From:	Erica Cermak Intertek
To:	Morissette, Rachel; Hywel Griffiths
Cc:	West-Barnette, Shayla
Subject:	RE: follow-up to phone call for GRNs 000776 and 000777
Date:	Friday, August 24, 2018 11:41:30 AM
Attachments:	image002.png image007.png image020.png GRN 000776 and GRN 000777 supplement August 24 2018.docx Appendix 1 Comparative Fatty Acid Analysis – ITERG report.pdf Appendix 2 Processing Aid Certificates.pdf

Dr. Morissette,

On behalf of Fermentalg, we respectfully submit this additional information in support of GRAS Notifications 000776 and 000777 in response to your questions received by email on June 17, 2018. It is our belief that this additional information provided as part of this notification adequately addresses the majority of your questions. As noted in your email below, we anticipate receipt of the sterol analysis by close of business next Friday, August 31st, and will provide the remaining responses upon receipt of this data.
My contact information is provided below. Please feel free to again contact me by phone or e-mail if you have any questions regarding this information.

Thank you,

Erica Cermak
Manager, Regulatory and Toxicology - Food & Nutrition
Health, Environmental & Regulatory Services (HERS)

Direct +1 908-290-7201
Skype erica.cermak.intertek
www.intertek.com

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Intertek, New Jersey, USA

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, August 24, 2018 10:15 AM
To: Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>; West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: follow-up to phone call for GRNs 000776 and 000777

Dear Dr. Griffiths,

Thank you for your phone call today to discuss the status of the responses to our questions for GRNs 000776 and 000777. You mentioned that the reason for the delay in responding to our questions is because the laboratory that you hired to test the sterols failed to provide those results in a timely manner; therefore, you have contracted with a separate company to perform those analyses. You mentioned that you can send the responses to the other questions now, excluding the sterol analyses, but that you anticipate having the sterol results by close of business (EST) next Friday, September 31. I agreed that sending what you have now would be best, with the expectation that we will receive the sterol response next week. If something changes, I requested that you contact Dr. Shayla West-Barnette, as I will be away next week. She will alert the review team and advise you on the next steps. You also mentioned that you would be amenable to withdrawing these notices should that become necessary. Please let me or Shayla know if you have any questions. We appreciate your keeping us apprised of the situation as it unfolds. I will look for your initial responses today. Please cc Dr. West-Barnette on that email as well.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION [fda.gov]

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Friday, August 24, 2018 9:51 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: Re: response requested for GRNs 776 and 777

Dear Dr Morissette,

Do you have time for a quick 5 minute call? If so is there a number on which I could reach you?

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

Fermentalg

On 24 Aug 2018, at 2:11 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dr. Griffiths,

I am following up on our conversation from last week. I have not received the responses to our questions for GRNs 776 and 777. Are you still planning to submit those responses by COB today? Withdrawing your notices and resubmitting them as I outlined below is still an option. If I do not hear back from you, we will need to assume that you are not planning to respond and will proceed with drafting no basis letters for these GRAS notices. Please let me know your intentions as soon as possible. I will be out of the office all next week. Dr. Shayla West-Barnette will be handling this matter while I'm away. Please cc her on all correspondence starting at 3 pm today EST. Email email address is Shayla.westbarnette@fda.hhs.gov. I hope to hear from you today about your intentions for these GRAS notices so that we can meet the 180-day mark.

Regards,

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Morissette, Rachel
Sent: Thursday, August 16, 2018 11:06 AM
To: 'Hywel Griffiths' <hgriffiths@fermentalg.com>
Subject: RE: questions for GRNs 000776 and 000777 (DHA algal oil)

Hi Hywel,

Thanks for your reply. Since we are already four weeks out from receipt of the questions, and typically 10 business days is the allowable time frame for responses from notifiers, early next week is preferable. If you don't think you'll be able to meet that timeframe, we'll have to discuss other options at this point, including withdrawing the notices and resubmitting revised versions that incorporate the questions that were raised in these notices, if necessary. I'll look out for your email next week.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Thursday, August 16, 2018 10:57 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: Re: questions for GRNs 000776 and 000777 (DHA algal oil)

Hi Rachel,

Thanks for your email. As you will have gathered from my out of office, I was away on vacation until today. The time it will take to review the response prepared by Intertek and check that we've collated all the data requested means I'm targeting next week for the reply. I hope this is acceptable.

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

<image007.png>

Tel. +335 57 250 252 | Mobile +337 61 33 37 96 | www.fermentalg.com [fermentalg.com] | Fermentalg – 4 Rue Rivière – 33500 Libourne |

> On 14 Aug 2018, at 8:48 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Hi Dr. Griffiths,
>
> I just wanted to check in and see when you anticipate sending your responses to our questions for GRNs 000776 and 000777?
>
> Thanks,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

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rachel.morissette@fda.hhs.gov

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Wednesday, July 18, 2018 11:31 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>
Subject: Re: questions for GRNs 000776 and 000777 (DHA algal oil)

Dear Ms Morissette,

Thank you for the letter. We will attempt to answer all questions within 10 business days, although with it already being holiday season in France we may have to ask for an extension for some of the questions requiring detailed technical responses.
In copy of this email is Erica Cermak of Intertek who was involved in the construction of the notifications and who may communicate on our behalf.

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

<image008.png>

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> On 17 Jul 2018, at 9:34 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dr. Griffiths,

Please see attached a letter with questions to be addressed for GRNs 000776 and 000777 (DHA algal oil). We request a response within 10 business days. Please let me know if you have any questions at this time.

Best regards,

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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<7-17-18 GRN776_777 Questions for Notifier.pdf>

1) For both GRNs 000776 and 000777, please clarify the intended use to indicate that the DHA oil will be used with a safe and suitable source of arachidonic acid (ARA) within the range indicated from 1:1 to 1:2 DHA:ARA.

Fermentalg confirms that its DHA algal oil is intended for use as a direct ingredient in exempt (pre-term) and nonexempt (term) infant formula (ages from birth to 12 months), in accordance with current good manufacturing practices (cGMP), and in combination with a source of arachidonic acid (ARA). The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is similar to all other approved uses for incorporation of DHA in infant formula.

2) For both GRNs 000776 and 000777, the DHA algal oil derived from *Schizochytrium* sp. that is the subject of these notices does not meet the specifications for fatty acid levels (with the exception of DHA), expressed in area %, as listed in the Food Chemicals Codex (FCC) 11 monograph for this ingredient. Please address this issue for both GRNs.

The upper and lower limits of fatty acids present in the FCC monograph are narrow and inconsistent with the values reported in GRAS Notices for other DHA algal oils intended for use in infant formula. In several cases, the levels of various fatty acids present in these oils likewise fall outside the ranges specified in the monographs.

Fatty acid	**Shorthand notation**	**Lower Limit (area %)**	**Upper limit (area %)**	**Martek DHASCO-B Lot # 08-6530 (GRN 553)**	**Mara Renewables Lot #16039 (GRN 677)**	**Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-1324 Lot # NF1 (GRN 776)**	**Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-3204 Lot # ITE_17_001 (GRN 777)**
Dihomo-gamma-linolenic acid	20:3 n-6	1.7	2.8	< 0.1	<0.1	0.1	0.1
Arachidonic acid	20:4 n-6	0.6	1.3	0.67	0.75	0.3	0.1
Eicosapentanoic acid	20:5 n-3	1.3	3.9	5.90	1.08	0.2	0.5
Docosapentaenoic acid	22:5 n-6	10.5	16.5	2.63	7.21	8.3	10.3
Docasahexaenoic acid	22:6 n-3	30	40	44.3	37.10	38.2	59.8

Fermentalg's oils are not intended as a source of dihomo-gamma-linolenic acid. With regards to arachidonic acid, although Fermentalg's oils are below the FCC monograph, this fatty acid will be added to infant formula separately as noted in the response to Question #1. Manufacturers will compensate to achieve the desired ratio in the finished product. Recommendations are to use less EPA than DHA in infant formula, as a result, levels below the monograph minimum are not considered to be detrimental. DHA550 is higher than FCC11 specification for DHA, but since incorporation of these oils into infant formula is by weight of DHA and not by weight of oil this autocontrols.

It is likely that the limits included in the monograph were representative of the oil produced by the industry member that originally petitioned FCC for the monograph and, as demonstrated in Table 1, are not

representative of all currently available DHA algal oils derived from *Schizochytrium*, including those marketed for use in infant formula.

3) In GRN 000776, Fermentalg provides a comparison of the fatty acid content of its oil with Martek's GRN 000137 oil. Fermentalg also notes:
"The substantial equivalence of Fermentalg's oil is supported by the decision of the Food Safety Authority of Ireland (FSAI), which considered Fermentalg's DHA 350 to be substantially equivalent to the Martek Biosciences Corporation's oil in terms of composition, nutritional value, metabolism, intended use and level of undesirable substances as set out in Article 3.4 of the novel food Regulation EC No 258/97 (EC, 1997; FSAI, 2014)."
However, the intended uses considered in GRN 000137 and by FSAI did not include infant formula. Please compare Fermentalg's DHA algal oil to oils currently used in infant formula or oils that were the subject of relevant developmental and clinical studies cited in the notice in order to support the claim of "substantial equivalence" for use in infant formula. If there are appreciable differences in the concentration of one or more fatty acids between Fermentalg's DHA algal oil and other oils used in infant formula or studies, explain why such differences are not a safety concern by discussing the absorption/distribution/metabolism/excretion of fatty acids and the requirement of fatty acids in infants, including pre-term infants.

As GRN 000137 did not include use in infant formula, Fermentalg presents the Table 2 below comparing the fatty acid profile of other DHA oils derived from *Schizochytrium* that were notified for use in formula, namely the oil produced by DSM Nutritional Products (GRN 000553) and the oil produced by Mara Renewables (GRN 000677).

