KELLER AND HECKMAN LLP
Serving Business through Law and Science®

1001 G Street, N.W.
Suite 500 West
Washington, D.C. 20001
tel. 202.434.4100
fax 202.434.4646

RECEIVED
OCT 10 2014
OFFICE OF
FOOD ADDITIVE SAFETY

GRN 000549

Writer's Direct Access
Melvin S. Drozen
(202) 434-4222
drozen@khlaw.com

October 3, 2014

Via FedEx (Tracking No. 771372498801)

Antonia Mattia, Ph.D.
Director, Division of Biotechnology and GRAS
Notice Review (HFS-225)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch
College Park, Maryland 20740

Re: GRAS Notification for Sodium Salts of *M. alpina* Oil

Dear Dr. Mattia:

We respectfully submit the attached GRAS Notification on behalf of our client, Jost Chemical Company (Jost) for the sodium salts of *M. alpina* oil (Sodium ARA) for use as a source of arachidonic acid (ARA) in term infant formula. As described in the GRAS Notification, Sodium ARA will be combined with other generally recognized as safe (GRAS) ingredients to produce a commercial blend, and the blend will be used at a maximum level of 5.00% of total dietary fat when used in combination with docosahexaenoic acid (DHA) at a maximum level of 0.50% of total dietary fat and at a ratio ranging from 1:1 to 1:2 (DHA: ARA). A use level of 5.00% of Jost's Sodium ARA blend as a percentage of total dietary fat supplies 0.75% ARA as a percentage of total dietary fat.

The attached GRAS Notification provides a review of the information related to the intended use and manufacturing of Sodium ARA, along with an explanation of this ingredient's substantial equivalence to other GRAS-notified *M. alpina* oil sources of ARA for use in infant formula. The GRAS Notification incorporates by reference the published data demonstrating the safety and GRAS status of ARA derived from *M. alpina* oil sources that were the subject of the following GRAS Notifications: GRN 41, GRN 80, GRN 94, and GRN 326. We have included three (3) hard copies of the GRAS Notification for your review.

Jost has determined that Sodium ARA is GRAS based on scientific procedures in accordance with 21 C.F.R. § 170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under proposed 21 C.F.R. § 170.36, 62 Fed. Reg. 18938 (Apr.

17, 1997). Therefore, the use of Sodium ARA in food as described in this GRAS Notification is exempt from the requirement of premarket approval as set forth in the Federal Food, Drug, and Cosmetic Act.

The analytical data, published studies, and information that are the basis for this GRAS determination are available for FDA review and copying at reasonable times at Keller and Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001, or will be sent to FDA upon request.

We look forward to FDA's review of this submission and would be happy to provide Agency officials with any information they may need to complete their assessment. Thank you for your attention to this matter.

Sincerely,

(b) (6)

Melvin S. Drozen

Enclosure

Form Approved: OMB No. 0910-0342; Expiration Date: 02/29/2016
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000549	
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

[x] New [ ] Amendment to GRN No._____ [ ] Supplement to GRN No. _____

2. [ ] All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3a. For New Submissions Only: Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

3b. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* [ ] Yes [ ] No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier			
Name of Contact Person John Siebert		Position Q.A. Supervisor	
Company *(if applicable)* Jost Chemical Company			
Mailing Address *(number and street)* 8150 Lackland Rd.			
City St. Louis	State or Province Missouri	Zip Code/Postal Code 63114	Country United States of America
Telephone Number 314-817-2552	Fax Number	E-Mail Address john.siebert@jostchemical.com	

1b. Agent or Attorney *(if applicable)*			
Name of Contact Person Melvin S. Drozen		Position Partner	
Company *(if applicable)* Keller and Heckman LLP			
Mailing Address *(number and street)* 1001 G Street NW			
City Washington	State or Province District of Columbia	Zip Code/Postal Code 20001	Country United States of America
Telephone Number 202-434-4222	Fax Number 202-434-4646	E-Mail Address drozen@khlaw.com	

000004

000005

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

Sodium salts of M. alpina oil

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Paper

☐ Electronic files on physical media with paper signature page

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes 1

Total number of pages

4. Does this submission incorporate any information in FDA's files by reference? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates by reference information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 41

☐ b) GRAS Affirmation Petition No. GRP

☐ c) Food Additive Petition No. FAP

☐ d) Food Master File No. FMF

☒ e) Other or Additional *(describe or enter information as above)* Also GRN 80, GRN 94, and GRN 326

6. Statutory basis for determination of GRAS status *(Check one)*

☒ Scientific Procedures *(21 CFR 170.30(b))* ☐ Experience based on common use in food *(21 CFR 170.30(c))*

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, see attached Designation of Confidential Information

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance *(e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).*

The sodium salts of M. alpina oil (Sodium ARA) will be used as a source of arachidonic acid (ARA) in term infant formula. Sodium ARA will be combined with other generally recognized as safe (GRAS) ingredients to produce a commercial blend, and the blend will be used at a maximum level of 5.00% of total dietary fat when used in combination with docosahexaenoic acid (DHA) at a maximum level of 0.50% of total dietary fat and at a ratio ranging from 1:1 to 1:2 (DHA: ARA). A use level of 5.00% of Jost's Sodium ARA blend as a percentage of total dietary fat supplies 0.75% ARA as a percentage of total dietary fat.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? *(Check one)*

☐ Yes ☒ No

000006

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
1	Sodium salts of *m. alpina*oil				
2					
3					

[1] Include chemical name or common name. Put synonyms *(whether chemical name, other scientific name, or common name)* for each respective item (1- 3) in Item 3 of Part V *(synonyms)*

[2] Registry used e.g., CAS *(Chemical Abstracts Service)*and EC *(Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))*

2. Description

Provide additional information to identify the notified substance*(s)*, which may include chemical formula*(s)*, empirical formula*(s)*, structural formula*(s)*, quantitative composition, characteristic properties *(such as molecular weight(s))*, and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source *(e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source), and include any known toxicants that* could be in the source.

Sodium ARA is produced from the reaction of sodium hydroxide (NaOH) with a GRAS *M. alpina*oil source to generate the sodium salts of the *M. alpina*oil, which include sodium arachidonate, sodium myristate, sodium palmitate, sodium palmitoleate, sodium stearate, sodium oleate, sodium linoleate, sodium-*gamma*-linoleate, sodium-homo-*gamma*-linoleate, sodium arachidate, sodium behenate, and sodium lignocerate.

3. Synonyms

Provide as available or relevant:

1	
2	
3	

000007

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete – check all that apply)

- [x] Any additional information about identity not covered in Part V of this form
- [x] Method of Manufacture
- [x] Specifications for food-grade material
- [x] Information about dietary exposure
- [ ] Information about any self-limiting levels of use *(which may include a statement that the intended use of the notified substance is not-self-limiting)*
- [ ] Use in food before 1958 *(which may include a statement that there is no information about use of the notified substance in food prior to 1958)*
- [x] Comprehensive discussion of the basis for the determination of GRAS status
- [ ] Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

[ ] Yes [x] No

Did you include this other information in the list of attachments?

[ ] Yes [ ] No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Jost Chemical Company *(name of notifier)*

has concluded that the intended use*(s)*of Sodium salts of M. alpina oil *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. [x] Jost Chemical Company *(name of notifier)* agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

Jost Chemical Company *(name of notifier)* agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

Keller and Heckman LLP, 1001 G Street NW, Suite 500W, Washington, DC 20001
(address of notifier or other location)

Jost Chemical Company *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

[ ] The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. ----------
(GRAS Affirmation Petition No.)

