

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Kirk E. Apt et al.
U.S. Patent No.: 10,757,959 B2 Attorney Docket No.: 55973-0004IP1
Issue Date: September 1, 2020
Appl. Serial No.: 16/132,992
Filing Date: September 17, 2018
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: 22nd August 2023

By: 
Jonathan M. Curtis, Ph.D.

TABLE OF CONTENTS

I.	EXPERIENCE AND QUALIFICATIONS.....	4
A.	Materials Considered	6
B.	Overview of Conclusions Formed.....	10
C.	Interpretation of the '959 Patent Claims.....	11
D.	Legal Standards	12
	1. Terminology.....	12
	2. Legal Standards for Anticipation.....	12
	3. Legal Standards for Obviousness.....	13
E.	Background Knowledge One of Skill in the Art Would Have Had Prior to the Priority Date of the '959 patent	18
	1. Composition of Microbial Oils	18
	2. Extraction of Microbial Oils	25
II.	SUMMARY OF THE '959 PATENT	29
A.	Brief Description	29
B.	Summary of the Prosecution History of the '959 Patent	31
C.	The Effective Priority Date of the Claims of the '959 Patent.....	32
D.	Level of Ordinary Skill in the Art.....	33
III.	THE CHALLENGED CLAIMS ARE UNPATENTABLE.....	34
A.	GROUND 1: Claim 1 is anticipated by Radianingtyas	34
	1. Overview of Radianingtyas.....	34
	2. Analysis.....	36
B.	GROUND 2: Claims 1-14 are obvious over Radianingtyas in view of Kralovec.....	42
	1. Overview of Radianingtyas.....	42
	2. Overview of Kralovec.....	42
	3. Combination of Radianingtyas and Kralovec	44
	4. Analysis.....	50
C.	GROUND 3: Claims 1-14 are obvious over Bijl in view of Nichols-I and Kralovec.....	72
	1. Overview of Bijl.....	72
	2. Overview of Nichols-I	73
	3. Overview of Kralovec	75
	4. Combination of Bijl and Nichols-I	75
	5. Combination of Bijl, Nichols-I, and Kralovec.....	78
	6. Analysis.....	81
D.	GROUND 4: Claims 1-4, 6-14 are obvious over Bijl + Komazawa....	95
	1. Overview of Bijl.....	95
	2. Overview of Komazawa	95

3.	Combination of Bijl and Komazawa	97
4.	Analysis.....	100
IV.	CONCLUSION.....	111

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 will continue my association with this department as Professor Emeritus on 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 4th 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. 10,757,959 (“the ’959 patent”) and prior art references relating to its subject matter. I have reviewed the ’959 patent, and relevant excerpts of the prosecution history of the ’959 patent. In addition to any materials cited in the present declaration, I have also reviewed the following references:

- MARA-1002: Prosecution History of the ’959 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*, 13:5 Australasian Biotechnology 26 (Oct./Nov. 2003) (“Nichols-I”)
- MARA-1010: *Single Cell Oils: Microbial and Algal Oils* (1st Ed. 2005, Cohen & Ratledge, eds.)
- MARA-1015: U.S. Pat. App. Pub. No. 2004/0161831 to Komazawa et al. (“Komazawa”)
- MARA-1018: US Pat. App. Pub. No. 2005/0273885 to Singh et al. (“Singh”)
- MARA-1019: U.S. Pat. App. Pub. No. 2006/0094089 to Barclay (“Barclay”)
- MARA-1020: Peter D. Nichols et al., *Omega-3 oils down under—An update*, 15:6 Inform 383 (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, USA (5th ed. 2017)
- MARA-1024: *Food Lipids*, C.C. Akoh and D.B. Min (Eds.), Marcel Dekker, New York, USA (2002)

- MARA-1025: A.M. Burja et al., Isolation and characterization of polyunsaturated fatty acid producing *Thraustochytrium* species: screening of strains and optimization of omega-3 production, *Appl. Microbiol. Biotechnol.* 72: 1161-1169 (2006)
- MARA-1027: Edwin N. Frankel, *Lipid Oxidation*, The Oily Press (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*, *Mar Biotechnol* 10:631-640 (2008)
- MARA-1034: U.S. Pat. App. Pub. No. 2003/0161864 to Tanaka et al. (“Tanaka”)
- MARA-1035: W.M.N. Rainayake et al., *Preparation of Omega-3 PUFA Concentrates from Fish Oils via Urea Complexation*, 90 Jahrgang 10:381-386 (1988)
- MARA-1036: Fereidoon Shahidi and Priyatharini Ambigaipalan, *Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits*, *Annu. Rev. Food Sci. Technol.* 9:345-81 (2018)

- MARA-1037: King-Wai Fan et al., *Lipid Characterization of Mangrove Thraustochytrid*—Schizochytrium mangrovei, J. Agric. Food Chem. 55:2906-2910 (2007)
- MARA-1038: Owen P. Ward & Ajay Singh, *Omega-3/6 Fatty Acids: Alternative Sources of Production*, Process Biochemistry 40:3627-3652 (2005)
- MARA-1039: Anthony Bimbo, *Marine Oils*, AOCS Lipid Library, <https://lipidlibrary.aocs.org/edible-oil-processing/marine-oils> (last accessed August 21, 2023).

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

11. I have previously submitted an expert declaration on behalf of Mara in a proceeding challenging the validity of a different DSM patent, U.S. Pat. No. 10,392,578 (IPR2023-1167). I reviewed additional materials in that proceeding. I have endeavoured to include here all of the materials I rely on for this proceeding, but the additional materials cited in the IPR2023-01167 declaration may also have informed some of my opinions here.

12. Counsel has informed me that I should consider these materials through the lens of one of ordinary skill in the art related to the '959 patent as of March 2009. I have done so. The '959 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 ’959 patent are entitled, and I have therefore used that Critical Date in my analysis below.

13. In writing this Declaration, I have considered the following: U.S. Patent No. 10,757,959 (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 publications and materials, in addition to any other materials I may cite in my declaration.

14. My opinions, as explained below, are based on my education, experience, and expertise in the fields relating to the ’959 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 ’959 patent and the prior art discussed below.

B. Overview of Conclusions Formed

15. 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 claim 1 of the '959 patent is anticipated by Radianingtyas;
- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion claims 1-14 of the '959 patent are obvious in light of Radianingtyas and Kralovec;
- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion claims 1-14 of the '959 patent are obvious in light of Bijl, Nichols-I, and Kralovec;
- Based upon my knowledge and experience and my review of the prior art publications listed above, in my opinion claims 1-4 and 6-14 of the '959 patent are obvious in light of Bijl and Komazawa.

C. Interpretation of the '959 Patent Claims

16. 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).

17. 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

18. 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 '959 patent as the point in time for claim interpretation purposes (the Critical Date).

2. Legal Standards for Anticipation

19. 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.

20. 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.

21. 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

22. 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.

23. 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.

24. 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.

25. 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."

26. 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."

27. 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.

28. 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.

29. 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.

30. 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.

31. 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.

32. 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.

33. 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.

34. 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.

35. 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).

E. Background Knowledge One of Skill in the Art Would Have Had Prior to the Priority Date of the '959 patent

36. 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

37. 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.

38. The two most important components of marine lipids that are associated with these benefits, are the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic 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 6th 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.

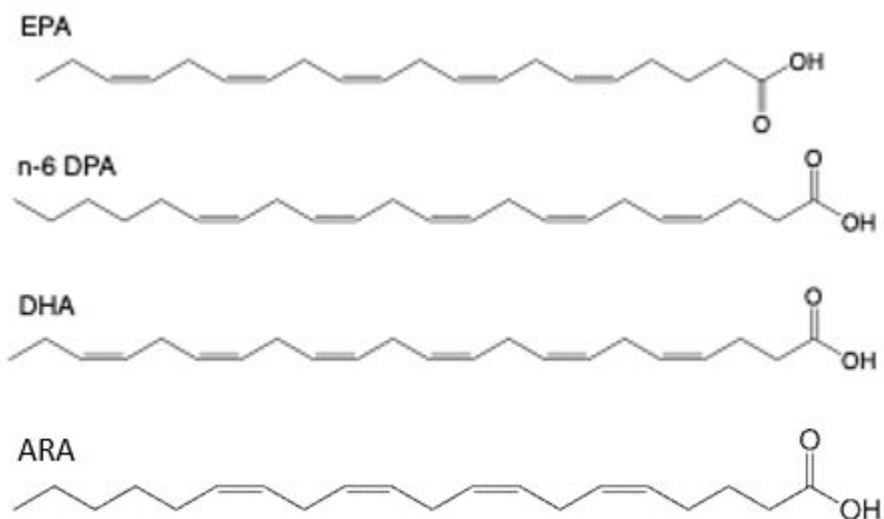


Figure 1 EPA, DPA n-6, DHA, ARA structures

39. 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.

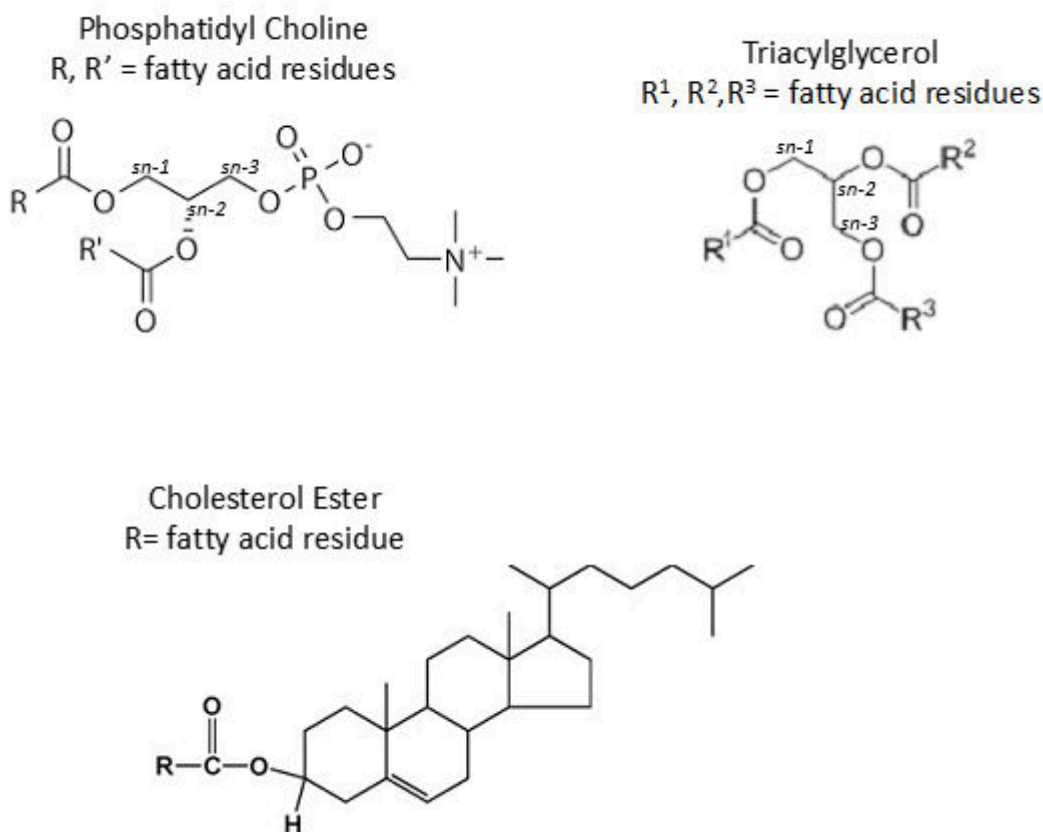


Figure 2 TAG (triacylglycerides), PC, cholesterol ester structures.