Fatty Acid	DSM DHASCO-B Lot # 08-6530 (GRN 553)	Mara Renewables Lot #16039 (GRN 667)	Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-1324 (DHA 350) Lot # NF1 (GRN 776)	Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-3204 (DHA 550) Lot # ITE_18_023 (GRN 777)
12:0	<0.1	0.92	0.2	0.1
14:0	1.30	12.30	4.0	1.1
14.1	<0.1	<0.10		0.3
15:0	0.24	0.68		0.1
16:0	13.9	22.67	42.8	18.7
17:0	<0.1	0.15		0.1
16:1 n-7	<0.1	6.16	0.1	0.2
17:0				0.1
17:1				0.1
18:0	1.64	0.77	1.1	0.7
18:1 n-9	24.5	7.49	0.6	0.7
18:1 n-7	0.22		Sum with oleate	
18:2 n-6	2.05	0.34	0.8	0.1
20:0	0.31	<0.1		0.1
18:3 n-3	0.10	0.24		0.2
18:3 n-6				0.1
18:4 n-3		0.24	0.2	0.3
20:1 n-9	<0.1	<0.1		
20:2 n-6	0.12			
20:3 n-6	<0.1	<0.1	0.1	0.2
22:0	0.32	<0.1		0.1
20:3 n-3	<0.1			
20:4 n-6	0.67	0.65	0.3	0.1
20:4 n-3			0.5	0.6
20:5 n-3	5.90	1.08	0.2	0.6
24:0	0.12	<0.1		0.1
22:1				0.9
22:2 n-6	0.54			
24:1 n-9	<0.1			0.1
22:4 n-3	<0.1			
22:4 n-6				
22:5 n-6	2.63	7.21	8.3	11.2
22:5 n-3			0.1	0.2
22:6 n-3	44.35	37.10	39.2	63.1

As shown in Table 2, the overall composition of DHA350 is similar to that of Mara Renewable's oil (GRN 000677). With the exception of some fatty acids present at low levels (≤1%), Fermentalg's oils do not contain fatty acids not present in other sources of DHA algal oils used in infant formulas. These other fatty acids are naturally present in either human breast milk (Yuhas *et al.* 2006) or infant formula components such as cow's milk (Blaško *et al.* 2010).
DHA550 contains similar fatty acids to the other oils but the polyunsaturates are present at higher levels, whereas the saturates are reduced. The ratio of DHA:DPAn-6 and DHA to other polyunsaturates is very similar between DHA550, DHA350 and Mara Renewable's oil. Levels of incorporation of the oil into infant formula are mandated by DHA level and not oil level and so the levels of polyunsaturates provided from DHA550 will be nearly identical to that provided by DHA350 and Mara Renewable's oil. The higher level of DHA in DHA550 will result in the use of less oil and lower levels of saturates incorporated from the DHA550. However, the milk component, not the DHA oil, is the major source of lipids in infant formula.

DHA, along with the other fatty acids present in Fermentalg's DHA 350 and DHA 550, are present in breast milk. The triglyceride structures of the fatty acids that are chemically equivalent to those delivered to infants from mother's milk, and as a result, the metabolic fate of the fatty acids present in DHA 350 and DHA 550 is similar. As reviewed by Kroes *et al.* (2003), dietary triglycerides, regardless of the source, undergo enzymatic hydrolysis in the upper intestine to free fatty acids and 2-monoglycerides, which are integrated into bile acid micelles for diffusion into the interior of the intestinal epithelial cells and subsequent incorporation into new or reconstituted triglycerides.

Comprehensive summaries of the clinical study literature regarding DHA or long-chain polyunsaturated fatty acids (LC-PUFA) relevant to supplementation of infant formula from fish and algal oil sources have been included in previous GRAS Notices (FDA, 2011, 2014, FDA 2015). As reviewed in GRN 553 (US FDA, 2015a) , there are no adverse effects associated with 1% DHA in infant formulas, with some studies suggesting a benefit to stature and body composition. Preterm infants fed 1.0% DHA and weighing ≥ 1250 g at birth were up to 1.7 cm longer at 18 months corrected age compared to infants fed 0.35% DHA. In those weighing <1250 g at birth, head circumference was greater in response to 1% DHA at expected delivery date (Collins *et al*., 2011). Pittaluga and co-workers (2011) note that preterm infants fed 0.25-0.12% DHA throughout the first year of life are leaner and have lower fasting insulin levels at 1 and 2 years of age than infants consuming formula devoid of tong chain polyunsaturated fatty acids (LCPUFA). Data on supplementation of DHA up to 1% in term and preterm infants appears to improve certain cardiovascular and respiratory health outcomes. Improvements in visual acuity and mental acuity have also been reported in infants fed DHA supplemented formulas.

4) Please provide the source of the data presented in Table 2.4.4-1 on pp. 12-13 of GRN 000776 for "Martek Oil Analysis (Composition by Area %)". The data does not appear to match the composition data for fatty acids provided in GRN 000137. The mean reported for 20:4 n3 appears to be in error compared to the individual data points. Please confirm these values.

The fatty acid composition performed on Fermentalg's DHA-rich oil and on the approved Martek DHA-rich oil presented in table 2.4.4-1 were analyzed for Fermentalg at an accredited external laboratory located in Europe (ITERG, the French Institute for Fats and Oils). Results of this analysis are provided as Appendix 1 to this response. This was an analysis performed by the same laboratory at the same time as the Fermentalg batches NF1-NF3 on a sample of oil obtained commercially. Part of the difference in values between Table 2.4.4-1 of our notice and Table 3 of GRN000137 may arise from the different methods of expressing content (the first is as % area – effectively the % of total fatty acids, the second is as mg/g which generally gives a lower value (x10) since it takes into account other components of the oil such as unsaponifiables).

Furthermore, while Fermentalg cannot comment on other differences in detail since this is not our oil, there has been a tendency within the industry towards higher and higher DHA contents in basic oils, and subsequent dilution with high-oleic sunflower oil back to specification. This may explain the presence of higher levels of C18:1 and C18:2.

The mean value for 20:4n3 should be 0.4 rather than 0.2 as originally indicated in the Notice.

5) Fermentalg provides a comparison of the sterol content of its GRN 000776 DHA algal oil with DSM Nutritional Product's (GRN 000553) oil and Mara Renewables Corporation's (GRN 000677) oil. For GRN 000776, there is a statement in the notice on p. 14 as follows:

"…the slight differences in the relative proportions of various sterols between Fermentalg's DHA350 and other DHA oil products are not expected to be [sic] affect safety."
Table 2.4.4-3 shows that (1) the level of total sterols in Fermentalg's DHA algal oil is higher than the total sterols in the GRN 000553 oil (0.56% w/w) and the GRN 000677 oil (0.23% w/w), and (2) the major sterols are not the same for GRNs 000776, 000553, and 000677. Please provide additional discussion and references to support the conclusion that these differences are not a safety concern for the intended use of Fermentalg's DHA algal oil in infant formulas for term and pre-term infants. Further, only a single batch analysis for sterols was reported for GRN 000776, with the comment that it is a representative batch. Please provide the results of a minimum of three non-consecutive batch analyses for sterols in order to characterize the sterol content of Fermentalg's DHA algal oil and to show typical levels of individual and total sterols.

Response to follow pending receipt of additional analytical data.

6) Sterols are not addressed in GRN 000777 beyond a general comment that there are:

"…slight differences in the relative proportions of various sterols which are not expected to be affect [sic] safety."
Please provide the results of sterol analyses from three non-consecutive batches for the DHA algal oil that is the subject of GRN 000777 and provide additional discussion explaining the aforementioned statement.

Response to follow pending receipt of additional analytical data.

7) Fermentalg does not provide a comparison of the fatty acid or sterol content of its GRN 000777 DHA algal oil with the *Schizochytrium* sp.-derived DHA algal oils that were the subjects of published studies cited in the notice. Please provide this comparison and a discussion comparing the identity of the subject of GRN 000777 to *Schizochytrium* sp.-derived DHA-algal oils currently used in infant formulas for term and pre-term infants or oils that were the subject of relevant developmental and clinical studies cited in the notice.

Response to follow pending receipt of additional analytical data.

8) For both GRNs 000776 and 000777, Fermentalg notes the trade name of the antifoam reagent. Please provide the chemical identity of the antifoam reagent and a statement regarding its safety for the intended use.

Biospumex 153K is a proprietary mix of modified polyalkoxyesters which are nonionic and contain no silicone. The product is used in a wide range of food processes including fermentation and extraction. A data sheet and certificate regarding its safety in the production of foodstuffs are included in Appendix 2.

9) For the optional clarification by filtration step, please cite an effective Food Contact Notification or relevant regulation for the filtration material.

If filtered, the oils are mixed with a filter-aid Clarcel DICB (a diatomaceous earth) and then filtered on a Fibrafix filter plate (bleached cellulose and perlite). Certificates confirming the suitability of these materials for contact with foods are included in Appendix 2.

10) On p. 9 of GRN 000776, the method of manufacture flow chart shows refining (discoloration/deodorization) as a single step. Please clarify the following: Is the oil degummed before refining? Is the oil alkali refined? Is the oil subjected to a separate bleaching step or is it partially decolorized by heat bleaching during the deodorization process? If bleaching clay or other material is used, please briefly describe.

No degumming step is carried out on the oils as the levels of gums are sufficiently low that they can be removed during the bleaching step without need for a separate process. The bleaching step is carried out prior to, and separately from the deodorization step. Bleaching earths Trisyl and Tonsyl are used. Certificates confirming the suitability of these materials for contact with foods are included in Appendix 2

11) The enzyme used when extracting the DHA algal oil was not identified in GRN 000777, but was identified as "alcalase" (from Novazyme) in GRN 000776. We note that this appears to be a trade name. Please indicate the accepted or recommended name of this enzyme, including the production organism and EC or CAS number for this enzyme. Is the same enzyme used in the production of both GRNs 000776 and GRN 000777 DHA algal oils? Is this the same enzyme preparation that was described in GRN 000667 (and used in accordance with 21 CFR 184.1027)?

The enzyme is a protease (subtilisin) produced by *Bacillus licheniformis*. The CAS number is 9014-01 -1 and It is used in accordance with 21 CFR § 184.1027. It would appear to be the same enzyme used in GRN000667.