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
(b) (6)	Melvin S. Drozen	10/03/2014

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Cover letter to Antonia Mattia	N/A
	GRAS Notification for Sodium Salts of M. alpina oil	Pages 1-15
	Appendix A	Pages 16-20
	Appendix B	Pages 21-27
	Appendix C	Pages 28-30

000009

OMB Statement: Public reporting burden for this collection of information is estimated to average 150 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, 1350 Piccard Drive, Room 400, Rockville, MD 20850. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

000010

000011

000012

GRAS Notification for

Sodium Salts of *M. Alpina* Oil

Prepared for: U.S. Food and Drug Administration
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Prepared by: Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20001

Date: October 3, 2014

Table of Contents

Page

I. Introduction 1

II. Administrative Information 1
- A. Claim Regarding GRAS Status 1
- B. Name and Address of the Notifier 1
- C. Common or Usual Name of the Subject Substance 2
- D. Intended Use and Level of Use of GRAS Substance 2
- E. Statement of Availability of Data and Information 2

III. Criteria for GRAS Status 2

IV. Detailed Information about the Identity of the Notified Substance 3
- A. Identity 3
- B. Physical Attributes 3
- C. Composition and Specifications 4
- D. Manufacturing Process 7
  - 1. Phase I: Saponification of the triglyceride ARA source to the free fatty acids 8
  - 2. Phase II: Conversion of the free fatty acids into the sodium salts 8
  - 3. Phase III: Addition of other ingredients and drying 8

V. Basis for GRAS Determination 9
- A. Substantial Equivalence of Sodium ARA to *M. alpina* Oil 9
- B. Substantial Equivalence in Intended Use Levels of Sodium ARA and *M. alpina* Oil in Infant Formula 12
- C. Safety of ARA in Term Infant Formula 13
  - 1. ARA Component 13
  - 2. Sodium Component 13

VI. Summary Basis for GRAS Determination 14

VII. Conclusion 15

Tables

Table 1	Fatty Acid Compositional Profile for Sodium ARA and GRAS-Notified *M. alpina* oil sources
Table 2	Chemical and Heavy Metal Specifications for Sodium ARA
Table 3	Microbial Specifications for Sodium ARA
Table 4	Analysis of Five Batches of Sodium ARA
Table 5	Composition of Commercial Sodium ARA Blend

I. Introduction

Keller and Heckman LLP submits the enclosed information on behalf of our client Jost Chemical Company (Jost) in support of this Notification that the sodium salts of *M. alpina* oil (Sodium ARA) are Generally Recognized as Safe (GRAS) for use as a source of arachidonic acid (ARA) in term infant formula.[1] The determination of GRAS status is based on scientific procedures, in accordance with 21 C.F.R. § 170.30(b), and conforms to the guidance issued by the Food and Drug Administration (FDA) under *proposed* 21 C.F.R. § 170.36, 62 Fed. Reg. 18938 (Apr. 17, 1997).

We submit information in the following areas:

- Identity of the substance.
- The production of Sodium ARA.
- Substantial equivalence of Sodium ARA to GRAS *M. alpina* oil sources of ARA.
- Substantial equivalence of use levels for Sodium ARA blend and GRAS *M. alpina* oil sources of ARA in term infant formula.
- Relevant published safety data on *M. alpina* oil sources of ARA.

It is our expectation that FDA will concur that the information presented fully supports the determination that Sodium ARA as produced by Jost is GRAS for use in term infant formula.

II. Administrative Information

A. Claim Regarding GRAS Status

Jost hereby notifies the agency of its determination that Sodium ARA is GRAS based on scientific procedures for use as a source of ARA in term infant formula.

B. Name and Address of the Notifier

Jost Chemical Company
8150 Lackland Rd.
St. Louis, MO 63114
U.S.A.

All communications on this matter are to be sent to Counsel for the Notifier:

---

[1] A term infant is an infant that has completed 37-42 weeks gestation. National Institutes of Health (NIH), Medline Plus, "Gestational age," available at: http://www.nlm.nih.gov/medlineplus/ency/article/002367.htm.

Melvin S. Drozen
1001 G Street, NW
Suite 500W
Washington, DC 20001
Telephone: (202) 434-4222
Facsimile: (202) 434-4646
Email: drozen@khlaw.com

C. Common or Usual Name of the Subject Substance

Sodium salts of *M. alpina* oil (abbreviated to "Sodium ARA" for the purpose of this Notification)

D. Intended Use and Level of Use of GRAS Substance

Sodium ARA is proposed for use in term infant formula as a source of ARA. Sodium ARA will be combined with other GRAS ingredients (described in detail below) to produce a commercial blend, and the blend will be used at a maximum level of 5.00% of total dietary fat when used in combination with docosahexaenoic acid (DHA) at a maximum level of 0.50% of total dietary fat and at a ratio ranging from 1:1 to 1:2 (DHA: ARA). A use level of 5.00% of Jost's Sodium ARA blend as a percentage of total dietary fat supplies 0.75% ARA as a percentage of total dietary fat.[2]

E. Statement of Availability of Data and Information

The analytical data, published studies, and information that are the basis for this GRAS determination are available for FDA review and copying at reasonable times at Keller and Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001. These documents will be sent to FDA upon request.

III. Criteria for GRAS Status

FDA previously has evaluated GRAS Notifications submitted for *M. alpina* oil sources of ARA. Specifically, FDA has issued "no questions" letters in response to the following GRAS Notifications:

- GRN 41 – ARASCO (arachidonic acid-rich single-cell oil) produced from fungal source (*M. alpina*)[3]
- GRN 80 – ARASCO (use extension of GRN 41)[4]

---

[2] These statements assume that infant formula is the sole food source for the term infant.

[3] GRN 41, Martek Biosciences, available at: http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm266169.pdf.

- GRN 94 – Arachidonic acid-rich oil from *Mortierella alpina* (AA-rich fungal oil)[5]

- GRN 326 – Refined Arachidonic Rich Oil (RAO) from *M. alpina* strain I_{49}-N_{18}[6]

It is respectfully submitted that this Notification establishes GRAS status for Sodium ARA for use in term infant formula based on: (1) the substantial equivalence of Sodium ARA to *M. alpina* oil as sources of ARA; (2) the fact that the intended use level for the Sodium ARA blend is substantially equivalent to the use levels for GRAS-notified *M. alpina* oil sources of ARA; and (3) the published safety data supporting the GRAS status of *M. alpina* oil.

IV. Detailed Information about the Identity of the Notified Substance

A. Identity

Chemical Name:	Sodium salts of *M. alpina* oil (namely: sodium arachidonate, sodium myristate, sodium palmitate, sodium palmitoleate, sodium stearate, sodium oleate, sodium linoleate, sodium-*gamma*-linolenate, sodium-homo-*gamma*-linolenate, sodium arachidate, sodium behenate, sodium lignocerate)
Source:	Reaction of sodium hydroxide (NaOH) with GRAS *M. alpina* oil source

B. Physical Attributes

Appearance:	Off white to cream colored powder
Odor and taste:	Characteristic and free from rancidity
Solubility:	Water soluble
Foreign matter:	Absent of any foreign matter

---

[4] GRN 80, Mead Johnson Nutritionals, available at: http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm266650.pdf (part I) and http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm266652 (part II).

[5] GRN 94, Ross Products Division, Abbott Laboratories, available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm266878 (part I) and http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm266881 (part II).

[6] GRN 326, Cargill, Inc., available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269507.

C. Composition and Specifications

Table 1 below provides the basic fatty acid compositional profile for Sodium ARA, as compared to the fatty acid compositional profiles provided for the *M. alpina* oil sources that were the subjects of GRN 41, GRN 80, GRN 94, and GRN 326. Because Sodium ARA will only be produced from a GRAS *M. alpina* oil source, the fatty acids and fatty acid ranges present in Sodium ARA necessarily will not deviate significantly from the profile of the GRAS triglyceride source.

Table 1 Fatty Acid Compositional Profile for Sodium ARA and GRAS-Notified *M. alpina* oil sources

Fatty Acid	Sodium ARA (present as sodium salts of the fatty acids)	ARASCO (GRN 41 and GRN 80)[7]	AA-rich oil, SUNTGA40S (GRN 94)[8]	RAO (GRN 326)[9]
Arachidonic acid (C20:4 n-6)	Minimum 15%	42.69-44.26%	38.6-42.4%	41.15-45.55%
Myristic acid (C14:0)	0.1-0.5%	0.34-0.58%	0.4-0.6%	0.23-0.35%
Palmitic acid (C16:0)	4.3-8.1%	7.17-9.59%	11.3-15.1%	5.43-6.87%
Palmitoleic acid (C16:1 n-9)	0.0-0.4%	NS*	ND**	0.02-0.04%
Stearic acid (C18:0)	4.2-7.6%	7.7-10.2%	7.5-8.0%	4.48-5.98%
Oleic acid (C:18:1 n-9)	3.4-9.5%	15.95-23.35%	6.2-6.7%	4.33-5.76%
Linoleic acid (C18:2 n-6)	3.8-15.2%	5.56-7.62%	NS	6.92-8.78%

[7] See GRN 41 at Table 7 (Analytical Specification of four lots of ARASCO).

[8] See GRN 94 at Table II-3 (Fatty acid analysis of AA-rich oil: SUNTGA25 and SUNTGA40S) (including analytical data from three batches of SUNTGA40S) and Table II-4 (Analysis of AA-rich oil: SUNTGA25 and SUNTGA40S) (including analytical data from three batches of SUNTGA40S).