40. 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.

41. 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.

42. 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.

43. 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, 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 50-59% 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 16.2-31.4 as a percentage of the TFA. MARA-1025, 1166-1167.

44. 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. Thus, refined microbial oils are entirely suitable for human nutrition.

45. 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.

46. 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

47. 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.

48. 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 since 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 '959 PATENT

A. Brief Description

49. My review of the '959 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.

50. In particular, I note that the '959 patent identifies thraustochytrids on deposit under ATCC Accession Numbers PTA-9695, PTA-9696, PTA-9697,

and/or PTA-9698. MARA-1001, 3:22-24. The '959 patent also describes “mutant, variant or recombinant” strains related to those ATCC-deposited microorganisms. MARA-1001, 6:11-23.

51. Microorganisms of the purported invention are described as containing varying fatty acids, including a triglyceride fraction (TAG fraction) of at least about 40% by weight, with varying amounts of docosapentaenoic acid n-6 (DPA n-6), eicosapentaenoic acid, heptadecanoic acid, arachidonic acid (ARA), and/or docosahexaenoic acid (DHA). MARA-1001, 3:1-10, 3:43-47, 4:1-52. Mutant/variant strains produce differing amounts of these same fatty acids. MARA-1001, 6:23-29.

52. My review of the '959 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) and then their oils are extracted. MARA-1001, 4:41-52. Those conditions are known in the art and encompass “conventional medium for growth of thraustochytrids.” MARA-1001, 6:60-64; MARA-1019.

53. I note that the '959 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, 10:31-42. The ’959 patent states that these oils may then be applied in compositions, such as in “food products, pharmaceutical compositions, cosmetics, and industrial compositions.” MARA-1001, 15:19-21.

54. The ’959 patent includes 14 claims, of which claim 1 is independent.

B. Summary of the Prosecution History of the ’959 Patent

55. My review of the ’959 patent indicates that it issued on September 1, 2020 from U.S. Patent Application No. 16/132,992 (“the ’992 application”), filed on September 17, 2018 as a divisional application. *See* MARA-1002.

56. I understand from counsel that a divisional application is an application in the same patent family as an earlier patent. I further understand from counsel that a “patent family” constitutes a series of patents that have a common disclosure. I also understand from counsel that, as a divisional application, the ’959 patent’s prosecution history includes statements made in parent applications.

57. My review of the ’959 patent’s prosecution history indicates little back-and-forth between the Patent Examiner and the applicant.

58. I understand that the Examiner rejected all claims except for then-pending claim 55 over Fichtali et al., U.S. Pat. Pub. No. 2007/0003686. In that

rejection, the Examiner noted that Fichtali “does not mention heptadecanoic acid,” and suggested that “the claims may be amended around the cited reference by adding to claim 51 the limitation that the amount of heptadecanoic acid is between 0.1% and 5% by weight (i.e., not zero).” MARA-1002, 158-159. Further, the Examiner noted claim 55, the only claim alleged to be “free of the prior art,” because in the prior art “the amount of ARA [arachidonic acid] is not 1.5% or less by weight.” MARA-1002, 158.

59. I understand that in response to that rejection, Patentee amended claim 51 (issued claim 1) to recite less than 1.5% of arachidonic acid. MARA-1002, 194. I also note that Patentee argued that Fichtali did not disclose fatty acids in the triglyceride form or in an amount claimed by the patent. MARA-1002, 192.

60. It is my understanding that, following Patentee’s amendments and arguments, the Examiner allowed the claims. MARA-1002, 198-204.

C. The Effective Priority Date of the Claims of the ’959 Patent

61. My review of the ’959 patent indicates that it issued from U.S. Application No. 16/132,992, filed on September 17, 2018. The ’959 patent’s face reveals that it is part of a series of patents and patent applications and traces its history back to U.S. Patent Application No. 12/407,687, filed on March 19, 2009, which is now U.S. Patent No. 8,207,363.

62. Claim 1 of the '959 patent is an independent claim. Claims 2-14 depend directly or indirectly from claim 1. I have been asked by counsel to assume, and I have assumed, that claims 2-14 cannot enjoy an effective filing date earlier than that of claim 1 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 '959 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 above, it is my opinion that the prior art discloses each of the elements of the Challenged Claims, and claims 1-14 of the '959 patent are unpatentable.

A. GROUND 1: Claim 1 is anticipated by Radianingtyas

1. Overview of Radianingtyas

66. It is my opinion that Radianingtyas, like the '959 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 1:27-32, 1:63-2:18, 2:33-52, 10:16-14:24. 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 as well as low levels of arachidonic acid. MARA-1007, 11:4-9, 99:22-24, 99:29-32. 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 modifying the “medium of an oil producing microbe with a fatty acid production antagonist” will increase the “ratio of omega n-3 and/or n-6 oils to omega n-9 oils,” as will manipulation of carbon-to-nitrogen sources and “by the addition of certain chemical compounds which impact upon fatty acid production.” MARA-1007, 27:15-28. More specifically, Radianingtyas teaches that increases in agitation, temperature, or dissolved oxygen during culturing decrease DHA content, while additions of acetate, trace elements, metal, and vitamins to the production medium increase DHA content. MARA-1007, 28:14-20, 30:15-17. These modifications were known to a PHOSITA as of 2009.

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.” 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).” MARA-1007, 26:29-34. ONC-T18 can also produce antioxidants. See MARA-1007, 89:32-90:25.

2. Analysis

72. I discuss below why, in my opinion, Radianingtyas anticipates claim 1 of the '959 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 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”).

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 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. I note that, in particular, 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 docosaheptaenoic acid content of the triglyceride fraction is at least 45% by weight,

81. In my opinion, Radianingtyas discloses that the triglyceride fraction is at least 45% by weight DHA. I understand this to mean that at least 45% of the fatty acids in triglyceride form are DHA.

82. I note that Radianingtyas discloses examples that result in the “[o]ptimized production of [DHA] by strain ONC-T18” and show the “[o]ptimal time for harvesting of ONC-T18 for maximal DHA production.” MARA-1007,

87:6-89:10. Further, 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).”¹ MARA-1007, 26:29-34.

83. Because the fatty acid component that ONC-T18 produces is “at least 95%” triglyceride, a PHOSITA would also have understood the majority of the PUFAs in Radianingtyas would be present in the triglyceride form. In my opinion, a PHOSITA would have found it unlikely that DHA would comprise a significant portion of the non-triglyceride fraction because DHA is generally found in triglyceride form when extracted from microbes.

84. Additionally, in a composition containing 60% by weight DHA (as contemplated by Radianingtyas), because the fatty acid component produced is “at

¹ I note that this paragraph identifies “a lipid composition comprising from about 25% to about 40% of an omega-3 fatty acid, such as n-3 DHA.” MARA-1007, 26:27-27:3. Immediately thereafter, a parenthetical states “for example, at least 15%, 20%, 25%, 30%, 35%, 40%. 45%, 50%, 55%, or 60% by weight[.]” 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.”

least 95%” triglyceride, even if the remaining 5% fatty acid component that was not in triglyceride form was entirely DHA, an oil containing 55% DHA in triglyceride form would still meet [1b].

85. Finally, Radianingtyas also teaches 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.”). As I discussed above in Section I(E), this purification uses methods known to a PHOSITA that would result in highly concentrated quantities of DHA.

86. In my opinion then, Radianingtyas teaches a triglyceride fraction that is at least 45 wt.% DHA.

[1c] and wherein the oil comprises 1.5% by weight or less of arachidonic acid.

87. In my opinion, Radianingtyas discloses a microbial oil that comprises 1.5 wt.% or less of arachidonic acid.

88. I note that Radianingtyas describes an Example 13, titled “Isolation and characterization of [PUFA] producting[sic] *Thraustochytrium* sp. ONC-T18: screening in order to identify strain and optimization of production therein,” which discloses that “[i]n addition to the omega-3 oils produced, approximately 80% of all isolates synthesized the omega-6 PUFAs, arachidonic acid (AA)...at

concentrations varying between 1 and 18%.” MARA-1007, 96:1-3, 99:29-32, Fig. 11.

89. 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 1-18% quantity of arachidonic acid anticipates that portion of the ’959 patent, claim 1.

90. Additionally, I note that Radianingtyas also discloses that its oil producing microbes can produce a wide range of oils that all consistently contain low amounts of arachidonic acid. *See* MARA-1007, 34:4-37:14 (showing arachidonic acid ranging from 0.13% to 0.90% as percentage of total fatty acid). In some embodiments, arachidonic acid is not disclosed at all. *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 arachidonic acid). It is also possible that arachidonic acid is present in these oils, but below detectable levels. I understand, however, from reviewing the prosecution history of the ’959 patent, and from counsel, that a patent claim requiring “1.5% by weight or less” can include compositions with 0% arachidonic acid. Therefore, in my opinion, whether Radianingtyas’s oils contain 0% arachidonic acid or low levels of arachidonic acid, Radianingtyas discloses a microbial oil composition with “1.5% by weight or less” of arachidonic acid within the meaning of this claim limitation.