12) Please provide a brief statement about the removal or inactivation of the alcalase enzyme after it is used to extract the DHA algal oil from the biomass.

The vast majority of the (water soluble) enzyme is expected to be separated from the oil immediately after the lysis reaction along with the cellular material and aqueous fractions. Any proteinaceous compounds remaining associated with the crude oil are removed during the standard processes of refining, but if any doubt remains, the enzymatic activity would be destroyed by the elevated temperatures to which the oil is exposed during deodorization.

13) Fermentalg states that there are no reports of toxin production by the *Thraustochytriaceae* family, but they provide the results of analysis for a number of algal toxins, including: azaspiracids, pectinotoxins, yessotoxin, okadaic acid, domoic acid, diarrhetic shellfish poison, and paralytic shellfish poison. GRN 000137 noted that two toxic compounds are produced in Chromista (domoic acid in *Pseudo-nitzschia* and prymnesin in *Prymnesium* spp.). Please comment on the reasoning behind the selection of algal toxins that Fermentalg presented in GRNs 000776 and 000777. Are they all lipophilic? Are they resistant to removal by common oil refining processes? Please provide a brief

statement on the method(s) of analysis for these toxins in GRN 000776 (this information is found in GRN 000777, but not GRN 000776).

The toxins tested are the complete list of toxins in the standard tests for seafood and analysis was performed by an independent laboratory (Eurofins) using method BVL L 12.03/04-4 of the German Federal Office of Consumr Protection and Food Safety

The complete range was tested for completeness but none would be expected a) to be found in the production organism or b) to be resistant to the refining process.

14) PCB and dioxin analyses are presented in GRN 000777. We would not anticipate finding these substances in algal oil produced in accordance with current good manufacturing practices. Please provide the reasoning behind including these analyses and whether Fermentalg expects to find these substances in the final product.

Fermentalg does not anticipate finding these substances in the algal oil, but wished to confirm and demonstrate this fact. Fermentalg will not be analyzing each batch for the absence of these contaminants, but will continue to perform periodic verification as this is a standard demand of the market place

15) For both GRNs 000776 and 000777, please verify that validated analytical methods were used for the "internal methods" mentioned for arsenic, mercury, and cadmium specifications.

The analytical methods used for the analysis of arsenic, mercury and cadmium were based on the method EN NF 15763 (French Standards Agency) but minor variation from the Standard cause this to be noted as a "internal method". The subcontractor who carried out the analyses validated that the deviation from the Standard did not significantly impact the result.

16) The FCC 11 monograph includes limits for anisidine value (NMT 20.0), free fatty acids (NMT 0.4%), and total oxidation value (NMT 26). For both GRNs 000776 and 000777, what is the limit for free fatty acids in Fermentalg's DHA algal oil? Are the limits in the FCC monograph for anisidine value and total oxidation met?

Specifications for the anisidine value (NMT20.0) and total oxidation (NMT 26) defined by the FCC monograph are respected. Our specification for free fatty acids (Acid value of NMT 0.5mg KOH/g, which is roughly equivalent to 0.25% free fatty acids) is ~~more strict~~stricter than the monograph.

17) While stability data is provided in GRN 000776, it is not provided in GRN 000777. Rather, there is a statement in GRN 000777 that the stability analysis is ongoing. Also, Fermentalg notes:

"Due to the high level of DHA present in DHA 550, this oil may be sensitive to oxidation compared to other available algal oils; however, under proper packaging and storage conditions, exposure to oxygen is limited and this should not present a significant real-world risk."
Please provide data from the accelerated storage studies for GRN 000777 if they are available.
Please provide a statement that total oxidation products arising from the use of this DHA algal oil are the same as those for other DHA-containing algal oils currently used in infant formula.

Preliminary data for the accelerated storage study of three batches of DHA 550 are provided in Table 5. Product remains within specification after 4 months in accelerated conditions, which is the recommended shelf-life at 4°C. Results at -20°C (the preferred storage temperature) appear consistent with a shelf-life of at least 12 months based on comparison with other commercial algal oils.

Batch	Parameter	Specification	Start	Accelerated stability studies carried out at 25°C and 60% RH			Study at -20 °C
			Time (weeks)				
			0	8	14	16	16
0403019	DHA (%)	Min 55	71.4	67.7	67.1	69.3	69.3
	Peroxide Value (meq KOH/g)	< 5	<1	< 1	< 1	< 1	< 1
	Anisidine Value	< 20	3.9	10.7	6.8	12.5	5.1

Batch	Parameter	Specification	Start	Accelerated stability studies carried out at 25°C and 60% RH			Study at -20 °C
			Time (weeks)				
			0	8	14	16	16
0413022-A	DHA (%)	Min 55	72.1	69.4	69.4	70.5	70.9
	Peroxide Value (meq KOH/g)	< 5	<1	1.5	1	<1	1.1
	Anisidine Value	< 20	2.2	6.4	3.1	3.6	2.8

Batch	Parameter	Specification	Start	Accelerated stability studies carried out at 25°C and 60% RH			Study at -20 °C
			Time (weeks)				
			0	8	14	16	12
041028-A	DHA (%)	Min 55	68.7	67.1	67.9	-	68.0
	Peroxide Value (meq KOH/g)	< 5	2.2	2.0	<1	-	1
	Anisidine Value	< 20	4.6	6.8	7.0	-	2.8

The limits for total levels of oxidation products per gram of oil for the product of GRN 000777 are the same as those for other DHA-containing algal oils currently used in infant formula. Given that less oil will be used to provide the same amount of DHA, actual exposure to oxidation products could be reduced.

18) For both GRNs 000776 and 000777, Fermentalg provides an estimate of intake based on the percent DHA. Please provide an estimate of intake of the total DHA algal oil ingredient, based on the range of DHA in the oil. We note that no upper limit is indicated for the DHA algal oil ingredients, but this information would be relevant for an exposure estimate.

As noted in GRN 000776 and GRN 000777, it is assumed that infants consume about 100 to 120 kcal/kg body weight (bw)/day, of which fat constitutes approximately 50% of calories, or approximately 5.5 to 6.7 g fat/kg bw/day (1 g of fat is equivalent to 9 kcal). Assuming incorporation of the proposed DHA ingredient at a maximum use level of 0.5% of fatty acids, the intake of DHA would be 27 to 33 mg/kg bw/day. These levels would be associated with exposure to 77-94 mg of DHA350 and 49-60 mg of DHA 550

19) Please provide an estimate of total sterol intake from the intended uses in GRNs 000776 and 000777. Please discuss how these estimates compare to the exposure from consuming *Schizochytrium* sp. oils that are the subjects of published studies cited in GRNs 000776 and 000777 that are relevant to infant formula uses in term and pre-term infants.

Response to follow pending receipt of additional analytical data.

20) For GRN 000776, please provide more background information on the *Schizochytrium* sp. organism, including whether or not *Schizochytrium* sp. FCC-1324 is pathogenic or toxigenic.

The taxonomic classification of the source organism is as follows:
Kingdom: Chromista
Phylum: Bigyra
Class: Labyrinthulea
Order: Thraustochytriida
Family: Thraustochytriaceae
Genus: Schizochytrium

Schizochytrium is a genus of unicellular protist that belongs to the *Thraustochytriaceae* family. Initially, this family was composed of seven genera (*Althornia*, *Aplanochytrium*, *Diplophrys*, *Japonochytrium*, *Schizochytrium*, *Thraustochytrium* and *Ulkenia*). Recent studies based on genetic and phenotypic analysis proposed changes in the classification, with the erection of new genera like *Botryochytrium*, *Parietichytrium* and *Sicyoidochytrium*, emended from *Ulkenia* or *Aurantiochytrium* and *Oblongichytrium* emended from *Schizochytrium* (Yokohama, Honda 2007; Yokohama, Salleh, Honda 2007).

Fermentalg collected a *Schizochytrium*-related strain in estuarine environment and undertook a characterization at a genetic and biochemical level. This study revealed that this strain (FCC-1324) could be assigned to the genus *Schizochytrium*. An example of a phylogenetic tree that has been constructed by comparison of sequences of the small subunit of ribosomal DNA (18S SSU-rDNA) is depicted on figure 1.

There are no reports of pathogenicity or toxigencity associated with *Schizochytrium* FCC-1324 or the other related *Schizochytrium* strains used in the production of DHA algal oils.

21) For GRN 000777, please provide more background information on the Schizochytrium sp. organism, including whether or not Schizochytrium sp. FCC-3204 is pathogenic or toxigenic.

Schizochytium strain FCC-3204 is a natural variant of the strain FCC-1324, from which the DHA350 oil is produced. FCC-3204 was selected without the use of any mutagenic agents and has not been subjected to any form of deliberate genetic modification. The taxonomic classification of the source organism is as follows:
Kingdom: Chromista
Phylum: Bigyra
Class: Labyrinthulea
Order: Thraustochytriida
Family: Thraustochytriaceae
Genus: Schizochytrium