[9] See GRN 326 at Table 6 (Other lipid parameters evaluated in five lots of RAO) and Table 9 (Fatty acid compositions of five RAO lots using fatty acid methyl ester analysis).

gamma-Linolenic acid (C18:3 n-6)	1.7-2.7%	2.45-2.99%	1.35-2.7%	NS
Arachidic acid (C20:0)	0.6-1.0%	0.83-0.96%	0.7-0.8%	0.69-0.80%
Homo-*gamma*-Linolenic acid (C20:3 n-6)	3.0-5.0%	1.43-2.57%	3.1-3.4%	3.61-3.77%
Behenic acid (C22:0)	2.5-4.1%	1.98-2.02%	2.2-3.0%	3.00-3.24%
Lignoceric acid (C24:0)	7.8-12.6%	1.85-2.04%	5.1-9.1%	9.37-10.50%
trans-Fatty acids	≤ 0.5%	< 1.0%	ND	0.40-0.51%
Free Fatty acids	≤ 0.45%	0.10-0.27%	0.025-0.065%	0.03-0.09%

* NS: Not specified
** ND: Not detected

Table 2 below provides the chemical and heavy metal specifications for Sodium ARA.

Table 2 Chemical and Heavy Metal Specifications for Sodium ARA

Peroxide value	Maximum 10 meq / kg
Anisidine value	Maximum 30
Lead*	≤ 0.1 ppm
Mercury*	≤ 0.05 ppm
Cadmium*	≤ 0.1 ppm
Arsenic*	≤ 0.1 ppm

* Method: ICP-MS

Table 3 below provides the microbial specifications for Sodium ARA.

Table 3 Microbial Specifications for Sodium ARA

Total plate count	Maximum 1000 cfu / g
Yeast and mold	Maximum 100 cfu / g
Coliforms	Negative / g

Table 4 below provides the results of a five-batch analysis to demonstrate that Sodium ARA complies with the specifications described in this GRAS Notification.

Table 4 Analysis of Five Batches of Sodium ARA

	Specification	**Lot Number TLB-II-162**	**Lot Number TLB-II-166**	**Lot Number TLB-II-167**	**Lot Number TLB-II-168**	**Lot Number TLB-II-169**
Fatty Acid Analysis						
Arachidonic acid (C20:4 n-6)	Minimum 15%	21.9%	21.6%	22.1%	22.2%	21.7%
Myristic acid (C14:0)	0.1-0.5%	0.445%	0.436%	0.440%	0.441%	0.425%
Palmitic acid (C16:0)	4.3-8.1%	6.30%	6.12%	6.16%	6.19%	5.96%
Palmitoleic acid (C16:1 n-9)	0.0-0.4%	0.106%	0.112%	0.114%	0.110%	0.111%
Stearic acid (C18:0)	4.2-7.6%	6.91%	6.58%	6.58%	6.66%	6.41%
Oleic acid (C:18:1 n-9)	3.4-9.5%	6.16%	6.75%	6.52%	6.36%	6.45%
Linoleic acid (C18:2 n-6)	3.8-15.2%	9.44%	9.53%	9.60%	9.50%	9.42%
gamma- Linolenic acid (C18:3 n-6)	1.7-2.7%	2.29%	2.33%	2.36%	2.32%	2.30%

Arachidic acid (C20:0)	0.6-1.0%	0.968%	0.833%	0.830%	0.849%	0.896%
Homo-*gamma*-Linolenic acid (C20:3 n-6)	3.0-5.0%	3.75%	3.82%	3.84%	3.84%	3.87%
Behenic acid (C22:0)	2.5-4.1%	3.38%	3.33%	3.35%	3.24%	3.45%
Lignoceric acid (C24:0)	7.8-12.6%	9.54%	8.89%	8.92%	9.11%	8.89%
Chemical and Heavy Metal Analysis						
Peroxide value	Maximum 10 meq / kg	0.0 meq / kg	0.0 meq / kg	0.0 meq / kg	0.0 meq / kg	0.0 meq / kg
Anisidine value	Maximum 30	2.17	0.0	0.63	3.82	0.0
Lead*	≤ 0.1 ppm	< 0.01 ppm	< 0.01 ppm	< 0.01 ppm	< 0.01 ppm	< 0.01 ppm
Mercury*	≤ 0.05 ppm	< 0.05 ppm	< 0.05 ppm	< 0.05 ppm	< 0.05 ppm	< 0.05 ppm
Cadmium*	≤ 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm
Arsenic*	≤ 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm
Microbial Analysis						
Total plate count	Maximum 1000 cfu / g	< 1000 cfu / g	< 1000 cfu / g	< 1000 cfu / g	< 1000 cfu / g	< 1000 cfu / g
Yeast and mold	Maximum 100 cfu / g	< 100 cfu / g	< 100 cfu / g	< 100 cfu / g	< 100 cfu / g	< 100 cfu / g
Coliforms	Negative / g	Negative	Negative	Negative	Negative	Negative

D. Manufacturing Process

Sodium ARA is produced in accordance with current Good Manufacturing Practices (cGMP) for food (21 C.F.R. Part 110). The production of Jost's Sodium ARA commercial blend takes place in three phases: (1) saponification of the ARA triglycerides to the free fatty acids; (2) conversion of the free fatty acids into the sodium salts; and (3) addition of other ingredients and drying. We describe each phase of production in detail below.

1. Phase I: Saponification of the triglyceride ARA source to the free fatty acids

An ARA-rich oil derived from *M. alpina* –containing primarily ARA triglycerides along with other fatty acid triglycerides -- is added to a reactor with stirring, followed by the addition of deionized water and sodium hydroxide (NaOH).[10] The suspension is heated to approximately 50-80°C under nitrogen gas with stirring.[11] Once the suspension changes to a viscous paste, a sample of the mixture is tested to determine the completeness of the reaction. The sample is analyzed by Fourier transform infrared spectroscopy (FTIR) to verify the extent of the conversion of the triglyceride to the free fatty acids. The disappearance of the ester carbonyl vibration of the triglyceride at ~ 1742 cm^{-1} signifies that the reaction is complete. Then deionized water is added and the reaction mixture is cooled to about 30°C. Upon cooling, a dilute aqueous solution of sulfuric acid is added.[12] The suspension is then stirred for 1-2 hours. The pH of the suspension is checked to ensure that the pH range is 2-3. Once the pH is stable, stirring is discontinued and the aqueous and organic phases of the suspension are allowed to separate. The aqueous phase is removed from the suspension and the organic phase (consisting of the free fatty acids) is washed with deionizing water. The aqueous wash is removed. An antioxidant blend is added and the suspension is stirred until uniform. The free fatty acids are drained into a commercial blender with weighing capabilities.

2. Phase II: Conversion of the free fatty acids into the sodium salts

The weight of the free fatty acids is obtained from the scale. Sodium or calcium phosphate is then added and the mixture is stirred until uniform. Based on the weight and titer of the free fatty acid, an equimolar amount of sodium hydroxide is added. The reaction mixture will become viscous and lighten in color with stirring. Once the reaction mixture has a paste-like consistency, the sample is analyzed by FTIR to verify the extent of the conversion of the free fatty acids to the corresponding sodium salts. The disappearance of the carboxylic acid vibration of the free fatty acid at ~ 1707 cm^{-1} signifies complete conversion to the sodium salt (Sodium ARA).

3. Phase III: Addition of other ingredients and drying

Although the subject of this GRAS Notification is Sodium ARA, Jost intends to add other GRAS ingredients to Sodium ARA to create a commercial blend. The Sodium ARA blend will be the commercial product. Once the Sodium ARA has been produced using the methods described above, the following ingredients are added: an antioxidant blend, sodium or calcium phosphate (as an anticaking agent), maltodextrin, sodium ascorbate, sodium citrate, and sodium caseinate. The mixture of Sodium ARA and these additional ingredients is stirred for sufficient time to uniformly mix the batch. Finally, hot water is circulated through the water jacket of the

---

10 GRAS affirmed in 21 C.F.R. § 184.1763.

11 GRAS affirmed in 21 C.F.R. § 184.1540.

12 GRAS affirmed in 21 C.F.R. § 184.1095.

mixture and the product is dried at approximately 50°C under vacuum with stirring. Once dry, the product is dispensed into clean food-grade containers, and blanketed with nitrogen gas to ensure oxidative stability.

Table 5 below describes the composition of the commercial Sodium ARA blend.