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

B. GROUND 2: Claims 1-14 are obvious over Radianingtyas in view of Kralovec

1. Overview of Radianingtyas

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

2. Overview of Kralovec

93. I note that Kralovec relates to methods for modifying oil concentrations by concentrating polyunsaturated fatty acids. 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.

94. 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.

95. 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. 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.

96. 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.

97. 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

1) DHA as the preferred PUFA

98. 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. 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.

99. 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.

100. 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.

101. Similarly, Kralovec teaches that DHA was known to reduce “cardiovascular and heart disease risk” and crucial to the “prevention and/or treatment of inflammation, neurogenerative diseases, and cognitive development.” MARA-1030, 1:16-2:3. Kralovec also 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.

102. 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.

103. 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, 64:18-22.

104. Moreover, it is my opinion that a PHOSITA would have expected success in pursuing extracted oils with high levels of DHA given that it was already known that certain strains of microbes contained “very high levels of DHA (61%).” MARA-1009, 27. Radianingtyas’ disclosure of ONC-T18, a microbe producing high levels of DHA, would have further confirmed this likelihood of success.

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

105. 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., approximately 96-100%) and DHA (at least 60% by weight), 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, 33:1-2; MARA-1030, 1:9-11.

106. 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.

107. 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 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.

108. 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.

109. 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).

110. 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)”).

111. To me, a PHOSITA would have naturally considered “traditional” or “well-known” methods for modifying an oil such as Radianingtyas’s oil, including Kralovec’s methods or similar. Thus, a PHOSITA would have looked to refine Radianingtyas’s oils in accordance with Radianingtyas’s teachings by looking to Kralovec.

112. 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/algal in origin and so have similar characteristics. MARA-1007, 1:22-31; MARA-1030, 2:28-29. Both acknowledge their oils are specific to containing omega-3 PUFAs, and even more specifically DHA. MARA-1007, 1:22-31; MARA-1030, 19:7-12.

113. 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.

114. Therefore, it is my opinion that a PHOSITA would have combined the teachings of Radianingtyas and Kralovec and would expect success in doing so. I detail my opinion on how this combination renders claims 1-14 of the '959 patent obvious below.

4. Analysis

Claim 1

[1pre] A microbial oil comprising

115. 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.

116. 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.

117. 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,

118. 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.

119. 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”).

120. 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 (e.g., Singh) also disclosed triglyceride-rich oils. MARA-1018, [0294] (“oil comprising at least 75% or 85% triacylglycerols...said triacylglycerols comprising the LC-PUFA”).

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

[1b] wherein the docosahexaenoic acid content of the triglyceride fraction is at least 45% by weight,

122. It is my opinion that Radianingtyas and Kralovec disclose a triglyceride fraction that comprises at least 45% by weight DHA. As I described above in Section III(A)(2)(1b), Radianingtyas includes DHA-rich microbes that satisfy this limitation. I also described how a PHOSITA would know that methods could be employed to further purify oils to increase DHA content.

123. Kralovec's methods share the same goal as Radianingtyas: to increase the concentration of DHA in a final microbial oil product. It is my opinion, therefore, that a PHOSITA would have found it obvious to apply Kralovec's methods (or similar) to the Radianingtyas microbial oil to produce a DHA-enriched microbial oil containing at least 45 wt.% DHA or higher in triglyceride form.

[1c] and wherein the oil comprises 1.5% by weight or less of arachidonic acid.

124. It is my opinion that Radianingtyas and Kralovec disclose a microbial oil that comprises 1.5% by weight or less of arachidonic acid. As I described above in Section III(A)(2)(1c), Radianingtyas discloses examples in which microbial oils contained arachidonic acid in ranges under 1.5 wt.%, including some examples where no arachidonic acid was disclosed at all. I also described the

desirability of such a composition: it would achieve higher-purity oils and would therefore be sought after.

125. Additionally, I note that Kralovec espouses similar goals. Kralovec teaches that in an embodiment, after using Kralovec's method to increase DHA, arachidonic acid is present at no more than 1.4% of the glyceride fraction.

MARA-1030, 43-44, Table 9.1; *see also* MARA-1019, Table 3 (indicating 72.7% DHA and 0% arachidonic acid).

126. I also note that a PHOSITA would have understood that it was desirable to have low levels of arachidonic acid in a primarily DHA-containing oil. In my view, a PHOSITA would recognize that high-DHA, low-arachidonic-acid 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.").

127. Therefore, it is my opinion that Radianingtyas and Kralovec disclose a microbial oil that renders obvious claim 1 of the '959 patent.

Claim 2

[2] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight.

128. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [2]. In my opinion, a PHOSITA would have understood that the majority of Radianingtyas's microbial oils are made up of triglycerides (e.g., up to 100%). MARA-1007, 33:1-2. Therefore, the majority (i.e., at least 60%) of the PUFAs in Radianingtyas would be present in the triglyceride form, including the DHA.

129. Radianingtyas discloses a DHA content of at least 60% by weight of the triglyceride fraction because it teaches that the microorganisms produce lipid compositions where DHA is present at up to 60 wt.% of the microbial oil. MARA-1007, 26:29-27:3. One such example results in the "[o]ptimized production of [DHA] by strain ONC-T18" and shows the "[o]ptimal time for harvesting of ONC-T18 for maximal DHA production." MARA-1007, 87:6-89:10. 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.

130. The fatty acid component that ONC-T18 produces is “at least 95%” triglyceride, so in an oil produced by ONC-T18 containing 60% DHA (as contemplated by Radianingtyas), even if the remaining 5% fatty acid component that was not in triglyceride form was entirely DHA, the oil would still contain 55%--which is nearly 60% DHA—DHA in triglyceride form and would still meet [2].

131. Additionally, I note that 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. Radianingtyas generally discloses “oil producing microbes...preferably *Thraustochytrium*...having the ability to produce lipids...unsaturated fatty acids...such as...DHA.” MARA-1007, 11:4-9. 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, [0016], [0051], [0116], Fig. 3; MARA-1009, 27; MARA-1032, 27-31.

132. I note that 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, 28:15-17, 27:13-21. To the extent, then, that 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. A PHOSITA would have known to use the methods Radianingtyas describes, as well as other methods, to accomplish that purification.

133. Additionally, a PHOSITA would also readily know how to increase the DHA content in triglyceride form using methods disclosed in references like Kralovec. In my opinion, a PHOSITA would have been motivated to apply Kralovec's oil modification techniques (or similar) to Radianingtyas's extracted microbial oil as a known way to increase its DHA content.

134. 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.

135. In my opinion then, 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.

136. In my view, 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 at 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 at 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 at 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.

Claim 3

[3] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 80% by weight.

137. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [3].

138. To me, Radianingtyas discloses microorganisms that produce lipid compositions where DHA is present in at least 60 wt.% of the microbial oil.

MARA-1007, 26:29-27:3.

139. Specifically, I note that Radianingtyas discloses examples that result in the “[o]ptimized production of [DHA] by strain ONC-T18” and show the “[o]ptimal time for harvesting of ONC-T18 for maximal DHA production.”

MARA-1007, 87:6-89:10. 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 . . . 40%, 45%, 50%, 55% or 60% by weight).” MARA-1007, 26:29-34.

140. Because the fatty acid component that ONC-T18 produces is “at least 95%” triglyceride, in the unlikely circumstance that the remaining 5% fatty acid component that was not in triglyceride form was entirely DHA, I note that an oil containing 60% DHA (as contemplated by Radianingtyas) would still contain 55% DHA in triglyceride form. But a PHOSITA would also have understood that because the majority of Radianingtyas’s microbial oils are made up of triglycerides (e.g., up to 100%), the majority of the PUFAs in Radianingtyas would be present in the triglyceride form, including the DHA.

141. Additionally, it is my opinion that compositions having a DHA content of the triglyceride fraction that is at least 80% by weight were also known to a PHOSITA based on the prior art that was available before the Critical Date.

142. I note that Radianingtyas generally discloses “oil producing microbes...preferably *Thraustochytrium*...having the ability to produce lipids...unsaturated fatty acids...such as...DHA.” MARA-1007, 11:4-9. 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 id.*, [0016], [0051], [0116], Fig. 3; MARA-1009, 27; MARA-1032, 27-31. 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, 28:15-17, 27:13-21.

143. To the extent the DHA content in the triglyceride fraction in Radianingtyas's microbial oil is determined to be less than 80%, Radianingtyas itself discloses 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.

144. 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 in Section III(B)(3), 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.

145. 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.

146. I therefore conclude that a PHOSITA would have 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 80 wt.% DHA in triglyceride form.

147. As I describe above in Section III(B)(4)(2), a PHOSITA would have understood that having higher levels of DHA in triglyceride form was desirable for reasons of commercial acceptability and stability.

Claim 4

[4] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 90% by weight.

148. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [4].

149. It appears to me that 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.

150. Furthermore, Radianingtyas discloses examples that result in the "[o]ptimized production of [DHA] by strain ONC-T18" and show "[o]ptimal time for harvesting of ONC-T18 for maximal DHA production." MARA-1007, 87:6-89:10. 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 . . . 40%, 45%, 50%, 55% or 60% by weight).” MARA-1007, 26:29-34.

151. In my opinion, a PHOSITA would have understood that because the majority of Radianingtyas’s microbial oils are made up of triglycerides (e.g., up to 100%), the majority of the PUFAs in Radianingtyas would be present in the triglyceride form, including the DHA.

152. Additionally, 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, 28:15-17, 27:13-21. Alternately, a PHOSITA would also readily know how to increase the DHA content in triglyceride form using methods disclosed in references like Kralovec.

153. As discussed above in Section III(B)(3), 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.

154. In my opinion then, 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 90 wt.% DHA in triglyceride form.

155. As I describe above in Section III(B)(4)(2), a PHOSITA would have understood that having higher levels of DHA in triglyceride form was desirable for reasons of commercial acceptability and stability.

Claim 5

[5] The microbial oil of claim 1, further comprising an arachidonic acid content of the triglyceride fraction of between 0.1% and 1.5% by weight.

156. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [5].

157. To me, both Radianingtyas and Kralovec disclose microbial oils with arachidonic acid content between 0.1% and 1.5% by weight.

158. In Radianingtyas, 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 that “[i]n addition to the omega-3 oils produced, approximately 80% of all isolates

synthesized the omega-6 PUFAs, arachidonic acid (AA)...at concentrations varying between 1 and 18%.” MARA-1007, 96:1-3, 99:29-32, Fig. 11. A PHOSITA would have understood that the low end of this disclosed range falls within the claimed range of 0.1% and 1.5% arachidonic acid.