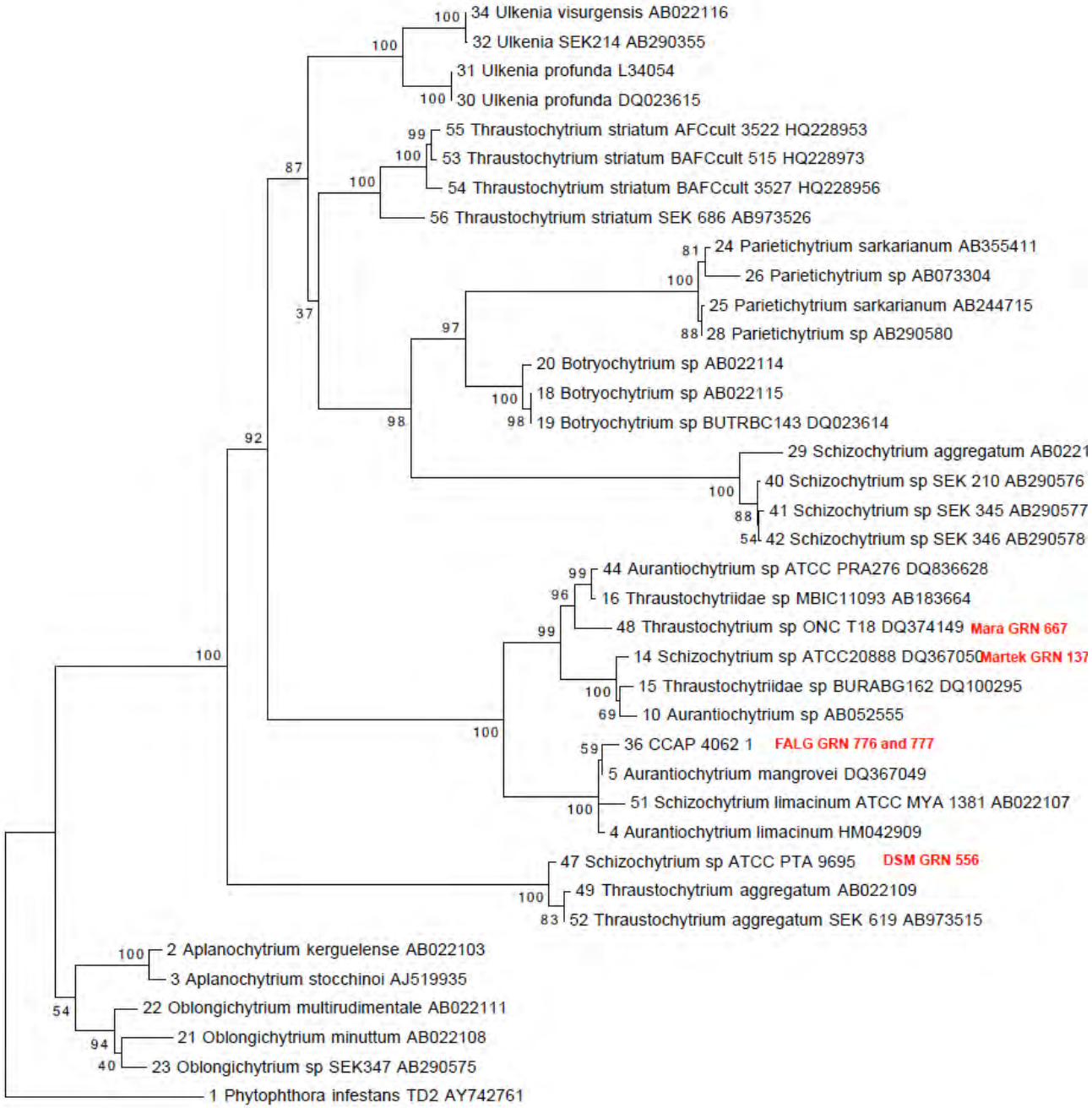
There are no reports of pathogenicity or toxigencity associated with *Schizochytrium* FCC-3204 or the other related *Schizochytrium* strains used in the production of DHA algal oils.

22) For both GRNs 000776 and 000777, please provide a narrative describing how the *Schizochytrium* sp. strains used in the toxicity studies you presented can be used to support the safety of your specific strains. The strains from your GRAS notices were not used in the mentioned toxicity studies and the connection between your strains and the published studies is not clear.

The source microalgae for all of these oils, *Schizochytrium*, are thraustochytrids, members of the kingdom Chromista (stramenopiles) which includes the heterokont algae. *Schizochytrium* sp. occurs widely in the aquatic environment and is an indirect component of the human food chain through indirect consumption of fish and other marine animals which feed on the microalgae. Figure 1 shows the phylogenic relationship between the strains used to produce other GRAS Notified DHA algal oils and the subject of the previous GRAS Notices covering infant formula.

The close taxonomic relationship between these species of micro-algae and Fermentalg's *Schizochytrium* strains is further evidenced by the close compositional similarity of the oil products derived from them. In addition, DHA 350 and DHA 550 are highly purified oils. Proximate analysis demonstrates that Fermentalg's DHA 350 is free from protein and carbohydrate (limit of detection of 0.1%), indicating that the remnants of production organism are not present in the purified oils.

Figure 1 **Updated Phylogeny of *Aurantiochytrium, Schizochytrium, Sicyiodochytrium* and *Thraustochytrium* Genera, Collectively Referred to as *Schizochytrium***



23) Please clarify how the presented safety studies in both GRNs 000776 and 000777 apply to the safe use of DHA algal oil in pre-term infant populations. Please specify which studies support the safe use of DHA algal oil in pre-term infants.

GRN 000776 and GRN000777 do not provide detail related to the safety of DHA algal oil in pre-term infants. However, such studies are available. For example, Carlson *et al.* (2013) reported no safety concerns following consumption of a marine algae-oil source of DHA (600 mg/day) from < 20 wk of gestation to birth. However, beneficial effects were observed. Specifically, DHA supplementation resulted in higher maternal and cord RBC-phospholipid-DHA, longer gestation duration (and greater birth weight, length, ad head circumference. In addition, there were fewer infants born at <34 wk of gestation and shorter hospital stays were required for infants born preterm in the DHA group compared to the placebo group.

Similarly, Clandinin *et al.* (2005) evaluated safety and benefits of feeding preterm infants formulas containing docosahexaenoic acid (DHA) and arachidonic acid (ARA) in pre-term infants. Preterm infants (n=361) were randomized across three formula groups: (1) control, no supplementation; (2) algal-DHA (DHA from algal oil, ARA from fungal oil); and (3) fish-DHA (DHA from fish oil, ARA from fungal oil). Term infants breast-fed <4 months (n = 105) served as a reference group. Infants receiving formula with algal DHA weighed significantly more than the control group from 66 to 118 weeks postmenstrual age (PMA) and significantly more than infants in the fish-DHA group at 118 weeks PMA. Likewise, the algal-DHA group was significantly longer than the control group at 48, 79, and 92 weeks PMA and the fish-DHA group at 57, 79, and 92 weeks. Weight and length were comparable to term infants. In addition, supplemented groups had higher Bayley mental and psychomotor development scores at 118 weeks PMA compared to the control group. Supplementation did not increase morbidity or adverse events. There were no differences in caloric intake from formula, daily gastric residuals, stool frequency, stool consistency, or abdominal distention among the preterm groups during hospitalization. Likewise, there were no differences among preterm groups with respect to parental reports of fussiness, diarrhea, or constipation. There were no adverse effects on hematology, serum glucose, cholesterol, high-density lipoproteins, triglyceride, mineral, and electrolyte measurements; and liver and kidney function tests. As noted in this supplement, the DHA algal oils used in these studies is comparable to that of Fermentalg's oils.

24) In both GRNs 000776 and 000777, two different dates are provided for when an updated literature search was conducted. Please clarify the correct date through which an updated literature search was done for these two notices.

Both GRN 000776 and 00077 state that the published scientific literature was reviewed in several previous GRAS Notices through May of 2017. In addition to reviewing information contained in these GRAS Notices, Fermentalg performed an updated search of the published scientific through August 2017.

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Collins CT, Makrides M, Gibson RA, et al. (2011). Pre- and post-term growth in pre-term infants supplemented with higher-dose DHA: a randomised controlled trial. Br J Nutr 105(11):1635-1643

Kroes R, Schaefer EJ, Squire RA, Williams GM. (2003) . A review of the safety of DHA45-oil. Food and Chemical Toxicology 41:1443-1446.

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U.S. FDA (2016 GRN 000677 [*Docosahexaenoic acid oil produced in Schizochytrium sp., Dartmouth (Canada) Mara Renewables Corporation.* Available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm530169.pdf

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Appendix 1 Comparative Fatty Acid Analysis – ITERG report

Appendix 2 Processing Aid Certificates

BIOSPUMEX 153 K

Technical Data Sheet

BPX0001

Revision date: 15/11/2017 Supersedes TDS of : 23/06/2017 TDS version: 1.5

DESCRIPTION

Defoamer BIOSPUMEX 153 K is a blend based on polyether polyol and a natural fatty acid.

PHYSICO-CHEMICAL HAZARD DATA

Appearance	Viscous Colourless,light yellow
Relative density	≈ 1
Viscosity, dynamic	≈ 800 mPa.s 20°C
Solubility	In water, the material disperses.
Active matter	< 100 %

APPLICATION

Defoamer BIOSPUMEX 153 K is recommended to cure the foaming problems in aqueous media.
It can be used in various processes such as:
- Sugar
- Yeast

SAFE HANDLING ADVICE

Our technical team is at your disposal to optimize the point of introduction and dosage.
It can be implemented continuously or localy, either manually operated or by metering pump.
The expected maximum dose is of 80 g/T cossettes for transforming sugar beets in white crystallised sugar.
In general it is advisable to use it at 50 to 500 ppm for fermentation process. For other process at a level not higher than is necessary to achieve the intended purpose.

ADDITIONAL TECHNICAL DATA

The French order dated 19th October 2006 regarding use of processing aids in foodstuffs manufacture allows components of BIOSPUMEX 153 K mixture to be used as defoaming agent for processing :
yeast
sugar

PURITY CRITERIA	Yes	Heavy Metals : Pb<5ppm, As<1ppm, Cd<1ppm, Cr<1ppm, Hg<1ppm
	Yes	Residual monomers (EO+PO) <25ppm
CONFESSIONAL STATUTE	Yes	Kosher product: only upon request
	Yes	Halal product: only upon request
	Yes	This product doesn't contain ingredients of animal origin (including oils, grease or gelatin) or ethyl alcohol.
CONTAMINANTS	Yes	Do not contain BSE/TSE
	Yes	Do not contain pesticides.
	Yes	Have not been treated by ionizing radiation.
	Yes	Doesn't contain nanomaterial according to definition in recommandation 2011/696/EU.
GMO STATUS	Yes	Does not contain any genetically modified organism and is not produced from genetically modified organisms.