Table 5 Composition of Commercial Sodium ARA Blend[13]

Ingredient	FDA Regulatory Status	Quantity
Sodium arachidonate	GRAS per current Notification	15% minimum as ARA
Sodium salts of other fatty acids	GRAS per current Notification	Maximum 70%
Antioxidant blend (rosemary extract, mixed tocopherols, soybean oil, sunflower lecithin, ascorbyl palmitate)	Mixture of GRAS additives, e.g., 21 C.F.R. § 182.3890 ("tocopherols"); 21 C.F.R. § 182.3149 ("ascorbyl palmitate") and GRAS ingredients (rosemary extract, soybean oil, sunflower lecithin)	Maximum 0.5%
Sodium phosphate	GRAS per 21 C.F.R. § 182.1778	Maximum 35%
Calcium phosphate	GRAS per 21 C.F.R. § 182.1217	Maximum 2%
Maltodextrin	GRAS affirmed in 21 C.F.R. § 184.1444	Maximum 20%
Sodium caseinate	GRAS per 21 C.F.R. § 182.1748	Maximum 30%
Sodium ascorbate	GRAS per 21 C.F.R. § 182.3731	Maximum 3%
Sodium citrate	GRAS affirmed in 21 C.F.R. § 184.1751	Maximum 3%

V. Basis for GRAS Determination

A. Substantial Equivalence of Sodium ARA to *M. alpina* Oil

The primary basis for Jost's determination that Sodium ARA is GRAS is the fact that it is "substantially equivalent" to other GRAS sources of ARA, namely to GRAS-notified *M. alpina* oil sources of ARA.

FDA's 1997 GRAS Notice proposed rule included some discussion of the concept of "substantial equivalence" between ingredients. Specifically, FDA cited a 1996 report of a joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) consultation which noted as follows:

[13] The contents of this table represent the current composition of Jost's Sodium ARA commercial blend. Should Jost make any changes to the blend in the future, the company will use only safe and suitable ingredients that have an appropriate regulatory status for their intended use.

> [s]ubstantial equivalence embodies the concept that if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e. the food or food component can be concluded to be as safe as the conventional food or food component). Account should be taken of any processing that the food or food component may undergo as well as the intended use and the intake by the population.[14]

Also in the proposed rule, FDA indicated that it "believes that in certain instances the concept of substantial equivalence may have applicability to the technical element of a GRAS determination."[15] In practice, FDA applied a similar concept to its evaluation of the "safety of foods derived from new plant varieties, including the safety of newly introduced substances in the food (primarily proteins, carbohydrates, and fatty acids) and unintended changes in the food's composition."[16] Further, FDA applied substantial equivalence principles in affirming the GRAS status of "several microbially-derived chymosin (i.e., rennet) enzyme preparations...and several animal-and plant-derived enzyme preparations."[17]

FDA cautioned that the concept of substantial equivalence would not be particularly relevant where the differences between two substances outweigh the similarities. As a result, the key feature in applying the concept of substantial equivalence is the comparison of any difference in composition or characteristic properties between the substances. Most relevant to the current evaluation of Sodium ARA, FDA noted that:

> the product resulting from the chemical reaction of two or more GRAS substances is a discrete new substance that may have properties that are distinctly different from the individual GRAS substances from which it is synthesized or from a simple mixture of those GRAS substances. The concept of substantial equivalence may not be relevant here <u>unless the reaction product is widely recognized to be metabolized in the same way as the individual components from which it is synthesized</u>.[18]

Sodium ARA is a product resulting from the chemical reaction of two GRAS substances: sodium hydroxide and *M. alpina* oil. The only difference between the fatty acids in Sodium ARA and those in *M. alpina* oil is that the former are present in the form of their sodium salts.[19] Although the production of Sodium ARA produces a "discrete new substance," the various fatty acid salts dissociate to liberate arachidonate and the other fatty acid ions, each of which is

---

14 62 Fed. Reg. at 18944.

15 *Id.* at 18945.

16 *Id.*

17 *Id.* (internal citations omitted).

18 *Id.* (emphasis added).

19 As we describe in Part V.C.2, below, the presence of sodium ultimately does not affect the safety or GRAS status of the substance.

metabolized in the same way as the original fatty acids. In support of this conclusion, Jost has developed *in vitro* dissociation data which demonstrate that Sodium ARA easily dissociates to liberate the ARA free fatty acid under gastric conditions and has the same fatty acid composition as the source *M. alpina* oil.[20] These data demonstrate that Sodium ARA is bioavailable and will effectively deliver ARA to healthy infants in a manner directly comparable to the delivery of ARA by *M. alpina* oil.[21] Jost also analyzed five batches of Sodium ARA and compared their fatty acid profiles to those of the *M. alpina* oil starting material used to produce the sodium salts.[22] These data demonstrate the substantial comparability of the complete fatty acid profile between the starting material and Jost's Sodium ARA.

As further support of the substantial equivalence concept, FDA historically has not objected to companies' determinations that common salts of GRAS substances are GRAS based on their substantial similarity to a GRAS substance. Specifically, FDA has issued "no questions" letters in responses to GRAS Notifications covering sodium bisulfate,[23] potassium bisulfate,[24] sodium potassium hexametaphosphate (SKMP),[25] and magnesium dihydrogenpyrophosphate (MDPP).[26] In each case, the GRAS determination was based on the fact that the notified substances were substantially equivalent common salts of GRAS substances.

---

20 Eric A. Bruton, Ph.D., Jost Chemical, "Dissociation of Sodium Arachidonate" (2014), attached as Appendix A.

21 Currently, several major marketed infant formula products contain *M. alpina* oil as a source of ARA for infants. Infant formula manufactured by Abbott Nutrition and Nestle, S.A. identify the ARA ingredient as "*M. alpina* oil*" whereas Mead Johnson Nutrition's infant formulas identify it as "*Mortierella alpina* oil*". For both versions, the asterisk refers to an additional label statement declaring, "A source of arachidonic acid (ARA)". When healthy infants consume these products, the triglyceride source of ARA is cleaved in the gut by pancreatic lipases to liberate ARA as a free fatty acid. The ARA then enters the gut wall and is absorbed into the bloodstream.

22 Teresa Bandrowsky, Ph.D., Jost Chemical, "Fatty Acid Analysis of Five Replicate Formulation Batches of Sodium Arachidonate Salt Compared to the Same Fatty Acid Analysis of the Starting Cargill ARA Triglyceride" (2014), attached as Appendix B.

23 GRN 3, Jones Hamilton Co., available at: http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm264384.pdf (reasoning that sodium bisulfate is GRAS based on its similarity to sulfuric acid, which is GRAS under 21 C.F.R. § 184.1095).

24 GRN 60, Jones Hamilton Co., available at: http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm266359.pdf (reasoning that potassium bisulfate is GRAS based on its similarity to sodium bisulfate, which was the subject of GRN 3).

25 GRN 316, ICL Performance Products LP, available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269487 (reasoning that SKMP is GRAS due to its similarity to sodium hexametaphosphate and potassium hexametaphosphate, both of which FDA determined to be GRAS on the basis of a SCOGS report on phosphates).

26 GRN 383, Chemische Fabrik Budenheim KG, available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm274406 (reasoning that MDPP is GRAS due to its "significant equivalence" to existing GRAS phosphates).

The ability to rely on a GRAS position for a related substance depends on the extent and significance of the differences between the two substances. In the instant case, the evidence indicates that the differences between Sodium ARA and *M. alpina* oil sources of ARA are minor and immaterial to considerations of safety or GRAS status. Therefore, the GRAS positions described in the Notifications for other *M. alpina* oil sources of ARA in term infant formula support a comparable GRAS determination for Sodium ARA.

B. Substantial Equivalence in Intended Use Levels of Sodium ARA and *M. alpina* Oil in Infant Formula

As noted above, FDA already has evaluated GRAS Notifications for *M. alpina* oil sources of ARA in term infant formula. Table 4 below provides the intended use level of Jost's Sodium ARA blend, as compared to the use levels of *M. alpina* oil sources that were the subjects of GRN 41, GRN 80, GRN 94, and GRN 326. The use level for Jost's Sodium ARA blend is consistent with the use levels set forth in GRAS Notifications for *M. alpina* oil sources that similarly are used to supply ARA in term infant formula.

Table 4 Use Levels of Sodium ARA and GRAS-Notified *M. alpina* oil sources

	Sodium ARA blend	**ARASCO (GRN 41)[27]**	**ARASCO (GRN 80)[28]**	**AA-rich oil, SUNTGA40S (GRN 94)[29]**	**RAO (GRN 326)[30]**
Amount of ARA supplied in term infant formula	Maximum of 0.75% of total dietary fat	Maximum of 0.5% of total dietary fat	Maximum of 0.75% of total dietary fat	Maximum of 0.4% of total dietary fat	Maximum of 0.75% of total dietary fat
DHA to ARA ratio	1:1 – 1:2	1:1 – 1:2	1:1 – 1:2	1:2.7	1:1– 1:2.7

[27] See Letter from Alan M. Rulis, Ph.D., CFSAN/Office of Food Additive Safety to Henry Linsert, Jr., Martek Biosciences Corporation (May 17, 2001), available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154126.htm.

[28] See Letter from Alan M. Rulis, Ph.D., CFSAN/Office of Food Additive Safety to Thomas L. Ferguson, Mead Johnson Nutritionals (Dec. 11, 2001), available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154201.htm.