159. Moreover, I note that Radianingtyas discloses that its oil producing microbes can produce a wide range of oils that all consistently fall within the claimed range of arachidonic acid. *See* MARA-1007, 34:4-37:14 (showing arachidonic acid ranging from 0.13% to 0.90% as percentage of total fatty acid). Because Radianingtyas discloses that its compositions include triglycerides from “96 to about 100%” of the neutral lipids, which “can be at least 95% by weight of total lipids,” a PHOSITA would have understood that the arachidonic acid was likely to be found in the triglyceride fraction. MARA-1007, 32:17-33:5; *see also* MARA-1037, 2908, Table 2 (disclosing oil similar to Radianingtyas with 96% triglycerides in the neutral lipids and 0.37% arachidonic acid in the triglyceride fraction). Thus, I conclude that a PHOSITA would understand that arachidonic acid in the triglyceride fraction would not deviate significantly from the 0.13-0.90% disclosed in Radianingtyas, and therefore would fall within the claimed range of 0.1-1.5%.

160. Additionally, I note that Kralovec also discloses an example showing that after using Kralovec’s method for increasing DHA, arachidonic acid (i.e. fatty

acid 20:4) is present at no more than 1.4% of the glyceride fraction. *See* MARA-1030, 43-44 (Table 9.1).

161. As I discussed previously in Section III(A)(2)(1c), a PHOSITA would have understood that it was desirable to have low levels of arachidonic acid in a primarily DHA-containing oil and would have sought out microbial oils containing low amounts of arachidonic acid.

Claim 6

[6] The microbial oil of any one of claims 1 to 5, wherein said microbial oil further comprising an effective amount of at least one added antioxidant to provide oxidative stability.

162. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [6].

163. I note that Radianingtyas teaches that the oil “compositions can contain any antioxidants.” MARA-1007, 37:30, 11:10-13. Radianingtyas also discloses compositions that contain “lipid with antioxidant.” MARA-1007, 40:22-26, 12:3-7.

164. A PHOSITA would have understood that the addition of antioxidants was desirable because it would provide oxidative stability. As the name suggests, antioxidants prevent oxidation. *See* MARA-1019, [0038], [0042].

165. To that end, Radianingtyas discloses that “the addition of antioxidants” is utilized to “enhance production of omega-3 fatty acids.” MARA-1007, 12:20-22 (referencing U.S. Pat. No. 5,130,242 to Barclay, MARA-1019).

The Barclay patent that Radianingtyas cites describes “adding an antioxidant compound...in order to prevent degradation of the omega-3 HUFAs” such as DHA. MARA-1019, 5:35-40; *see also id.*, 12:45-48, 14:16-17 (noting antioxidants “protect the fatty acids from oxidation” and “improve their stability”).

166. In my opinion, the stabilizing qualities of antioxidants were well known to a PHOSITA; it was noted “that many microbial oils contain relatively large quantities of their own antioxidants,” meaning that those oils were “much more stable than corresponding oils [extracted] from plants and marine animals.” MARA-1010, 204. It was a “standard process” as of 2009 to add antioxidants to microbial oils “to prolong shelf life.” MARA-1038, 3645-46. Indeed, microbial oils are “stabilized by adding antioxidants” including “ascorbyl palmitate and tocopherols.” MARA-1010, 213. Therefore, the “standard commercial practice” was “to add further antioxidants to the oils in order to assure the customer that all the necessary steps have been taken to ensure complete stability of the oil.” MARA-1010, 204.

Claim 7

[7] A food product composition for animals or humans, comprising the microbial oil of any one of claims 1 to 6.

167. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [7].

168. Radianingtyas states that “disclosed are the fatty acids and carotenoids produced by the disclosed oil producing microbe and any progeny (genetically modified or otherwise), various feedstuffs, nutraceuticals, pharmaceuticals and foods supplemented with the lipids and antioxidants, as well as a process for utilizing these compounds as an additive for various feedstuffs and foods.” MARA-1007, 12:3-7.

169. Radianingtyas also states that “[a]fter refining, the oils can be used directly as a feed or food additive to produce omega-3 and/or omega-6 enriched products.”) MARA-1007, 42:22-23; *see also* MARA-1007, 40:20-26, 42:7-9, 43:6-8, 72:4-17.

Claim 8

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

170. It is also my opinion that the combination of Radianingtyas and Kralovec discloses and/or renders obvious [8].

171. Radianingtyas discloses that the “compositions can be used in food products (e.g., baby milk).” MARA-1007, 13:15-16.

172. Radianingtyas also describes “an infant formula comprising the composition (lipid, lipid with antioxidant and antioxidant alone).” MARA-1007, 40:25-26. Radianingtyas discloses that “[t]hese lipids and antioxidants are targeted for use in animal feeds, pharmaceuticals, nutraceuticals (especially infant formula) as well as in the industry.” MARA-1007, 67:18-19, 79:12-17, claim 38.

Claim 9

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

173. To me, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [9].

174. Radianingtyas discloses that the “compositions can be used in food products (e.g., baby milk).” MARA-1007, 13:15-16.

175. Radianingtyas also discloses that “[i]n another aspect, the foodstuff is...milk...fruit juice, a dry beverage powder...” MARA-1007, 72:15-17, 79:11-13 (“Also disclosed are foodstuffs that comprises any of the compositions, delivery devices, or microcapsules described above. The foodstuff can be ... a milk product”); 79:19-21, claim 40.

Claim 10

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

176. To me, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [10].

177. Radianingtyas discloses that its compounds “are useful as or within nutraceuticals, ***food additives***, pharmaceuticals or industry.” MARA-1007, 11:18-21; 12:3-7 (“disclosed are the fatty acids and carotenoids produced by the disclosed oil producing microbe and any progeny (genetically modified or otherwise), various feedstuffs, nutraceuticals, pharmaceuticals and ***foods***”).

supplemented with the lipids and antioxidants, as well as a process for utilizing these compounds as *an additive for various feedstuffs and foods.*”), 42:24-25 (“After refining, the oils can be used directly as a *feed or food additive* to produce omega-3 and/or omega-6 enriched products.”), 43:6-8 (“Resulting in the concentration of the remaining PUFA compounds and used as a nutritional supplements for humans, as a *food additive*, or as pharmaceutical applications.”).

Claim 11

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

178. It is my opinion that the combination of Radianingtyas and Kralovec discloses and/or renders obvious [11].

179. Radianingtyas discloses that its compounds “are useful as or within *nutraceuticals*, food additives, pharmaceuticals or industry.” MARA-1007, 11:18-21; 12:3-7 (“disclosed are the fatty acids and carotenoids produced by the disclosed oil producing microbe and any progeny (genetically modified or otherwise), various feedstuffs, *nutraceuticals*, pharmaceuticals and foods supplemented with the lipids and antioxidants, as well as a process for utilizing these compounds as an additive for various feedstuffs and foods.”), 43:6-8 (“Resulting in the concentration of the remaining PUFA compounds and used as a *nutritional supplements* for humans, as a food additive, or as pharmaceutical applications.”), 59:16 (“Also disclosed herein are *nutritional supplements*.”),

59:21-22 (“For example, a ***nutritional supplement*** can comprise any of the lipids disclosed herein.”).

Claim 12

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

180. In my opinion, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [12].

181. 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; 12:3-7 (“disclosed are the fatty acids and carotenoids produced by the disclosed oil producing microbe and any progeny (genetically modified or otherwise), ***various feedstuffs***, nutraceuticals, pharmaceuticals and foods supplemented with the lipids and antioxidants, as well as a process for utilizing these compounds as an additive for ***various feedstuffs*** and foods.”), 67:18 (“These lipids and antioxidants are targeted for use in ***animal feeds***”), claim 39.

Claim 13

[13] The animal feed of claim 12, wherein the animal feed is an aquaculture feed.

182. To me, the combination of Radianingtyas and Kralovec discloses and/or renders obvious [13].

183. 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; 79:11-18 (“Also disclosed are foodstuffs that comprises any of the compositions, delivery devices, or microcapsules described above. The foodstuff can also be a fish product, a companion pet food, a livestock or an *aquaculture feed*.”), claim 39.

Claim 14

[14] The animal feed of claim 12, wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

184. It is also my opinion that the combination of Radianingtyas and Kralovec discloses and/or renders obvious [14].

185. 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; 79:11-18 (“Also disclosed are foodstuffs that comprises any of the compositions, delivery devices, or microcapsules described above. The foodstuff can also be a fish product, a *companion pet food, a livestock* or an *aquaculture feed*.”), claim 39.

C. GROUND 3: Claims 1-14 are obvious over Bijl in view of Nichols-I and Kralovec

1. Overview of Bijl

186. It appears to me that Bijl and the '959 patent are directed to microbial oils. MARA-1001 1:27-32; 1:63-2:18; 2:33-52; 10:16-14:24; 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-1001 1:27-32; 1:63-2:18; 2:33-52; 10:16-14:24; MARA-1008, 12:22-37; 2:35-3:2.

187. 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.

188. 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, pasteurizing 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.

189. Bijl teaches using pasteurization 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 pasteurization 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. Bijl teaches that, in particular, “degradation is particularly high when the PUFA is [arachidonic acid].” MARA-1008, 3:25-27.

190. 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 Nichols-I

191. My read of Nichols-I is that it discloses newly-discovered sources of long-chain polyunsaturated fatty acids (“LC-PUFAs”) in microorganisms. Nichols-I describes new strains of single cell organisms that produce high quantities of LC-PUFAs. MARA-1009, 27.

192. Nichols-I also discloses that different fatty acids are obtained from different microorganisms: for example, EPA is derived from eustigmatophytes and diatoms; DHA from dinoflagellates and thraustochytrids; arachidonic acid from red

algae, thraustochytrids, and fungi; and GLA from blue-green algae. MARA-1009, 27 (Table 1). And Nichols-I also reports on the relative fatty acid concentrations of certain strains of thraustochytrids and their main omega-3 long-chain polyunsaturated fatty acids (LC-PUFA): DHA and EPA (strain A); DHA and arachidonic (AA) (strains C/H); and DHA (strain O) fatty acids. MARA-1009, 27 (Table 2).

193. I see in Nichols-I the description that Strain O is “particularly attractive” because it “contains very high levels of DHA (61%).” MARA-1009, 27. Strain O also contains low levels of arachidonic acid (0.7%). MARA-1009, 27.