ALLERGEN STATUS	PRESENCE	CROSS-CONTAMINATION
Cereals containing gluten	No	No

Crustaceans and products thereof	No	No
Eggs et products thereof	No	No
Fish and products thereof	No	No
Peanuts / Groundnut and products thereof	No	No
Soybeans and products thereof	No	No
Milk and products thereof	No	No
Nuts and products thereof	No	No
Celery and products thereof	No	No
Mustard and products thereof	No	No
Sesame and products thereof	No	No
Sulphur dioxide and sulfites >10 ppm	No	No
Lupin and products thereof	No	No
Molluscs and products thereof	No	No

HANDLING AND STORAGE

Before use, it is recommended to read the safety data sheet.
Protect from freeze. Store in dry, cool, well-ventilated area.
After a long storage time a little phase displacement could appear. Original properties could be recovered by simple mixing. Shelf life : 2 years

PACKAGING

- Bulk
- Container of 1000 litres
- Drums of 200 litres
- Can of 25 litres

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cognis
www.cognis.com

functionalproducts

Biospumex 153 K

Composition

Modified polyalkoxyesters - Non ionic.

Quality Control Data

(These data are used for quality release and are certified for each batch.)

Item		Value	Method / Remarks
Appearance:		At 25°C, clear colourless to yellow liquid - In 5% deionised water dilution : opalescent emulsion + cream after 15 minutes	
Acid Value:		< 3 mg KOH/g	ISO 660
Density:	20 °C	1.015 - 1.025 g/l	ISO 6883
Viscosity:	20 °C-2-12-SG	0 - 1500 mPas	ISO 2555 - Brookfield

Properties & Use

BIOSPUMEX 153 K is particularly suitable to eliminate foam that builds-up in food processes like fermentation & extraction. This product is mainly used in biochemical media.

Food industry:

- Starch extraction from corn flour.
- Protein extraction from vegetables.

Bio-chemistry:

- Production of citric and amino acids.
- Production of natural flavours and biomass.

BIOSPUMEX 153 K contains 100% of active matter. Its main features are as follows:

- has a very low toxicity towards a wide range of micro-organisms and does not interfere with their growth,
- does not affect the dissolved oxygen rate,
- is not affected by sterilisation (either neat or in aqueous emulsions),
- is economical because of its effectiveness/concentration,
- mixes easily in water/aqueous medium,
- is used at temperatures ranging from 0°C to 100°C,
- is totally silicone free.

Recipies & Dosage

BIOSPUMEX 153 K is generally used neat. When a dilution is needed, it has to be stirred during storage and introduced into the foaming medium at the last minute. In fermentation processes, the dosage usually varies from 50 to 500 ppm. The rate is 10 to 20 times lower in other food processes.

Additional Technical Data

The freezing point of BIOSPUMEX 153 K is below - 20°C.

Its viscosity ranges from 415 cSt. at 40°C, 670 cSt. at 30°C, 1155 cSt. at 20°C to 2120 cSt. at 10°C.

BIOSPUMEX 153 K is free of ethanol and animal origin product. It is Kosher approved.

BIOSPUMEX 153 K is compliant with the decree of 19 October 2006 concerning the application of technological auxiliaries in the manufacturing of certain foodstuffs **and in particular of sugar(semi-)white crystallized.**

Remarks

Handling & Safety:	Please refer to the safety data sheet for details.
Storage:	BIOSPUMEX 153 K properties are not affected by low temperatures. Nevertheless, it should be stored at room temperature.

Revision-No. 2.1-07.2008 Effective July 8, 2008

Made in Paris, on December 01, 2016
Expires on December 31, 2017

Food Chemical Codex Statement

La diatomite (terre de diatomées) est listée dans la X^{ème} édition du Food Chemical Codex (2016) en tant qu'auxiliaire technologique.

CHEMVIRON FRANCE, filiale du groupe CALGON CARBON, certifie par la présente que ses diatomées naturelles, calcinées et calcinées activées commercialisées sous la marque CLARCEL® respectent les critères de pureté décrits dans la monographie FCC correspondante, et notamment les teneurs maximales en Arsenic et Plomb mentionnées ci-dessous. Les Diatomées commercialisées ne sont pas des additifs alimentaires.

The diatomite (Diatomaceoussilica) islistedin the Xthedition of Food Chemical Codex (2016) as filteraids in foodprocessing.

CHEMVIRON FRANCE, a subsidiary of CALGON CARBON corporation, hereby certifies that its natural diatomite, calcined and flux-calcined diatomite marketed under the trademarks CLARCEL® comply with the specifications of the FCC monograph, in particular the following maximum content in Arsenic and Lead. The Marketed Diatomite are not food additives.

Impurities	Typical content	Acceptance criteria NMT
Arsenic	< 8 mg / kg	10 mg / kg
Lead	< 3 mg / kg	10 mg / kg

Product Manager

Laurent Bertrand

(b) (4)

Regulatory Affairs & Product engineer

Mara Campagnolle

(b) (4)

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CLARCEL: 78 CBL, CBL3, CBR, CBR3, F, FD, DIC, DICB, DICS, DIC3, DIFBO, DITR, DIT2R, DIT3R, DIFB, DIFN, DIFD, DIFC, DIFR

Disclaimer

See the product's safety data sheet (SDS) for health & safety considerations.
The statements, technical information and recommendations contained herein are specific to this product and are believed to be accurate as of the date hereof. Since the conditions and methods of use of the product and of this information are beyond our control, we expressly disclaims any and all liability as to the consequences resulting from or relating to their use or reliance. No warranty or guarantee whether on performance, suitability, merchantability, fitness for purpose, compliance to laws or to end-use requirements or otherwise, is made concerning the product, its applications or the information that is provided by this document.
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05 août 2003

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FILTROX AG
Moosmühlestrasse 6
9001 St. Gallen

Confirmation relative à la conformité des couches filtrantes aux lois sur les denrées alimentaires

Suivant l'examen du dossier déposé concernant les couches filtrantes identifiées ci-après, nous arrivons à la conclusion qu'après un rinçage adéquat avant la première utilisation, une contamination des boissons filtrées par des substances insalubres n'est pas identifiable à l'état actuel des connaissances.

Les filtres sont conformes à la recommandation XXXVI/1 du BgVV et satisfont aux exigences de la Lebensmittel- und Bedarfsgegenständegesetz LMBG [loi sur les denrées alimentaires et les objets usuels], en particulier §§5, 30 et 31. Les produits peuvent être utilisés comme papiers filtres d'eau chaude et bouillante et couches filtrantes pour denrées alimentaires.

Les paramètres de test sont basés sur ces dispositions et les directives de la loi suisse sur les denrées alimentaires.

Cette confirmation concerne les filtres suivants:

FibraFix:							TecnaFix:
AF 6	AF 30	AF 21H	AF Steril 110	W-Steril	AF 03	AF 103	TS 4
AF 9	AF 50	AF 41H	AF Steril 130	W-Steril S	AF 23	AF 113	TS 5
AF 15	AF 70	AF 71H	AF Steril 140	FKV	AF 43	AF 133	TS 6
AF 15 S	AF 100	AF 71S	AF Steril 150	FKS	AF 73	AF 143	TS 15
AF 20	U3	AF 101 H WS				AF 153	TS 30
							TS 70

AMT FÜR LEBENSMITTELKONTROLLE
[OFFICE DU CONTRÔLE DES DENRÉES ALIMENTAIRES]
ST. GALLEN

(b) (4)

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Dr. P. Kolbener
Leiter Abt. Chemie
[Direction Dép. Chimie]

K. Luczynski
Sachbearbeiter
[Coll.compétent]

FILTROX AG, CH-9001 St.Gallen / Switzerland

St. Gallen, 24.03.2014

Declaration of Conformity for Filter Sheets

To whom it may concern

FILTROX AG is a producer of filter sheets for applications in the food and beverage industry as well as in the pharmaceutical and chemical industry.

These filter sheets are manufactured of specially selected raw materials such as purified and bleached cellulose, inorganic natural filter aids, like Kieselguhr, Perlite and Polyamidoamine resin as wet strength agent.

The filter sheets are in line with recommendation XXXVI/1 of BfR and comply with the requirements of the "Lebensmittel-, Bedarfsgegenstände- und Futtermittelgesetzbuch LFGB" (German Food and Feed Code). The products also comply with the requirements of U.S.P. (Safety Test) as well as F.D.A. regulations CFR21, § 177.2260 e,f,g,h,i,j,k, and I. All our products are made according to the rules of Quality Management System EN ISO 9001 as well as to the Environmental Management System EN ISO 14001.

Furthermore, we confirm that the filter sheets are in conformation with the regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27th October 2004 on materials and articles intended to come into contact with food, as well as regulation (EC) No 2023/2006.

FILTROX filter sheets and all raw materials contain no live organism or animal based extracts. Therefore these filter sheets can be used for HALAL certified foodstuffs.

FILTROX filter sheets do not contain alcohol or raw materials that were in contact with alcohol. The raw materials of all products we supply are GMO free. There is no contact with any animal based material during the whole production process.

Best regards

FILTROX AG

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Markus Saurer
General Sales Manager Filter Media

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To Whom It May Concern

May 2, 2017
DRI/MZF
Version 002
replaces Version 001

Food Application Status

TRISYL® 300 Silica

for Edible Oil Refining

Table of Content

General information

TRISYL® 300 Silica for Edible Oil Refining consists of synthetic amorphous silicon dioxide with citric acid treatment. Synthetic amorphous silicon dioxide is manufactured from a controlled mixture of sulfuric acid with sodium silicate solution. The hydrogel is generated from an acid-catalyzed condensation reaction. During the subsequent washing process excess salts are removed. Thereafter the product is dried and milled.