[29] See Letter from Laura M. Tarantino, Ph.D., CFSAN/Office of Food Additive Safety to Claire L. Kruger, Ph.D., Environ International Corporation (Apr. 16, 2006), available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154630.htm.

[30] See Letter from Mitchell A. Cheeseman, Ph.D., CFSAN/Office of Food Additive Safety to Phillip L. Casterton, M.S., D.A.B.T., Cargill, Incorporated (Feb. 16, 2011), available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm245246.htm.

C. Safety of ARA in Term Infant Formula

1. ARA Component

As FDA is aware from its review of GRAS Notifications for *M. alpina* oil sources of ARA in term infant formula, a wealth of published data demonstrate the safety and GRAS status of ARA derived from *M. alpina* oil sources for use in term infant formula. As described in this Notification, due to the substantial similarities between Sodium ARA and *M. alpina* oil sources of ARA, we incorporate by reference the safety discussions in GRN 41, GRN 80, GRN 94, and GRN 326 as supportive of the safety of Sodium ARA. Those GRAS Notifications contain the following key safety data to support the GRAS status of Sodium ARA:

- Clinical data establishing the role and functional importance of long-chain polyunsaturated fatty acids in the infant diet, particularly with respect to ARA and DHA. Data show that where infant formula is a sole or supplemental source of infant food, addition of ARA and DHA to the formula may support optimal nutritional status of infants.

- Data establishing that the target ARA levels described in previous GRAS Notifications (and in the current GRAS Notification) result in dietary exposure that is comparable to the intake of ARA from human breast milk and from commercially available infant formula products in both the U.S. and in Europe.

- An extensive body of animal studies and *in vitro* data establishing the safety of *M. alpina* sources of ARA, e.g., genotoxicity studies (bacterial reverse mutation assay, chromosome aberration assay, gene mutation assay), acute, subacute, and subchronic toxicity studies in rats; toxicity studies in neonatal piglets (postulated to be a better surrogate model for human infants).

- Data from infant clinical trials confirming the safety of ARA-supplemented infant formula.

2. Sodium Component

As noted above, the only difference between Sodium ARA and the GRAS-notified *M. alpina* oil sources is the presence of the fatty acids in the form of their sodium salts. The modest contribution that Sodium ARA will make to term infant formula and to the infant's overall dietary exposure to sodium poses no concerns either from a regulatory or a safety standpoint, as described below.

When used in accordance with the intended use level, Jost's Sodium ARA blend will contribute only a modest amount of additional sodium to a term infant formula product. FDA's nutrient specifications for infant formula provide a fairly wide range for sodium content in the finished product: 20 mg / kcal at the minimum and 60 mg / kcal at the maximum.[31] Infant

[31] 21 C.F.R. § 107.100(a).

formula manufacturers are responsible for ensuring that finished products comply with FDA's nutritional requirements for infant formula. Finished product manufacturers will be able to quantify the modest amount of sodium that Jost's Sodium ARA blend contributes to the recipe and remain responsible for ensuring that the finished product does not exceed the maximum level for sodium.

From the standpoint of dietary exposure, the sodium contributed by Jost's Sodium ARA blend is below the baseline adequate intake level (AI) for sodium for the infant population. An infant under one year of age consumes approximately 30 grams of fat per day.[32] The proposed intake of Jost's Sodium ARA blend is 5.00% of the dietary intake of fat. This implies a daily intake of 5.00% x 30 g = 1.5 g / day.[33] Jost's specification for total sodium in the Sodium ARA blend ranges from 6% to 12%.[34] Therefore, an infant's maximum sodium exposure from the Sodium ARA blend would be:

30 g x 5.00% x 12% = 0.18 g / day or 180 mg / day

The AI for sodium for an infant under one year of age ranges from 120 to 370 mg / day.[35] Because the infant formula to which Jost's Sodium ARA blend will be added is assumed to be the sole source of food for the infant, 180 mg of sodium safely may be accommodated into the formula to stay within the AI for sodium.

VI. Summary Basis for GRAS Determination

Jost has determined that Sodium ARA is Generally Recognized as Safe (GRAS) based on the following:

- The fact that Jost will produce Sodium ARA using an *M. alpina* oil source that is substantially equivalent to the sources covered in GRN 41, GRN 80, GRN 94, and GRN 326, for which FDA had no questions regarding GRAS status;
- The substantial equivalence of Sodium ARA and GRAS *M. alpina* oil sources of ARA;

---

32 *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (2002/2005). Summary table available at: http://www.iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/5_Summary%20Table%20Tables%201-4.pdf.

33 This estimate conservatively assumes that all components of Jost's Sodium ARA blend will supply sodium ions, which is not the case because the blend contains ingredients other than the sodium salts (e.g., antioxidants).

34 See Jost Chemical Co., R&D Data Sheet (Aug. 21, 2014), attached as Appendix C.

35 *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (2002/2005). Summary table available at: http://www.iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/5_Summary%20Table%20Tables%201-4.pdf.

- The substantial equivalence of use levels for the Sodium ARA blend and for *M. alpina* oil in term infant formula;
- Relevant published safety data on ARA derived from *M. alpina* oil; and
- The fact that the only difference between Sodium ARA and GRAS-notified *M. alpina* oil sources of ARA with respect to the infant diet is a modest contribution of sodium content, the level of which poses no concerns from the standpoint of dietary exposure.

VII. Conclusion

Based on the documentation provided in this GRAS Notification, and as discussed above, Jost has concluded that Sodium ARA is GRAS via scientific procedures for use as a source of ARA in term infant formula.

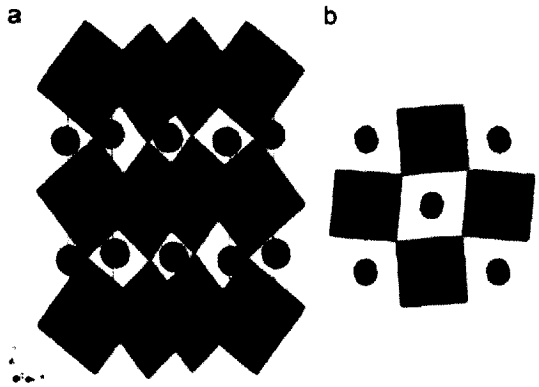
APPENDIX A

Eric A. Bruton, Ph.D., Jost Chemical, "Dissociation of Sodium Arachidonate" (2014)

2014

Jost Chemical

Eric A. Bruton, Ph.D.
Research Principal



[DISSOCIATION OF SODIUM ARACHIDONATE]

The sodium salt of arachidonic acid dissociates to the free fatty acid of arachidonic acid, along with the other free fatty acids present in the source oil, in simulated gastric environment without changing the fatty acid profile of the starting sodium arachidonate.

Introduction:

The polyunsaturated fatty acids (PUFA) arachidonic Acid (ARA) and docosahexaenoic Acid (DHA) have been shown to be essential in early child development.[1] There are several forms of PUFAs, free fatty acid (FFA), ethyl ester, triglyceride (Tg), or reesterified triglyceride, available as dietary supplements and pharmaceuticals. Several studies have examined the bioavailability of these forms side by side under different dietary conditions. The FFA forms of eicosapentaenoic acid (EPA) and DHA have been shown to have the same or better bioavailability as the Tg forms of EPA and DHA.[2,3]

Jost Chemical's sodium ARA salt, made from a fungal source of ARA, is a solid that is easy to handle and incorporate in foods and is able to be emulsified rapidly which makes it ideal for use in infant formula. The results of the work below will show that the simple sodium salt of ARA will dissociate in the acidic environments of the stomach to liberate the ARA as its FFA making it available for further digestion.

Conclusions:

The Na ARA easily dissociates to the ARA FFA and has the same fatty acid composition as the starting Na ARA showing that the gastric environment does not alter the ARA or any of the other fatty acids in the source oil.

---

[1] **a.** A. Sala-Vila, A.I. Castellote, C. Campoy, M. Rivero, M. Rodriguez-Palmero, M.C. López-Sabater, The Source of Long-Chain PUFA in Formula Supplements Does Not Affect the Fatty Acid Composition of Plasma Lipids in Full-Term Infants, J. Nutri 134 (2004) 868-873 **b.** K. Boswell. E.K. Koskelo, L. Carl, S. Glaza, D.J. Hensen, K.D. Williams, D.J. Kyle, Preclinical evaluation of single-cell oils that are highly enriched with arachidonic acid and docosahexaenoic acid, Food Chem. Toxicol. 34 (1996) 585-593 and references found therein.