194. Nichols-I also discloses uses and benefits of microbially-derived oils. Nichols-I teaches that omega-3 oils are beneficial to human health and are used to treat diseases and in development and protection of the human brain. MARA-1009, 26. Nichols-I further teaches that different omega-3 oils have different health benefits. DHA, for example, decreases the incidence of coronary heart disease. MARA-1009, 26.

195. Traditionally, and as I described above in Section I(E), omega-3 oils were derived from fish. MARA-1009, 26. As Nichols-I describes, however, “there is a need for new sources of these oils to be obtained” because “global wild fish stocks are declining.” MARA-1009, 26. So, not only does Nichols-I describe the use of microorganisms as sources of omega-3 oils, it also describes suggestions

for creating transgenic plants that contain genes of oil-producing microorganisms, to enable farmers to grow omega-3-producing plants. MARA-1009, 28.

3. Overview of Kralovec

196. I provide a description of Kralovec above in Section III(B)(2).

4. Combination of Bijl and Nichols-I

197. In my opinion, a PHOSITA would combine the teachings of Bijl, directed to microbial oils and processes of cultivating, processing, and extracting microbial oils from natural sources, with Nichols-I, which teaches using specific beneficial strains of microorganisms (e.g., *thraustochytrids*). I also believe that a PHOSITA would have found it obvious to implement Nichols-I's teachings of using a preferred strain of microorganisms (e.g., *thraustochytrids* of a strain O) as a microbial source for Bijl's extracted microbial oil.

198. First, it is my opinion that a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains (e.g., strain O *thraustochytrids*) as a microbial source for Bijl's extracted microbial oil because doing so "provide[s] an oil of a particularly high quality." MARA-1008, 9:5-7.

199. Second, in my opinion, a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains as a source for Bijl's extracted microbial oil because "[t]*hraustochytrids* are...single cell organisms that produce both high oil and LC-PUFA content." MARA-1009, 27.

200. Third, I believe that a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains as a source for Bijl's extracted oil because these strains were described as "a highly attractive, renewable and contaminant free, source of omega-3 oils" and "thraustochytrids can be grown in fermenters with an organic carbon source," which provides a renewable and contaminant free microbial source. MARA-1009, 27.

201. Fourth, it is my opinion that a PHOSITA would also have been motivated to use Nichols-I's preferred microbial strains as a source for Bijl's extracted oil because of the description that "[s]train O is particularly attractive as it contains very high levels of DHA (61%)." MARA-1009, 27. As Nichols-I notes, and as I described above, DHA is "beneficial against various human disorders." MARA-1009, 26. Moreover, there are many known benefits associated with DHA. MARA-1030, 1:16-2:3, 18:10-19:6; *see also* MARA-1010, 99, 182-186.

202. Fifth, I believe that a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains as a source for Bijl's extracted oil because Strain O contained only 0.7% arachidonic acid. MARA-1009, 27 (Table 2). Bijl teaches that "degradation is particularly high when the PUFA is [arachidonic acid]." MARA-1008, 3:25-27. Thus, it is my opinion that a PHOSITA would

have been motivated to use Strain O, which contained low levels of arachidonic acid.

203. Sixth, a PHOSITA would have been motivated to use Nichols-I's preferred microbial strains as a source for Bijl's extracted oil because doing so would have amounted to nothing more than the use of a known technology (e.g., identifying a microbial source) to improve similar oils (e.g., extracted microbial oils) in the same way and combining prior art elements according to known methods (e.g., apply Bijl's oil extraction methods) to yield predictable results (e.g., obtain an oil with desirable traits such as high PUFA levels). MARA-1010, 38-42, 47-48, 202-217.

204. I believe that a PHOSITA would also have a reasonable expectation of success in combining Bijl with Nichols-I. Bijl and Nichols-I both relate to the creation of microbial lipids using culturing techniques. MARA-1008, 7:2-11; MARA-1009, 27-28. Both references teach the same sources of those microbial lipids: algae, fungi, dinoflagellates, and other diatoms. MARA-1008, 12:22-37; MARA-1009, 27; *see also* MARA-1018, [0569]-[0570] (Table 3); MARA-1020, 384-385, Table 2. And both teach that the desired product is one of high enough quality for use in the human diet, including in infant formula. MARA-1008, 14:23-27; MARA-1009, 27-28. Furthermore, Bijl discloses that its processes are compatible with a wide range of natural sources, including *Thraustochytrium*,

which are also disclosed in Nichols-I. MARA-1008, 6:3-11; 13:19-31; MARA-1009, 27.

5. Combination of Bijl, Nichols-I, and Kralovec

205. As I noted above, Kralovec provides methods for obtaining compositions containing “triglycerides with higher DHA...contents than the starting triglycerides.” MARA-1030, 35:20-32. To me then, it would have been obvious for a PHOSITA to combine the teachings of Bijl-Nichols-I (directed to microbial oils having high levels of triglycerides (e.g., >97%) with a preferred PUFA (DHA)) with Kralovec (directed to methods for modifying an oil composition, to increase the concentration of that PUFA in the microbial oil). MARA-1008, 2:30-33, 4:24-28; MARA-1030, 1:9-11.

206. Specifically, I believe that a PHOSITA would have found it obvious to implement Kralovec’s oil modification techniques (or similar) to Bijl-Nichols-I’s method and extracted microbial oil to increase the amount of DHA.

207. First, it is my opinion that a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to the Bijl-Nichols-I 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 was well-known that decreasing the amount of unwanted components in a microbial oil was desirable because, as Kralovec describes, “[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.

208. Second, in my opinion, a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to the Bijl-Nichols-I 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. I also note that Kralovec suggests that “[s]uch oils can be incorporated into food products, nutraceuticals, pharmaceutical formulations, and microcapsules.” MARA-1030, 35:21-36:2.

209. Third, I believe that a PHOSITA would have been motivated to apply Kralovec’s oil modification techniques to the Bijl-Nichols-I 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 DHA).

210. It is further my opinion that a PHOSITA would have expected success in applying Kralovec's techniques to increase the concentration of the preferred PUFA (i.e., DHA) in the microbial oil. Bijl contemplates that its invention "can be subjected to one or more further refining steps." MARA-1008, 9:20-22. Thus, a PHOSITA would have looked to refine the Bijl-Nichols-I oils in accordance with Bijl-Nichols-I's teachings by looking to references such as Kralovec.

211. 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 Bijl-Nichols-I and Kralovec teach that their oils are microbial/algal in origin and so have similar characteristics. MARA-1008, 13:19-22; MARA-1030, 2:28-29. Bijl and Kralovec both acknowledge their oils are specific to containing omega-3 PUFAs, and even more specifically DHA. MARA-1008, 6:3-5; 7:18-19; MARA-1030, 19:7-12.

212. Furthermore, I note that both Bijl-Nichols-I and Kralovec acknowledge similar uses for their oils, such as human nutritional uses. Bijl, for example, teaches that its resultant oil can be used for nutritional purposes and can be added to human foods. MARA-1008, 9:30-36. Kralovec too discloses that its oils "can be incorporated into food products." MARA-1030, 35:31-36:2.

6. Analysis

Claim 1

[1pre] A microbial oil comprising

213. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [1pre].

214. 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. 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, like the ’959 patent, Bijl notes that “PUFA (oil) which has been extracted can be used” with or without further refining. MARA-1008, 9:20-22.

215. As I describe above in Section I(E), extracted microbial lipids were well known to a PHOSITA prior to the Critical Date. Bijl’s disclosures are consistent with a PHOSITA’s knowledge, in my view.

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

216. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [1a].

217. 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 greater than 95 wt.% and optionally higher than 97 wt.%. MARA-1008, 4:24-28.

218. Similarly, 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”).

219. I also note that triglyceride-rich oils were well known to a PHOSITA prior to the Critical Date. Other references (e.g., Singh) also disclosed triglyceride-rich oils. MARA-1018, [0294] (“oil comprising at least 75% or 85% triacylglycerols...said triacylglycerols comprising the LC-PUFA”).

[1b] wherein the docosaehaenoic acid content of the triglyceride fraction is at least 45% by weight,

220. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [1b].

221. It is my opinion that a PHOSITA would have applied the teachings of using a preferred microbial strain (e.g., thraustochytrid strain O), as taught by Nichols-I, to Bijl’s oil to yield an improved oil composition having higher amounts of a desired PUFA (e.g., DHA).

222. I also believe that a PHOSITA would have found it obvious that the DHA content of the triglyceride fraction is at least 45 wt.% in the proposed

combination because Nichols-I's preferred strain O of *Thraustochytrium*, when cultured, produces an oil composition with greater than 60 wt.% DHA. MARA-1009, 27, Table 2. Specifically, Nichols-I discloses that *Thraustochytrium* strain O contains "very high levels of DHA (61%)." MARA-1009, 27 (Table 2); MARA-1018, [0571]; MARA-1020, 384-385, Table 2.

223. Consistent with Nichols-I's disclosure, I note that 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 DHA); MARA-1015, [0107]-[0108], [0113]-[0114], [0016], Fig. 3; MARA-1019, Tables 3-4 (showing multitude of strains with >45% DHA (C22:6w3)). 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], [0016], Fig. 3.

224. In my view, a PHOSITA would have understood since the majority of Bijl's microbial oil is made up of triglycerides (e.g., > 97%) that the majority of the DHA (61%) in Bijl-Nichols-I is present in the triglyceride form. In fact, Bijl discloses that DHA is a preferred PUFA associated with the triglycerides present in

its oil: the invention “preferably comprises a triglyceride, such as one of the PUFAs previously mentioned,” such as DHA. MARA-1008, 6:3-5; 7:18-19.

225. Furthermore, it is my opinion that a PHOSITA would have known that microbial oils having a DHA content of approx. 60 wt.% in the triglyceride fraction would have been consistent with teachings of other prior art. Komazawa discloses exemplary *Thraustochytrium* strains (e.g., LFF1 strain) including DHA components in the triglyceride (TAG) form that range from “around 40%” to “80% or more,” depending on the culturing conditions. MARA-1015, [0016], Fig. 3.

226. Additionally, in my opinion Kralovec’s post-extraction methods could also be used to further increase the triglyceride fraction of the extracted microbial lipid composition. The method “produce[s] triglycerides of fatty acids,” which result in oils with “a high TG (triglyceride) content.” MARA-1030, 35:27-28, 37:22. From Bijl-Nichols-I’s starting oil, which has greater than 90% triglycerides, applying Kralovec’s methods would even further increase the amount and concentration of triglycerides. MARA-1030, 5:24-26.