Harmonized Tariff Schedule:	**38249996**
Nature of the raw materials:	Silicon dioxide: Inorganic Citric acid: Organic
Country of origin (product):	Germany

National Inventories

Synthetic amorphous silicon dioxide and Citric acid are registered as follows:

Inventory	**Silicon dioxide**	**Citric acid**
Australien, AICS CAS No.	7631-86-9	77-92-9
Canada, DSL CAS No.	7631-86-9	77-92-9
Canada, NDSL CAS No.	7631-86-9	77-92-9
China, IECSC CAS No.	7631-86-9	listed
EU, EINECS	231-545-4	201-069-1
EU, REACH	01-2119379499-16-XXXX	01-2119457026-42-XXXX
Japan, ENCS MITI No.	1-548	2-1318
Japan, ISHL	Not listed	Not listed
Korea, KECI (ECL) KE No.	KE-31032	KE-20831
New Zealand, NZIoC CAS No.	7631-86-9	77-92-9
Philippines, PICCS CAS No.	7631-86-9	77-92-9
Switzerland (Produkteregister Chemikalien)	Not applicable	Not applicable
Taiwan	EPEP4A01648271	EPEP4A01713947
Turkey EC No.	231-545-4	201-069-1
USA, TSCA CAS No.	7631-86-9	77-92-9

Nanomaterials registered (French- Décret No. 2012-232)	Synthetic amorphous silica (SAS) BK Notification Number: BK 484-2017-07665889	Not applicable

Europe

COMMISSION REGULATION (EU) No 231/2012
Silicon dioxide (E 551) and citric acid (E 330) meet the purity requirements according to COMMISSION REGULATION (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives, last amended by COMMISSION REGULATION (EU) 2017/234 of 24 February 2017. TRISYL® 300 Silica Gel is suitable for human consumption.

TRISYL® 300 Silica for Edible Oil Refining is used as a processing aid for the adsorptive cleaning of edible oils and fats. The clarification step ends with a filtration, where TRISYL® Silica for Edible Oil Refining is completely removed from the oil except for unintentional but technically unavoidable traces. Processing aids are particularly excluded from the European Regulation (EC) No 1333/2008 on food additives according to the scope and the definitions given therein. Since processing aids do not need to be approved or labeled in line with the current vertical EU provisions, horizontal and national legislations have to be considered as well.

Regulation (EU) No 1308/2013
Silicon dioxide and citric acid can be used in the processing of refined olive oil and refined olive-pomace oil according to Regulation (EU) No 1308/2013 establishing a common organization of the markets in agricultural products, last amended by COMMISSION DELEGATED REGULATION (EU) 2016/1226 of 04 May 2016.

Germany

According to the Guidelines on edible fats and edible oils (Leitsätze für Speisefette und Speiseöle) silicon dioxide and citric acid can be used as inert filter aids in the manufacturing process of cold pressed edible oil and refined edible fats.

For further information on the use of silicon dioxide and citric acid as processing aids for edible oils and edible fats please consider also national provisions and obligations.

USA

Silicon dioxide is approved as a direct food additive and as a stabilizer in the production of beer according to the Code of Federal Regulations 21, § 172.480 (revision date: April, 2016). Similarly it is referenced as a technological adjuvant for clarifying wine and juice in the Code of Federal Regulations 27, § 24.246 (revision date: April, 2016). Silicon dioxide meets the Food Chemicals Codex monograph requirements for INS 551, which are referenced by the U.S. Food and Drug Administration.

Citric acid is classified as Affirmed as Generally Recognized as Safe (GRAS) by the FDA (Food and Drug Administration) when used in accordance with 21 CFR, § 184.1033 and when used in accordance with good manufacturing practises.

Treatment with adsorptive materials is a common procedure for removing color producing substances from edible oil. The adsorbents have to be completely removed by filtration. Silicon dioxide can be considered as safe for this application.

TRISYL® 300 Silica for Edible Oil Refining can be applied as processing aid in refining of edible oil or fat. The before-mentioned product is appropriate to be used for, or be in contact with foodstuff and is not hazardous for human health.

Should further information be required on this subject, please do not hesitate to contact us via our local Grace Business Representative.

Yours sincerely,

Grace GmbH

(b) (4)

Dietmar Richter
Manager Toxicology and Regulatory Affairs

Disclaimer:
The above statement(s) are based on our current knowledge and experience and on legislation in effect on the date above. This compliance statement does not warrant against modifications of this product resulting from its processing or from the addition of other products, nor against any inadequate use and/or storage of this product or the materials and articles containing it. The present statement also does not warrant compliance with legislation changed after the date above.

Akkreditiertes Prüflabor gemäß DIN EN ISO / IEC 17025

Chemische und mikrobiologische Untersuchungen
von Lebensmitteln und Wasser
Beratung · HACCP-Konzepte

LABOR DR. BÖHM

Trinkwasseruntersuchungsstelle nach § 15 Abs. 4 der TrinkwV 200

Andreas Böhm, Staatl. geprüfter Lebensmittelchemiker

Telefon 089 / 14 71 83 - 0
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IBAN: DE93700202706410059361
SWIFT (BIC): HYVEDEMMXXX

Postbank München
IBAN: DE54700100800027757807
SWIFT (BIC): PBNKDEFF

UID: DE 294 230 008

Gerichtsstand München
Steuer-Nr. 144/154/01109

Labor Dr. Böhm · Inh. Andreas Böhm · Schragenhofstr. 35 · 80992 München

15. Januar 2018
AB/asw

Health Certificate

Article:

Tonsil Supreme 110 FF	Tonsil Standard 315 FF	Tonsil 7118-X FF
Tonsil Supreme 111 FF	Tonsil Standard 3151 FF	Tonsil 7120-X FF
Tonsil Supreme 112 FF	Tonsil Standard 316 FF	Tonsil 7125-X FF
Tonsil Supreme 113 FF	Tonsil Standard 317 FF	Tonsil 7127-X FF
Tonsil Supreme 114 FF	Tonsil Standard 318 FF	Tonsil 7130-X FF
Tonsil Supreme 115 FF	Tonsil Standard 510 FF	Tonsil 7132-X FF
Tonsil Supreme 116 FF	Tonsil Standard 512 FF	Tonsil 7134-X FF
Tonsil Supreme 117 FF	Tonsil 4110-X FF	Tonsil 7136-X FF
Tonsil Supreme 118 FF	Tonsil 4111-X FF	Tonsil 813-X FF
Tonsil Supreme 119 FF	Tonsil 4112-X FF	Tonsil 8114-X FF
Tonsil Supreme 516 FF	Tonsil 4114-X FF	Tonsil 8118-X FF
Tonsil Supreme 158 FF	Tonsil 4118-X FF	Tonsil 8120-X FF
Tonsil Optimum 208 FF	Tonsil 4120-X FF	Tonsil 8125-X FF
Tonsil Optimum 210 FF	Tonsil 4122-X FF	Tonsil 8132-X FF
Tonsil Optimum 212 FF	Tonsil 4124-X FF	Tonsil 919 FF
Tonsil Optimum 213 FF	Tonsil 4125-X FF	Tonsil 9191 FF
Tonsil Optimum 214 FF	Tonsil 4127-X	Tonsil 9192 FF
Tonsil Optimum 215 FF	Tonsil 413-X FF	Tonsil 9194 FF
Tonsil Optimum 216 FF	Tonsil 4130-X FF	Tonsil 9195 FF
Tonsil Optimum 217	Tonsil 4132-X FF	Tonsil 9196 FF
Tonsil Optimum 218 FF	Tonsil 4134-X FF	Tonsil 9198 FF
Tonsil Optimum 254 FF	Tonsil 4136-X FF	Tonsil EX 501
Tonsil Optimum 258 FF	Tonsil 4137-X FF	Tonsil EX 722
Tonsil Optimum 514 FF	Tonsil 4150-X FF	Tonsil EX 1707
Tonsil Optimum 515 FF	Tonsil 4192-X FF	
Tonsil Optimum 558 FF	Tonsil 713-X FF	
Tonsil Standard 310 FF	Tonsil 7110-X FF	
Tonsil Standard 312 FF	Tonsil 7112-X FF	
Tonsil Standard 314 FF	Tonsil 7114-X FF	

DAkkS Deutsche Akkreditierungsstelle D-PL-18257-01-00

Die Akkreditierung durch die DAkkS gilt für die Untersuchung von Lebensmitteln und Wasser für die in der Urkunde aufgeführten Prüfverfahren

Labor Dr. Böhm, zugelassen für
- Untersuchungen von amtlichen Gegenproben nach § 43 LFGB
- mikrobiologische Untersuchungen nach § 44 Infektionsschutzgesetz
- Trinkwasseruntersuchungen nach § 15 Abs. 4 TrinkwV 2001

After examination of the documents and dates given by the manufacturer we certify, that the above mentioned products can be used in food processing (especially refining vegetable and animal oils and fats).

As far as obvious out of the documents there are no health risks in using. Precondition is, that the products will be used appropriate and in accordance to the specific legal regulations.

(b) (4)

Andreas Böhm
General management, technical management

Labor Dr. Böhm
Schragenhofstraße 35
80992 München

From:	Erica Cermak Intertek
To:	West-Barnette, Shayla; Morissette, Rachel; Hywel Griffiths
Subject:	RE: follow-up to phone call for GRNs 000776 and 000777
Date:	Tuesday, September 04, 2018 3:44:42 PM
Attachments:	image001.png image014.png image020.png image021.png image027.png image003.png GRN 000776 and GRN 000777 sterol supplement September 4 2018.docx

Dr. Morissette,

Please find attached the remaining responses to the questions received by email on June 17, 2018.

Regards,

Erica Cermak
Manager, Regulatory and Toxicology - Food & Nutrition
Health, Environmental & Regulatory Services (HERS)

Direct +1 908-290-7201
Skype erica.cermak.intertek
www.intertek.com

Total Quality. Assured.

Intertek, New Jersey, USA

From: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Sent: Friday, August 31, 2018 1:57 PM
To: Erica Cermak Intertek <erica.cermak@intertek.com>; Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; Hywel Griffiths <hgriffiths@fermentalg.com>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Thank you, Ms. Cermak. We look forward to receiving your responses on Tuesday.

Regards,

Shayla West-Barnette, Ph.D.
Supervisory Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Shayla.WestBarnette@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION [fda.gov]

[facebook.com] [twitter.com] [youtube.com] [flickr.com] [fda.gov]

From: Erica Cermak Intertek <erica.cermak@intertek.com>
Sent: Friday, August 31, 2018 1:48 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Dr. Morissette,

Fermentalg received the sterol analysis this evening local time. We will prepare the response to the remaining questions related to sterols and anticipate providing these to you by email on Tuesday, September 4th.