[2] Review on bioavailability of EPA and DHA in their different forms: J.P. Schuchardt, A. Hahn, Bioavailability of long-chain omega-3 fatty acids, Prostaglandins Leukot. Essent. Fatty Acids 89 (2013) 1-8

[3] **a.** L.D. Lawson, B.G. Hughes, Human Absorption of Fish Oil Acids as Triglycerols, Free Acids, or Ethyl Esters Biochem. and Biophys. Research 152 (1988) 328-335; **b.** S.E. Boustani, C. Colette, B. Descomps, A. Crastes de Paulet, F. Mendy, Enteral Absorption in Man of Eicosapentaenoic Acid in Different Chemical Forms, Lipids 22 (1987) 711-714; **c.** J. Dyerberg, P. Madsen, J.M. Møller, I. Aardestrup, E.B. Schmidt, Bioavailability of marine n-3 fatty acid formulations, Prostaglandins Leukot. Essent. Fatty Acids 83 (2010) 137-141, **d.** M.H. Davidson, J. Johnson, M.W. Rooney, M.L. Kyle, D.F. Kling, A novel omega-3 free fatty acid formulation has dramatically improved bioavailability during a low-fat diet compared with omega-3-acid ethyl esters: The ECLIPSE (Epanova® compared to Lovaza® in a pharmacokinetic single-dose evaluation) study J. Clinc. Lipidol. 6 (2012) 573-584.

Results & Discussion:

Na ARA –

The dissociation of the unformulated Na ARA salt recovered 95% of the expected weight (0.89 g of the 0.94 g expected). The equation below, gives the theoretical weight of FFA in the Na ARA salt. The determined average molecular weight of the collection of FFAs from Cargill's fungal source of ARA was 319.9 g/mol FFA for the lot used in this experiment and thus the molecular weight of the corresponding Na FFA salts would be 341.9 g/mol.

$$1.00g \times \frac{mol}{341.9\ g} \times \frac{319.9g}{mol} = 0.94\ g$$

GC analysis of the FFA extracted in the dissociation process show the same components are present as in the sample prepared from the starting Na ARA (Figure 1~~Figure 1~~).

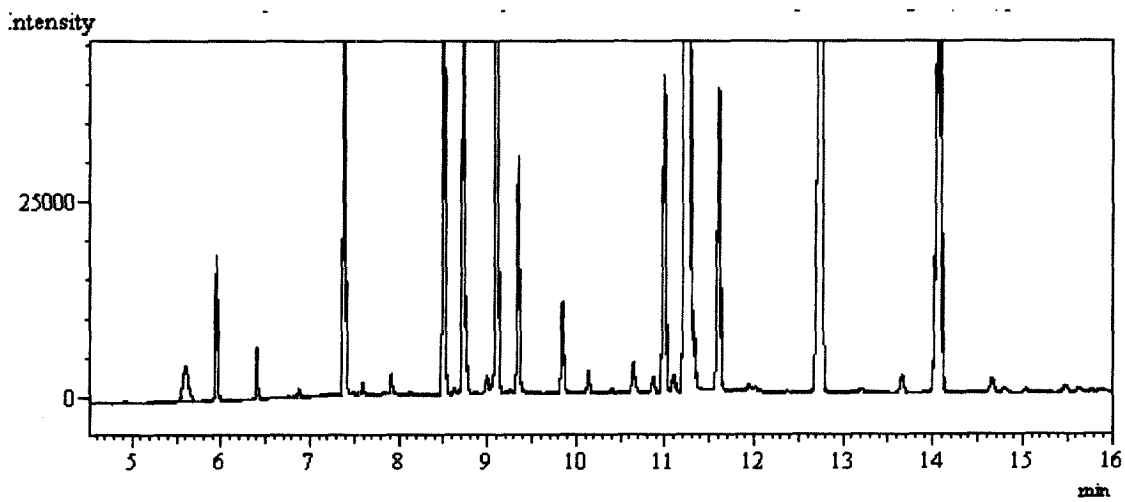


Figure 1: GC Chromatograms of dissociated Na ARA sample (red) and starting sample of Na ARA (blue)

Experimental:

Dissociation method and analysis

- *Dissociation in simulated gastric fluid*

  To a 250-mL round bottom flask 1.00 g of sodium salt of either ARA or DHA is added followed by 100 mL of simulated gastric fluid without pepsin. The flask is then put in a ~37°C water bath for 1 hour stirring at 350 rpm with a 1" magnetic stir bar. Once the solution has stirred for an hour, 25 mL of Isooctane is added the flask. The two phase solution is stirred for about 15 minutes to allow the isooctane to warm up and dissolve the free fatty acid. The contents of the flask are then transferred to a separatory funnel to separate the two phases. The 250-mL flask is rinsed with two aliquots of 20 mL isooctane which are transferred to the separatory funnel. The aqueous phase was dropped off and the organic layer was washed twice with DI water. The isooctane was transferred to a weighed 250-mL flask. The isooctane is then stripped off. The weight of the contents of the flask gives the organic content of the starting salt.

- *Simulated gastric fluid*

  USP Reagents Test Solutions: Gastric Fluid, Simulated, TS — 2.0 g of sodium chloride and 7.0 mL of hydrochloric acid were mixed and diluted to 1000 mL with DI water. For the purpose of this testing the pepsin was left out, which is commonly done for dissolution or disintegration of monograph materials when not needed.

- GC Method (See method JGM-601)

APPENDIX B

Teresa Bandrowsky, Ph.D., Jost Chemical, "Fatty Acid Analysis of Five Replicate Formulation Batches of Sodium Arachidonate Salt Compared to the Same Fatty Acid Analysis of the Starting Cargill ARA Triglyceride"

Teresa Bandrowsky, PhD

Fatty Acid Analysis of Five Replicate Formulation Batches of Sodium Arachidonate Salt Compared to the Same Fatty Acid Analysis of the Starting Cargill ARA Triglyceride

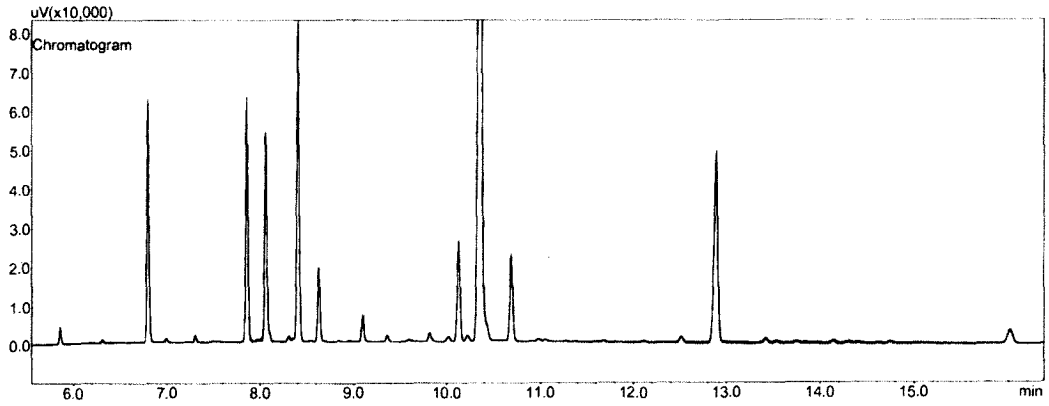
To determine and demonstrate the reproducibility of preparing formulated sodium arachidonate salts, a series of five batches of the salt have been prepared. Each batch was analyzed to establish a free fatty acid profile. Similarly, the starting material, Cargill arachidonic acid oil in the triglyceride form was analyzed.

The five sodium arachidonate salts were analyzed by gas chromatography (GC) of FAMES in order to elucidate their full fatty acid breakdown. The results indicate that conversion of the Cargill arachidonic acid oil (starting triglyceride) to the sodium arachidonate salt does not alter the free fatty acid profile. The full fatty acid breakdowns of the five replicate batches of the sodium arachidonate salt were identical to the Cargill arachidonic acid oil (starting triglyceride). The area percentages for each of the fatty acids within the sodium arachidonate salts were within the typical ranges described for the Cargill arachidonic acid oil (starting triglyceride) in the certificate of analysis.

The Cargill arachidonic acid oil (triglyceride) and the five sodium arachidonate salts were converted to the methyl esters and analyzed using a modification of GC method JGM-601. The Cargill arachidonic acid oil (triglyceride) was analyzed at the same time as the sodium arachidonate salts.

GC analyses of the five replicate batches of sodium arachidonate salt indicate that identical components are present in all five samples. This data is represented by Figure 1.

Figure 1. Overlay of the chromatograms of the five replicate batches of sodium arachidonate salt, TLB-II-162 through TLB-II-169



The area percentages for the individual fatty acids within the sodium arachidonate salts for each of the five replicate batches are given in Table 1. The area percentages for the fatty acids present in the Cargill arachidonic acid oil (starting triglyceride) that was used to prepare the sodium arachidonate salts are also provided for comparison. All the area

percentages for the individual fatty acids within the sodium arachidonate salts correspond to those of the Cargill arachidonic acid oil (starting triglyceride).