227. I conclude, then, that a PHOSITA would have therefore understood and/or found obvious to apply Kralovec’s (or similar) methods to the Bijl-Nichols-I microbial oil to produce a DHA-enriched microbial oil containing at least 45 wt.% DHA, or higher, such as at least 60 wt.% DHA or at least 80 wt.% DHA in the triglyceride fraction. That makes obvious [1b].

[1c] and wherein the oil comprises 1.5% by weight or less of arachidonic acid.

228. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [1c].

229. I understand from counsel that element [1c] is a “negative limitation.” I understand that to mean that an oil containing no arachidonic acid meets this limitation.

230. I also note that this is how the Examiner treated this limitation. During prosecution the Examiner determined that a claim which recited “less than 5% heptadecanoic acid” could be amended around “by adding to claim 51 the limitation that the amount of heptadecanoic acid is between 0.1% and 5% by weight (i.e., not zero).” MARA-1002, 158-159. To me, this means that art that does not disclose that the oil contains any heptadecanoic acid reads on the claim. Since the same language appears here for arachidonic acid, oil containing no arachidonic acid would meet this limitation.

231. It is my opinion that if a non-zero amount of arachidonic acid that is less than 1.5 wt.% is required, Bijl-Nichols-I-Kralovec discloses and/or renders obvious this limitation.

232. I note that Nichols-I discloses that Strain O contains 0.7% arachidonic acid total fatty acids. MARA-1009, 27. And Kralovec teaches that in an embodiment, after processing, arachidonic acid is present at no more than 1.4% of

the glyceride fraction. MARA-1030, 43-44, Table 9.1; *see also* MARA-1019, Table 3 (indicating 72.7% DHA and 0% arachidonic acid). These disclosures disclose and/or render obvious [1c].

233. As I also discussed above in Section III(A)(2)(1c), a PHOSITA would have understood that it was desirable to have low levels of arachidonic acid in a primarily DHA-containing oil because higher purity oils result in more efficient supplementation. Thus, a PHOSITA would have sought out microbial oils containing low amounts of arachidonic acid, as taught in Nichols-I and in Kralovec.

Claim 2

[2] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight.

234. For the same reasons I discuss above in Section III(C)(6)(1b), it is my opinion that this limitation is disclosed by Bijl-Nichols-I-Kralovec.

Claim 3

[3] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 80% by weight.

235. It is my opinion that the Bijl-Nichols-I-Kralovec combination discloses and/or renders obvious [3].

236. For the reasons discussed above in Sections III(C)(6)(1a)-(1b), Bijl-Nichols-I-Kralovec discloses a DHA content of at least 80% by weight of the triglyceride fraction.

237. Additionally, as described above in Sections III(C)(6)(1a) and III(C)(6)(1b), compositions having a DHA content of the triglyceride fraction that is at least 80% (or 90%) by weight were also known to a PHOSITA based on the prior art that was available before the Critical Date.

238. To the extent the DHA content in the triglyceride fraction is determined to be less than 80%, it is my opinion that a PHOSITA would have been further motivated to apply Kralovec's oil modification techniques (or similar) to increase DHA content, as I discuss above in Sections III(C)(6)(4)-(5). 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.

Claim 4

[4] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 90% by weight.

239. For the reasons given in Section III(C)(6)(1a), Section III(C)(6)(1b), and Section III(C)(6)(3), it is my opinion that the Bijl-Nichols-I-Kralovec combination renders this claim obvious.

Claim 5

[5] The microbial oil of claim 1, further comprising an arachidonic acid content of the triglyceride fraction of between 0.1% and 1.5% by weight.

240. In my opinion, the Bijl-Nichols-I-Kralovec combination discloses and/or renders obvious [5].

241. As I discuss above in Section III(C)(6)(1c), both Kralovec and Nichols-I disclose microbial oils with arachidonic acid content between 0.1% and 1.5% by weight. Because Bijl discloses that its compositions include triglycerides in over 97% of the total composition, it is my opinion that a PHOSITA would have understood that the arachidonic acid was likely to be found in triglyceride form.

242. Moreover, I believe that a PHOSITA would have understood that it was desirable to have low levels of arachidonic acid in a primarily DHA-containing oil because higher purity oils resulted in more efficient supplementation. Thus, a PHOSITA would have sought out microbial oils containing low amounts of arachidonic acid, as taught in Nichols-I and in

Kralovec. As I discussed previously in Section III(C)(6)(1c) and Section III(A)(2)(1c), this provides a further reason for why this claim is obvious.

Claim 6

[6] The microbial oil of any one of claims 1 to 5, wherein said microbial oil further comprising an effective amount of at least one added antioxidant to provide oxidative stability.

243. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [6]. Bijl teaches that antioxidants, including antioxidants such as tocopherol or ascorbyl palmitate, can be added to compositions. MARA-1008, 26:16-18. Its composition may also contain antioxidants. MARA-1008, 3:37-4:1. Bijl-Nichols-I-Kralovec therefore renders [6] obvious.

Claim 7

[7] A food product composition for animals or humans, comprising the microbial oil of any one of claims 1 to 6.

244. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [7]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula.” MARA-1008, 9:28-10:8. Nichols-I also teaches that “fish-derived LC-PUFA oils are being *incorporated into food products* and in infant formula...Indeed, the other components of microorganisms such as antioxidants, vitamins and protein

can offer a ***highly nutritious***, whole cell package.” MARA-1009, 27. Bijl-Nichols-I-Kralovec therefore renders [7] obvious.

Claim 8

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

245. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [8]. 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, 4:13-18, 14:23-27, 29:20-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as ***infant formula***.

MARA-1008, 9:28-10:8. Nichols-I also teaches that “fish-derived LC-PUFA oils are being ***incorporated into food products*** and ***in infant formula***...Indeed, the other components of microorganisms such as antioxidants, vitamins and protein can offer a ***highly nutritious***, whole cell package.” MARA-1009, 27. Table 1 further identifies thraustochytrid-derived DHA as good for “[a]quaculture, health supplements, [and] ***infant formula***.” MARA-1009, Table 1. Bijl-Nichols-I-Kralovec therefore renders [8] obvious.

Claim 9

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

246. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [9]. 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, 4:13-18, 14:23-27, 29:20-25, cls. 33-36. This includes uses in “*milk, health drinks*, and bread,” as well as *infant formula*.

MARA-1008, 9:28-10:8. Nichols-I also teaches that “fish-derived LC-PUFA oils are being *incorporated into food products* and *in infant formula*...Indeed, the other components of microorganisms such as antioxidants, vitamins and protein can offer a *highly nutritious*, whole cell package.” MARA-1009, 27. Table 1 further identifies thraustochytrid-derived DHA as good for “[a]quaculture, health supplements, [and] *infant formula*.” MARA-1009, Table 1. Because a PHOSITA would understand infant formula to include milks and therapeutic drinks, Bijl-Nichols-I-Kralovec therefore renders [9] obvious.

Claim 10

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

247. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [10]. 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, 4:13-18, 14:23-27, 29:20-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula.

MARA-1008, 9:28-10:8. Nichols-I also teaches that “fish-derived LC-PUFA oils are being *incorporated into food products* and in infant formula...Indeed, the other components of microorganisms such as antioxidants, vitamins and protein

can offer a highly nutritious, whole cell package.” MARA-1009, 27. Table 1 further identifies thraustochytrid-derived DHA as good for “[a]quaculture, health supplements, [and] infant formula.” MARA-1009, Table 1. Bijl-Nichols-I-Kralovec therefore renders [10] obvious.

Claim 11

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

248. It is my opinion that Bijl-Nichols-I-Kralovec discloses and/or renders obvious [11]. 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, 4:13-18, 14:23-27, 29:20-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula. MARA-1008, 9:28-10:8. Nichols-I also teaches that “fish-derived LC-PUFA oils are being ***incorporated into food products*** and in infant formula...Indeed, the other components of microorganisms such as antioxidants, vitamins and protein can offer ***a highly nutritious***, whole cell package.” MARA-1009, 27. Table 1 further identifies thraustochytrid-derived DHA as good for “[a]quaculture, ***health supplements***, [and] infant formula.” MARA-1009, Table 1. Bijl-Nichols-I-Kralovec therefore renders [11] obvious.

Claim 12

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

249. It is my opinion that Bijl-Nichols-I-Kralovec discloses and/or renders obvious [12]. 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, 4:13-18, 14:23-27, 29:20-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula. MARA-1008, 9:28-10:8. As to animals, the “granular particles or granules may find use as a food or ***feed composition or supplement.***” MARA-1008, 4:13-18. Nichols-I also teaches that “fish-derived LC-PUFA oils are being ***incorporated into food products*** and in infant formula...Indeed, the other components of microorganisms such as antioxidants, vitamins and protein can offer a highly nutritious, whole cell package.” MARA-1009, 27. Table 1 further identifies thraustochytrid-derived DHA as good for “[a]quaculture, health supplements, [and] infant formula.” MARA-1009, Table 1. Bijl-Nichols-I-Kralovec therefore renders [12] obvious.

Claim 13

[13] The animal feed of claim 12, wherein the animal feed is an aquaculture feed.

250. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [13]. Nichols-I teaches that “These unique microorganisms can be used ***in aquaculture*** to supply essential LC-PUFA to ***live feeds used for rearing larvae***,

and as components in *aquaculture feeds* (e.g. pellets) used for raising juvenile and adult finfish and crustaceans (prawns and lobsters). Just as the LC-PUFA are beneficial for human nutrition and well being, they are also *key ingredients for cultured marine and freshwater species.*” MARA-1009, 27. Table 1 further identifies thraustochytrid-derived DHA as good for “[a]quaculture, health supplements, [and] infant formula.” MARA-1009, Table 1. Bijl-Nichols-I-Kralovec therefore renders [13] obvious.

Claim 14

[14] The animal feed of claim 12, wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

251. In my opinion, Bijl-Nichols-I-Kralovec discloses and/or renders obvious [14]. 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, 4:13-18, 14:23-27, 29:20-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula. MARA-1008, 9:28-10:8. As to animals, the “granular particles or granules may find use as a food or *feed composition or supplement.*” MARA-1008, 4:13-18. Nichols-I teaches that “These unique microorganisms can be used in aquaculture to *supply essential LCPUFA to live feeds used for rearing larvae*, and as components in *aquaculture feeds* (e.g. pellets) used for raising juvenile and adult finfish and crustaceans (prawns and lobsters). Just as the LC-PUFA are beneficial

for human nutrition and well being, they are also *key ingredients for cultured marine and freshwater species*.” MARA-1009, 27. Table 1 further identifies thraustochytrid-derived DHA as good for “[a]quaculture, health supplements, [and] infant formula.” MARA-1009, Table 1. Bijl-Nichols-I-Kralovec therefore renders [14] obvious.