Regards,

Erica Cermak
Manager, Regulatory and Toxicology - Food & Nutrition
Health, Environmental & Regulatory Services (HERS)

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intertek
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Intertek, New Jersey, USA

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, August 24, 2018 11:45 AM
To: Erica Cermak Intertek <erica.cermak@intertek.com>; Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Thank you! I will forward this information to our review team.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Erica Cermak Intertek [mailto:erica.cermak@intertek.com]
Sent: Friday, August 24, 2018 11:40 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Dr. Morissette,

On behalf of Fermentalg, we respectfully submit this additional information in support of GRAS Notifications 000776 and 000777 in response to your questions received by email on June 17, 2018. It is our belief that this additional information provided as part of this notification adequately addresses the majority of your questions. As noted in your email below, we anticipate receipt of the sterol analysis by close of business next Friday, August 31st, and will provide the remaining responses upon receipt of this data.
My contact information is provided below. Please feel free to again contact me by phone or e-mail if you have any questions regarding this information.

Thank you,

Erica Cermak
Manager, Regulatory and Toxicology - Food & Nutrition
Health, Environmental & Regulatory Services (HERS)

Direct +1 908-290-7201
Skype erica.cermak.intertek
www.intertek.com

Intertek, New Jersey, USA

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, August 24, 2018 10:15 AM
To: Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>; West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: follow-up to phone call for GRNs 000776 and 000777

Dear Dr. Griffiths,

Thank you for your phone call today to discuss the status of the responses to our questions for GRNs 000776 and 000777. You mentioned that the reason for the delay in responding to our questions is because the laboratory that you hired to test the sterols failed to provide those results in a timely manner; therefore, you have contracted with a separate company to perform those analyses. You mentioned that you can send the responses to the other questions now, excluding the sterol analyses, but that you anticipate having the sterol results by close of business (EST) next Friday, September 31. I agreed that sending what you have now would be best, with the expectation that we will receive the sterol response next week. If something changes, I requested that you contact Dr. Shayla West-Barnette, as I will be away next week. She will alert the review team and advise you on the next steps. You also mentioned that you would be amenable to withdrawing these notices should that become necessary. Please let me or Shayla know if you have any questions. We appreciate your keeping us apprised of the situation as it unfolds. I will look for your initial responses today. Please cc Dr. West-Barnette on that email as well.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Friday, August 24, 2018 9:51 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>

Subject: Re: response requested for GRNs 776 and 777

Dear Dr Morissette,

Do you have time for a quick 5 minute call? If so is there a number on which I could reach you?

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

Fermentalg

Tel. +335 57 250 252 | Mobile +337 61 33 37 96 | www.fermentalg.com [fermentalg.com] | Fermentalg – 4 Rue Rivière – 33500 Libourne |

On 24 Aug 2018, at 2:11 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dr. Griffiths,

I am following up on our conversation from last week. I have not received the responses to our questions for GRNs 776 and 777. Are you still planning to submit those responses by COB today? Withdrawing your notices and resubmitting them as I outlined below is still an option. If I do not hear back from you, we will need to assume that you are not planning to respond and will proceed with drafting no basis letters for these GRAS notices. Please let me know your intentions as soon as possible. I will be out of the office all next week. Dr. Shayla West-Barnette will be handling this matter while I'm away. Please cc her on all correspondence starting at 3 pm today EST. Email email address is Shayla.westbarnette@fda.hhs.gov. I hope to hear from you today about your intentions for these GRAS notices so that we can meet the 180-day mark.

Regards,

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Morissette, Rachel
Sent: Thursday, August 16, 2018 11:06 AM
To: 'Hywel Griffiths' <hgriffiths@fermentalg.com>
Subject: RE: questions for GRNs 000776 and 000777 (DHA algal oil)

Hi Hywel,

Thanks for your reply. Since we are already four weeks out from receipt of the questions, and typically 10 business days is the allowable time frame for responses from notifiers, early next week is preferable. If you don't think you'll be able to meet that timeframe, we'll have to discuss other options at this point, including withdrawing the notices and resubmitting revised versions that incorporate the questions that were raised in these notices, if necessary. I'll look out for your email next week.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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<image002.jpg> [facebook.com] <image003.jpg> [twitter.com] <image004.jpg> [youtube.com] <image005.jpg> [flickr.com] <image006.jpg> [fda.gov]

From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Thursday, August 16, 2018 10:57 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: Re: questions for GRNs 000776 and 000777 (DHA algal oil)

Hi Rachel,

Thanks for your email. As you will have gathered from my out of office, I was away on vacation until today. The time it will take to review the response prepared by Intertek and check that we've collated all the data requested means I'm targeting next week for the reply. I hope this is acceptable.

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

<image007.png>

Tel. +335 57 250 252 | Mobile +337 61 33 37 96 | www.fermentalg.com [fermentalg.com] | Fermentalg – 4 Rue Rivière – 33500 Libourne |

On 14 Aug 2018, at 8:48 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Hi Dr. Griffiths,

I just wanted to check in and see when you anticipate sending your responses to our questions for GRNs 000776 and 000777?

Thanks,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Wednesday, July 18, 2018 11:31 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>
Subject: Re: questions for GRNs 000776 and 000777 (DHA algal oil)

Dear Ms Morissette,

Thank you for the letter. We will attempt to answer all questions within 10 business days, although with it already being holiday season in France we may have to ask for an extension for some of the questions requiring detailed technical responses.
In copy of this email is Erica Cermak of Intertek who was involved in the construction of the notifications and who may communicate on our behalf.

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

<image008.png>

Tel. +335 57 250 252 | Mobile +337 61 33 37 96 | www.fermentalg.com [fermentalg.com] | Fermentalg – 4 Rue Rivière – 33500 Libourne |

On 17 Jul 2018, at 9:34 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dr. Griffiths,

Please see attached a letter with questions to be addressed for GRNs 000776 and 000777 (DHA algal oil). We request a response within 10 business days. Please let me know if you have any questions at this time.

Best regards,

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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<7-17-18 GRN776_777 Questions for Notifier.pdf>

5) Fermentalg provides a comparison of the sterol content of its GRN 000776 DHA algal oil with DSM Nutritional Product's (GRN 000553) oil and Mara Renewables Corporation's (GRN 000677) oil. For GRN 000776, there is a statement in the notice on p. 14 as follows:

"…the slight differences in the relative proportions of various sterols between Fermentalg's DHA350 and other DHA oil products are not expected to be [sic] affect safety."
Table 2.4.4-3 shows that (1) the level of total sterols in Fermentalg's DHA algal oil is higher than the total sterols in the GRN 000553 oil (0.56% w/w) and the GRN 000677 oil (0.23% w/w), and (2) the major sterols are not the same for GRNs 000776, 000553, and 000677. Please provide additional discussion and references to support the conclusion that these differences are not a safety concern for the intended use of Fermentalg's DHA algal oil in infant formulas for term and pre-term infants. Further, only a single batch analysis for sterols was reported for GRN 000776, with the comment that it is a representative batch. Please provide the results of a minimum of three non-consecutive batch analyses for sterols in order to characterize the sterol content of Fermentalg's DHA algal oil and to show typical levels of individual and total sterols.

The sterol profile of 3 non-consecutive batches of DHA 350 is shown in Table 3

Table 3 Sterol Profile of DHA 350

Sterol	Lot #0403012-A	Lot #0403014-A	Lot #0303010	Mara Renewables GRN 000677* (Range)	DSM Nutritional Products GRN 000553* (Range)
Cholesterol	44.1%	50.4%	54.7%	12.6-32.9%	9.8-14.4%
Brassicasterol and/or Ergosterol[1]	7.8%	8.1%	8.1%	<0.1-6.5%	0.9-1.7%
Campesterol	<0.1%	<0.1%	<0.1%	1.2-3.9%	1.5-2.2%
Campestanol	<0.1%	<0.1%	<0.1%	<0.1%	0.1
Stigmasterol	1.7%	3.0%	1.9%	<0.1-23.1%	60.6-65.3%
Delta 7-Campesterol	<0.1%	<0.1%	<0.1%	<0.1-7.0%	0.4-0.6%
D5,23 Stigmastadienol	<0.1%	<0.1%	<0.1%	<0.1-7.7%	0.8-1.0%
Chlerosterol and/or fucosterol[1]	29.4%	25.9%	26.8%	6.3-19.3%	1.6%
Beta-sitosterol	4.4%	3.9%	3.4%	9.4-14.8%	9.7-14.6%
Sitostanol	<0.1%	<0.1%	<0.1%	<0.1-0.5%	0.5-0.6%
Delta5-Avenasterol	0.9%	0.4%	0.2%	1.2-5.7%	0.9-2.9%
Delta 5,24 Stigmastadienol	<0.1%	<0.1%	0.2%	3.9-7.0%	0.4-0.5%
Delta 7-Stigmasterol	7.7%	0.9%	1.2%	<0.1-26.1%	1.6-2.5%
Delta7-Avenasterol	0.4%	0.4%	0.3%	1.4-9.1%	0.3-3.2%
Sum of non-identified peaks[2]	3.6%	7.0%	3.2%	Not reported	Not reported
Total Sterol Content	9,377 mg/kg of fat	8,482 mg/kg of fat	10,011 mg/kg of fat	831-2310 mg/kg fat	5100-5600 mg/kg fat

*Mara Renewable's oil and DSM's oil also contained 24-methylene cholesterol. [1] Two sterol compounds that have the same retention time during analysis. [2] Non-identified peaks have not been seen in previous analyses such as those submitted with the original notification. It is probable that these are sterols that have been incompletely derivatized (AOCS DOI:10.21748/lipidlibrary/40384).

The sterols present in Fermentalg's DHA 550 oil and the inter-batch variation are comparable to those present in other DHA algal oils currently used in infant formula, and other ingredients used in the manufacture of infant formula. They are also present in human milk and in the human diet.