Table 1. GC FAME analysis of the starting material, the Cargill triglyceride, and each of the five sodium arachidonate salts.

Free fatty acid	Cargill (TG) Area %	TLB II 162 Area %	TLB II 166 Area %	TLB II 167 Area %	TLB II 168 Area %	TLB II 169 Area %
Myristic (C 14:0)	0.426	0.445	0.436	0.440	0.441	0.425
Palmitic (C 16:0)	6.220	6.303	6.124	6.166	6.195	5.967
Palmitoleic (C 16:1 *n*-9)	0.121	0.106	0.112	0.114	0.110	0.111
Stearic (C 18:0)	6.634	6.915	6.584	6.580	6.660	6.417
Oleic (C 18:1 *n*-9)	6.234	6.160	6.759	6.525	6.360	6.451
Linoleic (C 18:2 *n*-6)	9.628	9.445	9.533	9.601	9.502	9.426
gamma Linolenic (C 18:3 *n*-6)	2.347	2.295	2.334	2.363	2.323	2.308
Arachidic (C 20:0)	0.866	0.968	0.833	0.830	0.849	0.896
Homo-gamma Linolenic (C 20:3 *n*-6)	3.790	3.750	3.821	3.843	3.842	3.872
Arachidonic (C 20:4 *n*-6)	46.368	46.053	46.731	46.855	47.009	46.696
Behenic (C 22:0)	3.317	3.384	3.331	3.352	3.245	3.458
Lignoceric (C 24:0)	9.564	9.545	8.892	8.920	9.119	8.898

The range for the individual fatty acids from the original certificate of analysis for the Cargill arachidonic acid oil (starting triglyceride) is provided in the first column of Table 2. The data in Table 2 illustrates that the free fatty acid profiles for the Cargill triglyceride and the five batches of the sodium arachidonate salt all fall within the range. If even one of the component fatty acids in the sodium arachidonate salt batches falls out of the range, then it would indicate that the composition of the fatty acid mixture has been modified. The fact that the Cargill starting triglyceride fatty acid profile corresponds exactly with the fatty acid acid profile of each of the five replicate sodium salt batches shows that our chemistry did not change the composition of the Cargill starting triglyceride. The data also suggests that the sodium arachidonate salt should have the same nutritional properties as the starting triglyceride since the same components are present at the same concentrations.

Table 2. Standard deviation of the area percent of the individual fatty acids from the five sodium arachidonate salts.

Free fatty acid	Area % Range of the TG	Standard Deviation	Mean	% RSD
Myristic (C 14:0)	0.1 - 0.5	0.00764	0.437	1.74564
Palmitic (C 16:0)	4.3 - 8.1	0.12232	6.151	1.98864
Palmitoleic (C 16:1 *n*-9)	0 - 0.4	0.00297	0.111	2.68217
Stearic (C 18:0)	4.2 -7.6	0.18173	6.631	2.74053

Oleic (C 18:1 *n*-9)	3.4 -9.5	0.21983	6.451	3.40770
Linoleic (C 18:2 *n*-6)	3.8 -15.2	0.07033	9.501	0.74021
gamma Linolenic (C 18:3 *n*-6)	1.7 - 2.7	0.02606	2.325	1.12120
Arachidic (C 20:0)	0.6 -1.0	0.05821	0.875	6.65134
Homo-gamma Linolenic (C 20:3 *n*-6)	3.0 - 5.0	0.04599	3.826	1.20223
Arachidonic (C 20:4 *n*-6)	≥ 40	0.36545	46.669	0.78308
Behenic (C 22:0)	2.5 - 4.1	0.07767	3.354	2.31572
Lignoceric (C 24:0)	7.8 - 12.6	0.27914	9.075	3.07600

Figures 2 through 6 are the overlays of the GC chromatograms from the Cargill starting triglyceride and each of the five batches of the sodium arachidonate salt. The chromatograms indicate that identical components are present in both the Cargill triglyceride and the sodium arachidonate salts.

Figure 2. Chromatogram of TLB-II-162 (black) compared to the Cargill triglyceride (pink)

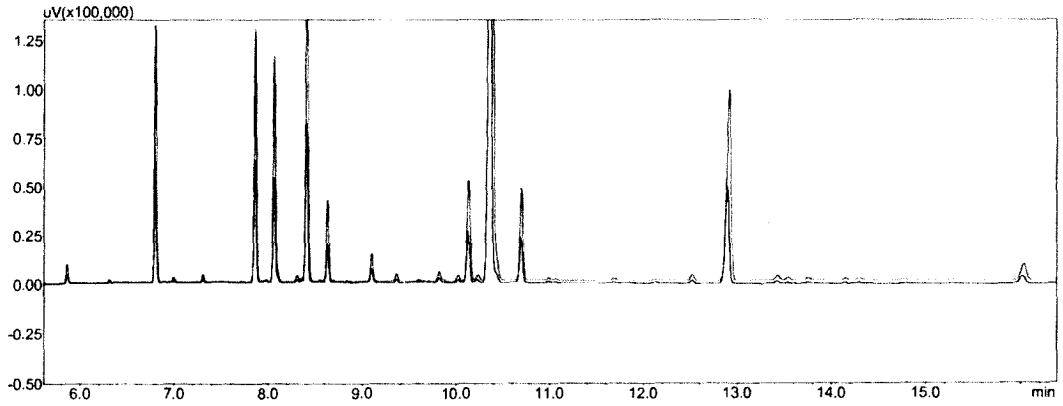


Figure 3. Chromatogram of TLB-II-166 (black) compared to the Cargill triglyceride (pink)

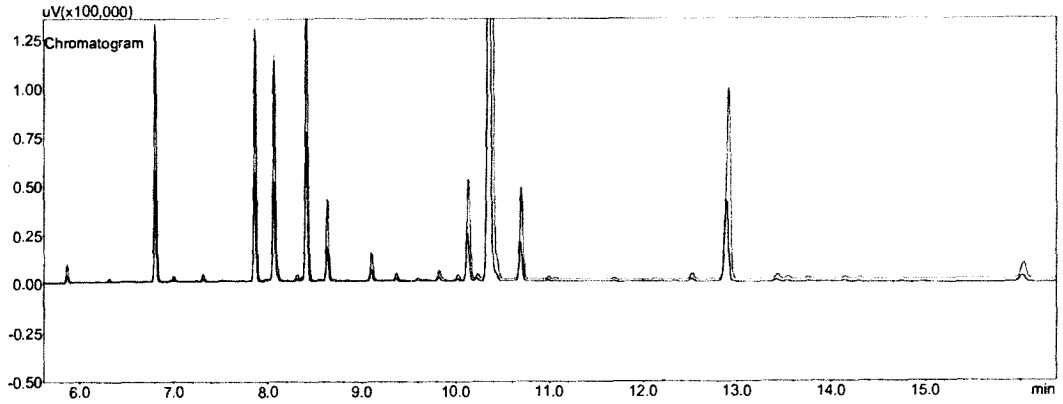


Figure 4. Chromatogram of TLB-II-167 (black) compared to the Cargill triglyceride (pink)

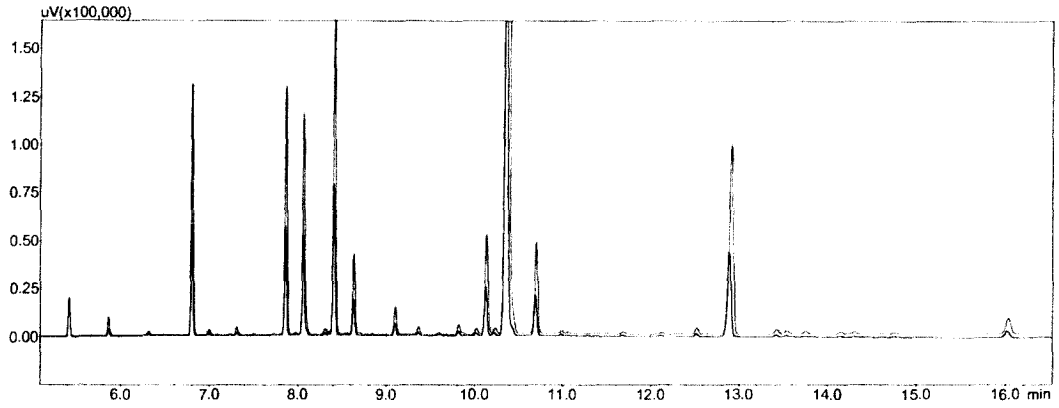


Figure 5. Chromatogram of TLB-II-168 (black) compared to the Cargill triglyceride (pink)