D. GROUND 4: Claims 1-4, 6-14 are obvious over Bijl + Komazawa

1. Overview of Bijl

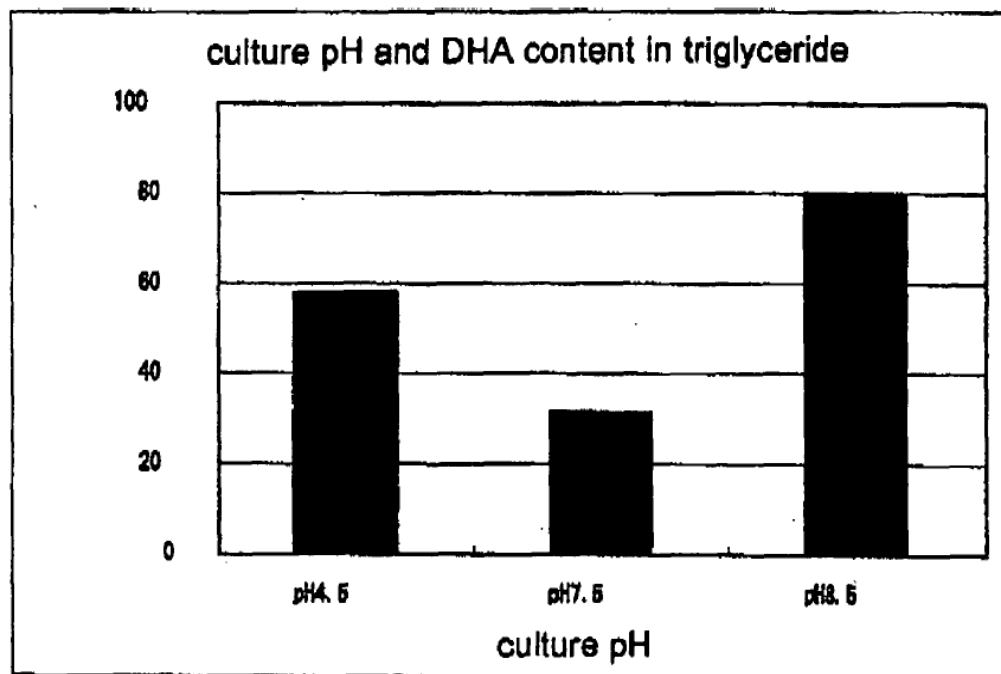
252. I provided an overview of Bijl above in Section III(C)(1).

2. Overview of Komazawa

253. I note that Komazawa “relates to a novel microorganism having an ability of producing docosahexaenoic acid (DHA) and the use of said microorganism.” MARA-1015, [0001]. In particular, Komazawa teaches “culturing the Thraustochytrium strain having an ability of producing docosahexaenoic acid in a medium, and collecting the docosahexaenoic acid-containing fat and oil from the culture product.” MARA-1015, [0012].

254. To me, Komazawa teaches that “to promote the production of docosahexaenoic acid, a docosahexaenoic acid precursor can be added to a medium.” MARA-1015, [0044]. Komazawa discloses that the “DHA component is around 40% when culturing was carried out at pH 7.5, but DHA component

becomes a high content of 80% or more, when culturing was carried out at pH 8.5.” MARA-1015, [0116]; *see also* FIG. 3.



MARA-1015, FIG. 3.

255. In my opinion, Komazawa teaches increased omega-3 fatty acid (DHA) concentrations in microbial oils. Specifically, Komazawa provides methods for “culturing the *Thraustochytrium* strain having an ability of producing docosahexaenoic acid in a medium, and collecting docosahexaenoic acid-containing fat and oil from the culture product.” MARA-1015, cl.3. Furthermore, Komazawa also teaches that “when docosahexaenoic acid is used in the field of medicines or the like, it is necessary that docosahexaenoic acid is separated and purified from docosahexaenoic acid-containing fat and oil” but “a variety of

polyunsaturated fatty acids having structures similar to that of docosahexaenoic acid are contained, and thus, separation and purification are difficult”—a problem that Komazawa sought to solve. MARA-1015, [0003]. In my opinion, a PHOSITA would have recognized that Komazawa discloses highly purified DHA-containing oils that would be understood to contain little or no arachidonic acid. To the extent any arachidonic acid was present, I also believe that a PHOSITA would have understood it to be below the limit of detection.

3. Combination of Bijl and Komazawa

256. It is my opinion that it would have been obvious for a PHOSITA to combine the teachings of Bijl, directed to microbial oils and processes of cultivating, processing, and extracting microbial oils from natural sources, with Komazawa, which teaches using specific beneficial strains of microorganisms (e.g., thraustochytrids) cultured under certain conditions to increase DHA. A PHOSITA would have found it obvious to implement Komazawa’s teachings of using a preferred strain of microorganisms (e.g., thraustochytrids of a strain LFF1) as a microbial source for Bijl’s extracted microbial oil.

257. First, it is my opinion that a PHOSITA would have been motivated to use Komazawa’s preferred microbial strains (e.g., strain LFF1 thraustochytrids) as a microbial source for Bijl’s extracted microbial oil because doing so “provide[s] an oil of a particularly high quality.” MARA-1008, 9:5-7.

258. Second, in my opinion, a PHOSITA would have been motivated to use Komazawa's preferred microbial strains as a source for Bijl's extracted microbial oil because the *Thraustochytrium* strain of Komazawa "can be easily cultured, and can produce fat and oil which contain a large amount of DHA." MARA-1015, [0009].

259. Third, I believe that a PHOSITA would have been motivated to use Komazawa's preferred microbial strains as a source for Bijl's extracted oil because when the LFF1 strain is used, the obtained "fat and oil is advantageous for concentration and separation of docosahexaenoic acid" because it "contains substantially no fatty acid having physiological activities similar or agonistic to that of docosahexaenoic acid." MARA-1015, [0058].

260. As Komazawa notes, DHA is "present specifically in the brain or retina of an animal" and "plays a major physiological role in those organs, and also has physiological activities such as an anti-inflammatory effect or an effect of lowering blood cholesterol level." MARA-1015, [0002]. Moreover, as I discussed above, there are many known benefits associated with DHA. MARA-1030, 1:16-2:3, 18:10-19:6; *see also* MARA-1010, 99, 182-186. Additionally, I note that Komazawa states that "application thereof is convenient in the fields of functional foods or medicines, and utilization thereof is particularly convenient in the

production of behenic acid which is used for photographic photosensitive materials.” MARA-1015, [0058].

261. Fifth, it is my opinion that a PHOSITA would have been motivated to use Komazawa’s preferred microbial strains as a source for Bijl’s extracted oil because Komazawa’s LFF1 strain contained no measurable amount of arachidonic acid. MARA-1015, [0112], Table 1. To the extent arachidonic acid was present in Komazawa’s oil, in my opinion a PHOSITA would have understood it to be below the limit of detection for the gas chromatography method used, meaning a level of less than 1%, and likely less than <0.1% arachidonic acid based on the disclosures in Table 1 that disclose percentages down to hundredths of a percent. As discussed above, it was desirable for a DHA-containing oil to have high purity, and a PHOSITA would have considered arachidonic acid to be a contaminant. Thus, in my opinion a PHOSITA would have been motivated to use the LFF1 strain, which contained low levels of arachidonic acid.

262. Sixth, in my opinion, a PHOSITA would have been motivated to use Komazawa’s preferred microbial strain as a source for Bijl’s extracted oil because doing so would have amounted to nothing more than the use of a known technology (e.g., identifying a microbial source) to improve similar oils (e.g., extracted microbial oils) in the same way and combining prior art elements according to known methods (e.g., apply Bijl’s oil extraction methods) to yield

predictable results (e.g., obtain an oil with desirable traits such as high PUFA levels). MARA-1010, 38-42, 47-48, 202-17.

263. I also believe that a PHOSITA would have a reasonable expectation of success in combining Bijl with Komazawa. Bijl and Komazawa both relate to the creation of microbial lipids using culturing techniques. MARA-1008, 7:2-11; MARA-1015, [0012], [0116], FIG. 3. Both references teach the same sources of those microbial lipids: algae, fungi, dinoflagellates, and other diatoms. MARA-1008, 12:22-37; MARA-1015, [0004]; *see also* MARA-1018, [0569]-[0570] (Table 3); MARA-1020, 384-385. Furthermore, Bijl discloses that its processes are compatible with a wide range of natural sources, including *Thraustochytrium*, which are also disclosed in Komazawa. MARA-1008, 6:3-11; 13:19-31; MARA-1015, [0004], [0009]-[0011]. And both teach that the desired product is one of high enough quality for use in the human diet, including in infant formula. MARA-1008, 14:23-27; MARA-1015, [0060].

4. Analysis

Claim 1

[1pre] A microbial oil comprising

264. In my opinion, Bijl-Komazawa discloses and/or renders obvious [1pre].

265. 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. 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, like the ’959 patent, Bijl notes that “PUFA (oil) which has been extracted can be used” with or without further refining. MARA-1008, 9:20-22.

266. Additionally, I note that Komazawa teaches the production of DHA-containing oil from a microbe—*Thraustochytrium*—and therefore, it discloses a microbial oil as well.

267. Further, as I described above in Section I(E), extracted microbial lipids were well known to a PHOSITA prior to the Critical Date.

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

268. In my opinion, Bijl-Komazawa discloses and/or renders obvious [1a].

269. As described above in Section III(C)(6)(1a), Bijl discloses a microbial oil “which has a triglyceride content of greater than 90%.” MARA-1008, 2:30-33. Bijl discloses a triglyceride content greater than 95 wt.% and optionally higher than 97 wt.%. MARA-1008, 4:24-28.