Nine sterols comprise at least 5% of at least one of the oils, (Cholesterol, Brassicasterol, Stigmasterol, Chlerosterol, Beta-sitosterol, Delta-7-stigmasterol, Delta-5,23-stigmasterol, Delta 5-avenasterol, and Delta-7-avenasterol). These sterols are ubiquitous in the food supply and commonly used as sources of essential fatty acids in infant formula including corn, palm, safflower, soybean, and sunflower oil.

Fermentalg's oil contains a significantly higher amount of Cholesterol and Chlerosterol when compared to the other two oils, whereas the other sterols are either found in roughly equivalent proportions or are found in higher levels in Mara's oil and/or DSM's oil.

Cholesterol is the most significant sterol in the DHA 350 oil at 44-55%. Human breast milk contains significant quantities of cholesterol, whereas infant formulas contain up to ten times less (Claumarchirant *et al.* 2015). Nonetheless, given the expected levels of incorporation of the algal oil into infant formula,

the amounts of cholesterol provided by the algal oil will not significantly increase the total amount of cholesterol provided by the infant formula since the sterols provided in the algal oil will represent 1/10th to 1/20th of the total sterol.

As reviewed in GRN 000553, the other sterols are also reported in human milk, infant formula, or common foods and dietary oils. Various plant stanols have been evaluated by competent authorities world-wide and approved for use in a variety of foods, beverages and dietary supplements (Cantrill and Kawamura, 2008).

In the event that the unidentified unsaponifiable components of the oil are *not* the result of a partial derivitization during analysis, they would represent, at maximum, 0.7% of sterols provided in the infant formula.

Fermentalg's specifications for unsaponifiables (max. 3.5%) is the same as that of similar DHA algal oils, including the oils notified in GRN 000553 and GRN 000667. While the level in Fermentalg' DHA 350 is higher than the values presented in the representative batches of these oils, the levels are within the specification for all oils. Under the intended conditions of use, the total sterol intake from DHA algal oil would be minimal.

6) Sterols are not addressed in GRN 000777 beyond a general comment that there are:

"…slight differences in the relative proportions of various sterols which are not expected to be affect [sic] safety."
Please provide the results of sterol analyses from three non-consecutive batches for the DHA algal oil that is the subject of GRN 000777 and provide additional discussion explaining the aforementioned statement.

The sterol profile of 3 non-consecutive batches of DHA 550 is shown in Table 4.

Table 4 Sterol Profile of DHA 550

Sterol	Lot # 0403019	Lot # 0419022	Lot #0419028-A	Mara Renewables GRN 000677* (Range)	DSM Nutritional Products GRN 000553* (Range)
Cholesterol	40.7%	49.9%	53.9%	12.6-32.9%	9.8-14.4%
Brassicasterol and/or Ergosterol[1]	10.4%	10.1%	9.2%	<0.1-6.5%	0.9-1.7%
Campesterol	< 0.1%	< 0.1%	<0.1%	1.2-3.9%	1.5-2.2%
Campestanol	< 0.1%	<0.1%	<0.1%	<0.1%	0.1
Stigmasterol	1.5%	5.2%	3.6%	<0.1-23.1%	60.6-65.3%
Delta 7-Campesterol	<0.1%	< 0.1%	< 0.1%	<0.1-7.0%	0.4-0.6%
D5,23 Stigmastadienol	<0.1%	<0.1%	< 0.1%	<0.1-7.7%	0.8-1.0%
Chlerosterol and/or fucosterol[1]	33.5%	21.9%	19.7	6.3-19.3%	1.6%
Beta-sitosterol	9.6%	5.2%	4.6%	9.4-14.8%	9.7-14.6%
Sitostanol	<0.1%	< 0.1%	< 0.1%	<0.1-0.5%	0.5-0.6%
Delta5-Avenasterol	0.3%	0.4%	0.6%	1.2-5.7%	0.9-2.9%
Delta 5,24 Stigmastadienol	<0.1%	<0.1%	<0.1%	3.9-7.0%	0.4-0.5%
Delta 7-Stigmasterol	0.6%	0.4%	0.8%	<0.1-26.1%	1.6-2.5%
Delta7-Avenasterol	0.4%	0.2%	0.6%	1.4-9.1%	0.3-3.2%
Sum of non-identified peaks[2]	3.0%	6.8%	7.1%	Not reported	Not reported
Total Sterol Content	20,381 mg/kg fat	12,894 mg/kg fat	10210 mg/kg fat	831-2310 mg/kg fat	5100-5600 mg/kg fat

*Mara Renewable's oil and DSM's oil also contained 24-methylene cholesterol. [1] Two sterol compounds that have the same retention time during analysis. [2] Non-identified peaks have not been seen in previous analyses. It is probable that these are sterols that have been incompletely derivatized (AOCS DOI:10.21748/lipidlibrary/40384).

The sterols present in Fermentalg's DHA 550 oil and the inter-batch variation are comparable to those present in other DHA algal oils currently used in infant formula, and other ingredients used in the manufacture of infant formula. They are also present in human milk and in the human diet.

Nine sterols comprise at least 5% of at least one of the oils, (Cholesterol, Brassicasterol, Stigmasterol, Chlerosterol, Beta-sitosterol, Delta-7-stigmasterol, Delta-5,23-stigmasterol, Delta 5-avenasterol, and Delta-7-avenasterol). These sterols are ubiquitous in the food supply and commonly used as sources of essential fatty acids in infant formula including corn, palm, safflower, soybean, and sunflower oil.

Fermentalg's oil contains a significantly higher amount of Cholesterol and Brassicasterol when compared to the other two oils, whereas the other sterols are either found in roughly equivalent proportions or are found in higher levels in Mara's oil and/or DSM's oil.

Cholesterol is the most significant sterol in the DHA 550 oil at 40-54%. Human breast milk contains significant quantities of cholesterol, whereas infant formulas contain up to ten times less (Claumarchirant *et al.* 2015). Nonetheless, given the expected levels of incorporation of the algal oil into infant formula,

the amounts of cholesterol provided by the algal oil will not significantly increase the total amount of cholesterol provided by the infant formula since the sterols provided in the algal oil will represent 1/10th to 1/20th of the total sterol.

As reviewed in GRN 000553, the other sterols are also reported in human milk, infant formula, or common foods and dietary oils. Various plant stanols have been evaluated by competent authorities world-wide and approved for use in a variety of foods, beverages and dietary supplements (Cantrill and Kawamura, 2008).

In the event that the unidentified unsaponifiable components of the oil are *not* the result of a partial derivitization during analysis, they would represent, at maximum, 0.7% of sterols provided in the infant formula.

Fermentalg's specifications for unsaponifiables (max. 3.5%) is the same as that of similar DHA algal oils, including the oils notified in GRN 000553 and GRN 000667. While the level in Fermentalg' DHA 550 is higher than the values presented in the representative batches of these oils, the levels are within the specification for all oils. Under the intended conditions of use, the total sterol intake from DHA algal oil would be minimal (see response to question #19).

7) Fermentalg does not provide a comparison of the fatty acid or sterol content of its GRN 000777 DHA algal oil with the *Schizochytrium* sp.-derived DHA algal oils that were the subjects of published studies cited in the notice. Please provide this comparison and a discussion comparing the identity of the subject of GRN 000777 to *Schizochytrium* sp.-derived DHA-algal oils currently used in infant formulas for term and pre-term infants or oils that were the subject of relevant developmental and clinical studies cited in the notice.

Please see the Table 2 in the response of August 24, 2018 in response to question #3, which compares the fatty acid profile of Fermentalg's DHA 550 to those marketed by DSM (GRN 553) and Mara Renewables (GRN 677). A comparison of the sterol profile is provided in Tables 3 and 4 in response to questions #5& 6 above.
Given the phylogenic relationship between strains used in the safety studies, along with the comparative fatty acid and sterol profile, Fermentalg's DHA 550 may be considered sufficiently similar to the other GRAS-notified oils such that the developmental and clinical studies safety data generated for these oils can be considered supportive of the safety of DHA 550.

19) Please provide an estimate of total sterol intake from the intended uses in GRNs 000776 and 000777. Please discuss how these estimates compare to the exposure from consuming *Schizochytrium* sp. oils that are the subjects of published studies cited in GRNs 000776 and 000777 that are relevant to infant formula uses in term and pre-term infants.

As noted in GRN 000776 and GRN 000777, it is assumed that infants consume about 100 to 120 kcal/kg body weight (bw)/day, of which fat constitutes approximately 50% of calories, or approximately 5.5 to 6.7 g fat/kg bw/day (1 g of fat is equivalent to 9 kcal). Assuming incorporation of the proposed DHA ingredient at a maximum use level of 0.5% of fatty acids, the intake of DHA would be 27 to 33 mg/kg bw/day. These levels would be associated with exposure to 77-94 mg of DHA350 and 49-60 mg of DHA 550.

The unsaponifiable content of the three batches of DHA 350 included in GRN 000776 ranged from 1.40% to 1.86%.

As a result, sterol consumption resulting from the proposed use of DHA 350 would range from 1.1 to 1.7 mg/kg bw/day, roughly half of which would be cholesterol.

The unsaponifiable content of the three batches of DHA 550 included in GRN 000777 ranged from 1.22% to 1.77%.
As a result, sterol consumption resulting from the proposed use of DHA 550 would range from 0.6 to 1.1 mg/kg bw/day, roughly half of which would be cholesterol.

In comparison, total sterol consumption from an infant formula diet would be around 15-30mg/kg bw/day (Claumarchirant *et al.* 2015), with higher levels possible in breast fed infants.

As the maximum specification for sterol content for DHA 350 and DHA 550 is the same as that for DSM and Mara's oils, maximum exposure to sterols would be the same.

References

Cantrill R and Kawamura Y. (2008). Phytosterols, phytostanols and their esters. Chemical and technical assessment for the 69th JECFA. Available online at: http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/69/Phytosterols.pdf

Claumarchirant, L.; Matencio, E.; Sanchez-Siles, L.M.; Alegria, A.; & Lagarda, M.J. (2015). Sterol Composition in Infant Formulas and Estimated Intake. J Agric Food Chem 63(32):7245-7251.