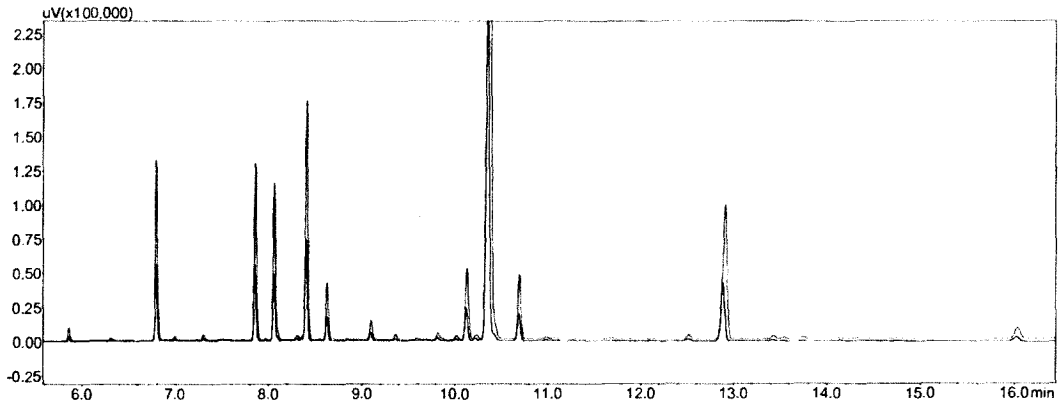
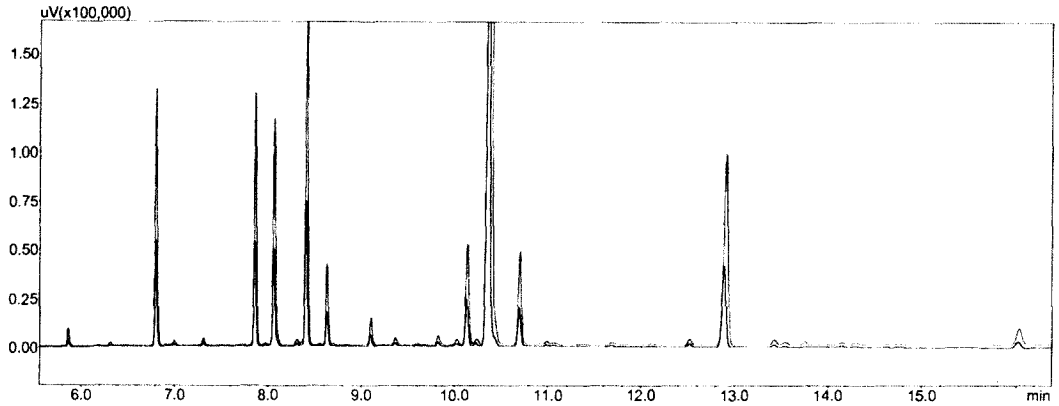


Figure 6. Chromatogram of TLB-II-169 (black) compared to the Cargill triglyceride (pink)

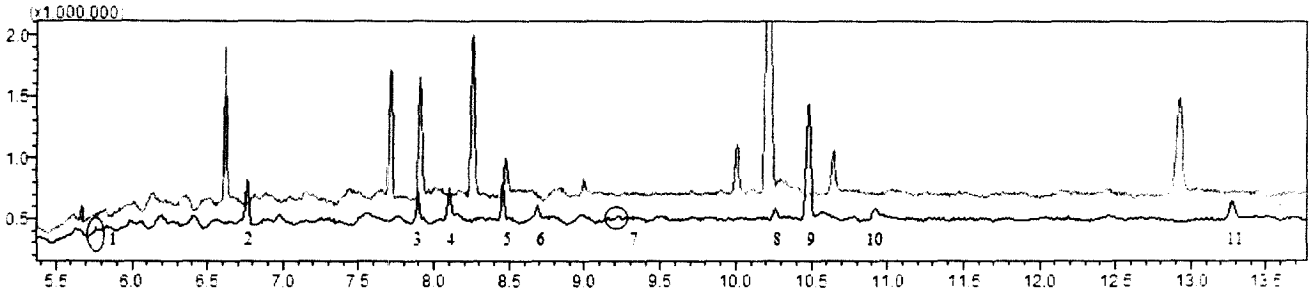


Gas chromatography-mass spectrometry (GC-MS) analysis was used to identify the individual components within the Cargill triglyceride and the five replicate batches of sodium arachidonate salt. Most compounds have a distinct fragmentation pattern that allows for positive identification of the chemical based on structure. There are twelve specific fatty acids listed on the Cargill arachidonic acid oil (starting triglyceride) certificate of analysis (eleven listed in the fatty acid profile plus arachidonic acid). Eleven of the twelve fatty acids have been identified in both the Cargill triglyceride and the sodium arachidonate salts by GC-MS. The twelfth fatty acid, palmitoleic acid, was not observed in either analysis as a result of its low concentration range (0 – 0.4 %). The palmitoleic acid was identified and quantified in the GC analysis using an authentic GLC standard of the acid purchased from Nu-Chek Prep, Inc.

In Figure 7, the chromatograms of one of the sodium arachidonate salts (TLB-II-169) and the Cargill triglyceride are off-set by 0.2 seconds and are representative of all the salts analyzed. GC-MS indicates that the same components are indeed present in the triglyceride and the sodium arachidonate salts.

The identification of the individual fatty acids along with the fragmentation patterns for the Cargill triglyceride and the sodium arachidonate salt (TLB-II-169) are provided in separate pdf files.

Figure 7. GC-MS of the Cargill triglyceride (pink) and a sodium arachidonate salt (TLB-II-169 black).



(b) (6)

Signature

APPENDIX C

Jost Chemical Co., R&D Data Sheet (Aug. 21, 2014)

JOST CHEMICAL CO.
8150 LACKLAND ST. LOUIS, MO 63114 TEL. 314-428-4300 FAX 314-428-4366
www.jostchemical.com

R&D Data Sheet

Effective Date: August 21, 2014

Section 1: Product Identification

Chemical name:	Sodium Arachidonate Powder
Vegetable oil from fungi:	Mortierella alpina

Section 2: Physical Attributes

Appearance:	Off white to cream colored powder
Odor and taste:	Characteristic and free from rancidity
Solubility:	Water Soluble
Foreign matter:	Absent of any foreign matter

Section 3: Composition / Information on Ingredients

Ingredients	**Amounts**
Antioxidant Blend	Max 0.5 %
Calcium phosphate	Max 2 %
Maltodextrin	Max 20 %
Sodium ascorbate	Max 3 %
Sodium arachidonate	Min 15 % , as arachidonic acid, (C20:4 *n*-6)
Sodium caseinate	Max 30 %
Sodium citrate	Max 3 %
Sodium phosphate	Max 35 %
Other sodium free fatty acids	Max 70 %

Section 4: Chemical Analysis

Peroxide value:	Max. 10 meq/kg
Anisidine value:	Max. 30
Moisture	Max. 8%

JOST CHEMICAL CO.
8150 LACKLAND ST. LOUIS, MO 63114 TEL. 314-428-4300 FAX 314-428-4366
www.jostchemical.com

Elemental Analysis	Method: ICP - MS
Lead	≤ 0.1 ppm
Mercury	≤ 0.05 ppm
Cadmium	≤ 0.1 ppm
Arsenic	≤ 0.1 ppm
Sodium	6 -12 %

R&D Data Sheet

Microbial Analysis	
Total plate count	< 1000 cfu / g
Yeast and Mould	< 100 cfu / g
Coliforms / g	Negative

Other Analysis Method: Gas Chromatography analysis of FAMES

Fatty acid Profile (Area Percent of Total Fatty Acid)	**Typical Results**
Myristic acid (C14:0)	0.1 - 0.5
Palmitic acid (C16:0)	4.3 - 8.1
Palmitoleic acid (C16:1 *n*-9)	0 - 0.4
Stearic acid (C18:0)	4.2 - 7.6
Oleic acid (C18:1 *n*-9)	3.4 - 9.5
Linoleic acid (C18:2 *n*-6)	3.8 - 15.2
gamma-Linolenic acid (C18:3 *n*-6)	1.7 - 2.7
Homo-*gamma*-Linolenic acid (C20:3 *n*-6)	3.0 - 5.0
Arachidic acid (C20:0)	0.6 - 1.0
Behenic acid (C22:0)	2.5 - 4.1
Lignoceric acid (C24:0)	7.8 - 12.6

Storage This product should be stored in the tightly closed original packaging in cool and dry conditions. It should be used as soon as possible after opening.

SUBMISSION END