270. Additionally, it is my opinion that a PHOSITA would also understand Komazawa to contain predominantly triglycerides because Komazawa teaches the

use of lipase. MARA-1015, [0021]. Lipase treatment is specific to triglycerides; other forms of fatty acids would require alternative enzymes such as phospholipase (for phospholipids), and in particular, fatty acids can be removed from phospholipids using enzymes such as phospholipase A₁ and A₂ which remove fatty acids from phospholipid *sn-1* and *sn-2* positions, respectively. Komazawa's specific disclosure of 1,3-selective lipase further indicates triglyceride-specific oils. MARA-1015, [0055]. This is because phospholipid fatty acids are not located at the *sn-3* position whereas triglyceride fatty acids are located at the *sn-1*, *sn-2*, and *sn-3* positions.

271. I note that other of Komazawa's disclosures also suggest predominantly triglyceride compositions to a PHOSITA. Komazawa describes its invention as "fat and oil," which to a PHOSITA predominantly refers to triglycerides (or neutral lipids). *See* MARA-1015, [0146]. If Komazawa meant to describe non-triglyceride fatty acids, Komazawa would have used terms such as "lipid," "total lipid," or "bound lipid" to refer to multiple lipid classes. But instead, Komazawa discloses DHA content *in triglyceride only*. MARA-1015, [0026], [0116], Fig.3. To a PHOSITA and to me, this implies that triglycerides are the main component of the fat and oil.

272. Indeed, I note that triglyceride-rich oils were also well known to a PHOSITA prior to the Critical Date. Consistent with the teachings in Bijl and

Komazawa, other references (e.g., Singh, Kralovec) also disclosed triglyceride-rich oils. MARA-1018, [0294] (“oil comprising at least 75% or 85% triacylglycerols...said triacylglycerols comprising the LC-PUFA”); MARA-1030, 35:20-27, 36:12-15, 37:22.

[1b] wherein the docosaheptaenoic acid content of the triglyceride fraction is at least 45% by weight,

273. In my opinion, Bijl-Komazawa discloses and/or renders obvious [1b].

274. In my opinion, a PHOSITA would have applied the teachings of using a preferred microbial strain (e.g., *thraustochytrid* strain LFF1), as taught by Komazawa, to Bijl’s oil to yield an improved oil composition having higher amounts of a desired PUFA (e.g., DHA). I also agree that a PHOSITA would have found it obvious that the DHA content of the triglyceride fraction is at least 45 wt.% in the proposed combination because Komazawa’s preferred strain LFF1 of *Thraustochytrium*, when cultured, produces an oil composition with greater than 60 wt.% DHA.

275. Additionally, I believe that a PHOSITA would generally have understood that *Thraustochytrium* microbes would have produced oil that was “preferably 50% by weight or more” DHA. MARA-1015, [0058], [0108]; MARA-1009, 27 (Table 2); 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). Moreover, Komazawa discloses that its culturing method can be used to produce oils having a “DHA component *in the*

triglycerides” with “a high content of 80% or more,” based on its culturing conditions. MARA-1015, [0116], FIG. 3. Because 80% DHA in the triglycerides is greater than 45%, Bijl-Komazawa teaches and/or renders obvious [1b].

[1c] and wherein the oil comprises 1.5% by weight or less of arachidonic acid.

276. In my opinion, Bijl-Komazawa discloses and/or renders obvious [1c].

277. As I discuss above in Section III(C)(6)(1c), I understand [1c] to be negative limitation, for which prior art disclosure is not necessary to meet the claim element.

278. Komazawa teaches an example of a “fatty acid composition of the extracted lipid” that contains no detectable amount of arachidonic acid. MARA-1015, [0112], Table 1 (showing 100% oils with no arachidonic acid). To the extent any arachidonic acid is present in Komazawa’s oil, it is below the limit of detection, which is lower than 1.5% based on the data in Table 1. Komazawa therefore discloses and/or renders obvious [1c].

279. As I also discuss above in Section III(A)(2)(1c), a PHOSITA would have understood that it was desirable to have low levels of arachidonic acid in a primarily DHA-containing oil because higher purity oils result in more efficient supplementation. Thus, a PHOSITA would have sought out microbial oils containing low amounts of arachidonic acid, as taught in Komazawa.

Claim 2

[2] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight.

280. For the reasons discussed in Section III(D)(4)(1b), it is my opinion that Bijl-Komazawa renders [2] obvious.

Claim 3

[3] The microbial oil of claim 1, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 80% by weight.

281. For the reasons discussed in Section III(D)(4)(1b), it is my opinion that Bijl-Komazawa renders [2] obvious.

Claim 4

[4] The microbial oil of claim 1 wherein said docosahexaenoic acid content of the triglyceride fraction is at least 90% by weight.

282. For the reasons discussed in Section III(D)(4)(1b), it is my opinion that Bijl-Komazawa renders [2] obvious. See MARA-1015, [0116] (stating that “DHA component in the triglycerides” can have “a high content of 80% or *more*,” based on its culturing conditions); *see also* MARA-1015, FIG. 3.

Claim 6

[6] The microbial oil of any one of claims 1 to 5, wherein said microbial oil further comprising an effective amount of at least one added antioxidant to provide oxidative stability.

283. In my view, Bijl-Komazawa discloses and/or renders obvious [6]. Bijl teaches that antioxidants, such as tocopherol or ascorbyl palmitate, can be added to compositions. MARA-1008, 26:16-18. Its composition may also contain

antioxidants. MARA-1008, 3:37-4:1. Bijl-Komazawa therefore renders [6] obvious.

Claim 7

[7] A food product composition for animals or humans, comprising the microbial oil of any one of claims 1 to 6.

284. In my view, Bijl-Komazawa discloses and/or renders obvious [7]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula.” MARA-1008, 9:28-10:8.

285. Komazawa also teaches that its oils can “be added to various foods and drinks, feed or bait. Examples of such foods include nutritional supplementary foods, modified milk for infants or premature babies, health foods, functional foods, baby foods, food for pregnant women, and foods for elderly people.” MARA-1015, [0060]. Bijl-Komazawa therefore renders [7] obvious.

Claim 8

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

286. In my opinion, Bijl-Komazawa discloses and/or renders obvious [8]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes

uses in “milk, health drinks, and bread,” as well as *infant formula*. MARA-1008, 9:28-10:8.

287. Komazawa also teaches that its oils can “be added to various foods and drinks, feed or bait. *Examples of such foods include* nutritional supplementary foods, modified *milk for infants or premature babies*, health foods, functional foods, *baby foods*, food for pregnant women, and foods for elderly people.” MARA-1015, [0060]. Bijl-Komazawa therefore renders [8] obvious.

Claim 9

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

288. In my opinion, Bijl-Komazawa discloses and/or renders obvious [9]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes uses in “*milk, health drinks*, and bread,” as well as *infant formula*. MARA-1008, 9:28-10:8.

289. Komazawa also teaches that its oils can “be added to various foods and *drinks*, feed or bait. *Examples of such foods include nutritional supplementary foods*, modified *milk* for infants or premature babies, health foods, functional foods, baby foods, food for pregnant women, and foods for elderly people.” MARA-1015, [0060]. Bijl-Komazawa therefore renders [9] obvious.

Claim 10

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

290. In my opinion, Bijl-Komazawa discloses and/or renders obvious [10]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula. MARA-1008, 9:28-10:8.

291. Komazawa likewise teaches that its oils can “***be added to various*** foods and drinks, ***feed*** or bait. Examples of such foods include nutritional supplementary foods, modified milk for infants or premature babies, health foods, functional foods, baby foods, food for pregnant women, and foods for elderly people.” MARA-1015, [0060]. Komazawa also teaches that its invention can be used in “livestock feeds for pigs or cows, poultry feeds for chickens, pet foods for dogs or cats, and feed for pisciculture.” MARA-1015, [0060]. Bijl-Komazawa therefore renders [10] obvious.

Claim 11

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

292. In my opinion, Bijl-Komazawa discloses and/or renders obvious [11]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula. MARA-1008, 9:28-10:8.

293. Komazawa also teaches that its oils can “be added to various foods and drinks, feed or bait. *Examples of such foods include nutritional supplementary foods*, modified milk for infants or premature babies, health foods, functional foods, baby foods, food for pregnant women, and foods for elderly people.” MARA-1015, [0060]. Bijl-Komazawa therefore renders [11] obvious.

Claim 12

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

294. In my opinion, Bijl-Komazawa discloses and/or renders obvious [10]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes uses in “milk, health drinks, and bread,” as well as infant formula. MARA-1008, 9:28-10:8. As to animals, the “granular particles or granules may find use as a food or *feed composition or supplement.*” MARA-1008, 4:13-18.

295. Komazawa also teaches that its oils can “*be added to various* foods and drinks, *feed* or bait. Examples of such foods include nutritional supplementary foods, modified milk for infants or premature babies, health foods, functional

foods, baby foods, food for pregnant women, and foods for elderly people.”

MARA-1015, [0060]. Komazawa also teaches that its invention can be used in “*livestock feeds* for pigs or cows, poultry feeds for chickens, *pet foods* for dogs or cats, and feed for pisciculture.” MARA-1015, [0060]. Bijl-Komazawa therefore renders [12] obvious.

Claim 13

[13] The animal feed of claim 12, wherein the animal feed is an aquaculture feed.

296. In my opinion, Bijl-Komazawa discloses and/or renders obvious [13]. Komazawa teaches that its invention can be used in “livestock feeds for pigs or cows, poultry feeds for chickens, pet foods for dogs or cats, and *feed for pisciculture.*” MARA-1015, [0060]. Komazawa also teaches that its invention may be used as “feeds for microorganisms (so-called animal plankton) that is *provided as bait for the aquafarming of seafood.*” MARA-1015, [0060]. Bijl-Komazawa therefore renders [13] obvious.

Claim 14

[14] The animal feed of claim 12, wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

297. In my opinion, Bijl-Komazawa discloses and/or renders obvious [14]. 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, 4:13-18, 14:23-27, 29:2-25, cls. 33-36. This includes

uses in “milk, health drinks, and bread,” as well as infant formula. MARA-1008, 9:28-10:8.

298. As to animals, the “granular particles or granules may find use as a food or *feed composition or supplement*.” MARA-1008, 4:13-18. Moreover, Komazawa teaches that its invention can be used in “*livestock feeds* for pigs or cows, poultry feeds for chickens, *pet foods* for dogs or cats, and feed for pisciculture.” MARA-1015, [0060]. Komazawa also teaches that its invention may be used as “feeds for microorganisms (so-called animal plankton) that is provided as bait for the aquafarming of seafood.” MARA-1015, [0060]. *Bijl-Komazawa* therefore renders [14] obvious.

IV. CONCLUSION

299. 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.

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