300. Bijl-Barclay discloses and/or renders obvious that the food product is an additive for animal or human food.

301. I note that Bijl teaches that the oil of its invention can “***be used in foods, food stuffs or food compositions or serve as a nutritional supplement, for humans as well as for animals.***” MARA-1008, 4:13-15; *see also* MARA-1008, 4:17-18, 9:28-10:8, 14:23-27, 29:20-25, claims 33-36. Barclay teaches “lipids with high concentrations of omega-3 highly unsaturated fatty acids (HUFA) suitable for human and animal consumption as ***food additives*** or for use in pharmaceutical and industrial products” and “purified omega-3 highly unsaturated fatty acids [to]...be used as a nutritional supplement for humans, as ***a food additive***, or for pharmaceutical applications.” MARA-1019, 1:23-28, 14:12-14.

Claim 12

***[12] The food product of claim 7, wherein the food product is a nutritional supplement.***

302. Bijl-Barclay discloses and/or renders obvious that the food product is a nutritional supplement.

303. I note that Bijl describes that the oil of its invention can “***be used in foods, foods stuffs or food compositions or serve as a nutritional supplement, for humans as well as for animals.***” MARA-1008, 4:13-15; *see also* MARA-1008, 4:17-18, 9:28-10:8, 14:23-27, 29:20-25, claims 33-36. Barclay teaches that “[t]he purified omega-3 highly unsaturated fatty acids can then be used as a ***nutritional supplement*** for humans, as a food additive, or for pharmaceutical applications.” MARA-1019, 14:12-14.

Claim 13

***[13] The food product of claim 7, wherein the food product is an animal feed.***

304. Bijl-Barclay discloses and/or renders obvious that the food product is an animal feed.

305. I note that Bijl teaches that the oil of its invention can “***be used in foods, foods stuffs or food compositions or serve as a nutritional supplement, for humans as well as for animals.***” MARA-1008, 4:13-15; *see also* MARA-1008, 4:17-18 (“granular particles or granules may find use as a food or ***feed composition or supplement***”), 9:28-10:8, 14:23-27, 29:20-25, claims 33-36.

306. I also note that Barclay teaches “lipids with high concentrations of omega-3 highly unsaturated fatty acids (HUFA) suitable for human and *animal consumption* as *food additives* or for use in pharmaceutical and industrial products” and “extracted omega-3 HUFAs can be used as a *feed* or food supplement.” MARA-1019, 1:23-28, 11:46-48.

Claim 14

*[14] The animal feed of claim 13, wherein the animal feed is an aquaculture feed.*

307. Bijl-Barclay discloses and/or renders obvious that the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

308. I note that Barclay discloses that “[t]he microbial product of the present invention is of value as a source of omega-3 highly unsaturated fatty acids for fish, shrimp and other products produced by *aquaculture*” and that “[t]he product can be added directly as a supplement to the feed or it can be fed to brine shrimp or other live feed organisms intended for consumption by the *aquacultured product*.” MARA-1019, 17:30-36.

#### Claim 15

***[15] The animal feed of claim 13, wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.***

309. Bijl-Barclay discloses and/or renders obvious that the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

310. I note that Bijl teaches that the oil of its invention can “***be used in foods, foods stuffs or food compositions or serve as a nutritional supplement, for humans as well as for animals.***” MARA-1008, 4:13-15; *see also* MARA-1008, 4:17-18 (“granular particles or granules may find use as a food or ***feed composition or supplement***”), 9:28-10:8, 14:23-27, 29:20-25, claims 33-36. Barclay discloses that “[t]he microbial product of the present invention is of value as a source of omega-3 highly unsaturated fatty acids for fish, shrimp and other products produced by ***aquaculture***” and that “[t]he product can be added directly as a supplement to the feed or it can be fed to brine shrimp or other live feed organisms intended for consumption by the ***aquacultured product.***” MARA-1019, 17:30-36.

#### **IV. CONCLUSION**

311. I reserve the right to supplement my opinions to address any information obtained, or positions taken, based on any new information introduced throughout this proceeding.

312. I declare under penalty of perjury that the foregoing is true and accurate to the best of my ability.