

GRN 000914

Morgan Lewis

Kathleen M. Sanzo

Partner
+1.202.739.5209
kathleen.sanzo@morganlewis.com



February 25, 2020

VIA FEDEX

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Dear Sir or Madam:

Pursuant to 21 C.F.R. § 170.205, attached please find one copy of completed and signed FDA Form 3667 submitted on behalf of Kao Corporation and GRAS Notice for Alpha-Linolenic Acid Diacylglycerol (ALA DAG) Oil for the Addition to Finished Food.

Please contact me if you have any questions.

Sincerely,

[Redacted signature area]

Kathleen M. Sanzo

Enclosures

c: Richard E. Bonnette, M.S., Division of Food Ingredients Center for
Food Safety and Applied Nutrition

Morgan, Lewis & Bockius LLP

1111 Pennsylvania Avenue, NW
Washington, DC 20004
United States

GRAS Notice for *Alpha*-Linolenic Acid Diacylglycerol (ALA DAG) Oil for the Addition to Finished Food

Submitted by:

Morgan, Lewis & Bockius LLP
1111 Pennsylvania Ave, NW
Washington, DC
20004-2541, USA

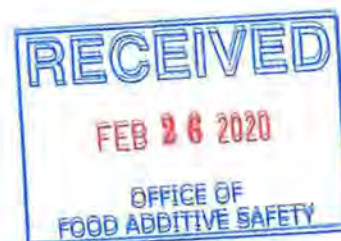
On behalf of:

Kao Corporation
2-1-3 Bunka
Sumida-ku, Tokyo
131-8501, Japan

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740

February 25, 2020



GRAS Notice for *Alpha*-Linolenic Acid Diacylglycerol (ALA DAG) Oil for the Addition to Finished Food

TABLE OF CONTENTS

PART 1.	SIGNED STATEMENTS AND CERTIFICATION (21 CFR § 170.225)	4
1.1	Name and Address of Notifier	4
1.2	Common Name of Notified Substance	5
1.3	Conditions of Use	5
1.4	Basis for GRAS.....	5
1.5	Availability of Information.....	5
PART 2.	IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT (21 CFR § 170.230)	6
2.1	Identity of the ALA DAG Oil.....	6
2.2	Composition of the ALA DAG Oil.....	6
2.3	Physical and Chemical Characteristics of the ALA DAG Oil.....	8
2.4	Method of Manufacture	8
2.5	Product Specifications	11
2.6	Batch Analyses.....	11
2.7	Additional Chemical Characterization.....	13
2.7.1	Glycidyl Esters.....	13
2.7.2	Saponification Value and Unsaponifiable Matter.....	13
2.8	Stability of the ALA DAG Oil.....	14
PART 3.	DIETARY EXPOSURE (21 CFR § 170.235)	15
3.1	Proposed Uses and Use Levels of ALA DAG Oil.....	15
3.2	Background Dietary Intakes of ALA DAG	15
3.3	Background Intakes of ALA	16
3.4	Estimated Daily Intakes of the ALA DAG Oil Based on the Intended Food Uses.....	18
PART 4.	SELF-LIMITING LEVELS OF USE (21 CFR § 170.240)	18
PART 5.	EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958 (21 CFR § 170.245).....	18
PART 6.	NARRATIVE (21 CFR § 170.250).....	18

6.1	Metabolic Fate of the ALA DAG Oil	19
6.1.1	Absorption and Distribution	19
6.1.2	Metabolism and Excretion	20
6.1.3	Interactions with Lipid-Soluble Vitamins.....	21
6.2	Toxicological Studies.....	23
6.2.1	Repeat-Dose Toxicity	23
6.2.2	Developmental and Reproductive Toxicity	27
6.2.3	Chronic Toxicity and Carcinogenicity.....	28
6.2.4	Mutagenicity and Genotoxicity.....	29
6.3	Human Studies.....	31
6.4	Allergenicity	39
6.5	Overall Conclusions Related to Safety	39
PART 7.	LIST OF SUPPORTING DATA AND INFORMATION (21 CFR § 170.255)...	41

List of Figures and Tables

Figure 2.1-1	Chemical Structure of the ALA DAG Oil.....	6
Figure 2.4-1	Schematic of the Manufacturing Process for the ALA DAG Oil.....	10
Table 2.2-1	Compositional Analysis of 3 Non-Consecutive Lots of ALA DAG Oil.....	7
Table 2.2-2	Identified Phytosterol Content of 3 Non-Consecutive Lots of ALA DAG Oil..	7
Table 2.2-3	Fatty Acid Distribution of 3 Non-Consecutive Lots of ALA DAG Oil.....	7
Table 2.3-1	Physical and Chemical Properties of 3 Non-Consecutive Lots of ALA DAG Oil and Flaxseed Oil	8
Table 2.5-1	Product Specifications for the ALA DAG Oil.....	11
Table 2.6-1	Results of Batch Analysis of 3 Non-Consecutive Lots of the ALA DAG Oil .	11
Table 2.7.1-1	Results of Analysis for Glycidyl Esters in 3 Non-Consecutive Lots of ALA DAG Oil	13
Table 2.7.2-1	Results of Analysis for Saponification Value and Unsaponifiable Matter in 3 Non-Consecutive Lots of ALA DAG Oil.....	13
Table 2.8-1	Results of the 1-Month Stability Study on the ALA DAG Oil	14
Table 2.8-2	Results of the 18-Month Shelf-Life Stability Study on the Final ALA DAG Product ^a	15
Table 3.2-1	Dietary Consumption of ALA DAG from Flaxseed, Flaxseed Oil, and Rapeseed Oil.....	16
Table 3.3-1	ALA Content of Various Foods ^a	17

Table 6.3-1 Summary of Clinical Studies on the ALA DAG Oil 34
Table 6.4-1 Results of Analysis for Protein Content in 3 Non-Consecutive Lots of ALA
DAG Oil 39

List of Appendices

Appendix A – Summary and Conclusions of the GRAS Panel


GRAS Notice for *Alpha*-Linolenic Acid Diacylglycerol (ALA DAG) Oil for the Addition to Finished Food

Part 1. Signed Statements and Certification (21 CFR § 170.225)

Kao Corporation (“Kao”), through its attorneys, Morgan, Lewis & Bockius LLP (“ML&B”) hereby informs the United States Food and Drug Administration (U.S. FDA) that Kao has concluded that *alpha*-linolenic acid diacylglycerol (ALA DAG) oil, as manufactured by Kao, meeting the specifications as described below, is Generally Recognized as Safe (GRAS) under the conditions of intended use as described below, and is therefore not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. In accordance with 21 C.F.R. § 170.30(a), this conclusion is based on the views of a convened panel of experts who are qualified by scientific training and experience and who, using scientific procedures, have reviewed all unfavorable and favorable information known to Kao that are pertinent to the safety evaluation of the ALA DAG oil for the addition to finished food.

All data and information presented in Parts 2 through 7 of this GRAS Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential. Therefore, the data and information that are presented herein are not exempt from the Freedom of Information Act (5 U.S.C. § 552).

Signed,


Kathleen M. Sanzo

Partner

Morgan Lewis & Bockius, LLP

kathleen.sanzo@morganlewis.com

on behalf of Kao Corporation

2-25-2020
Date

1.1 Name and Address of Notifier

Kao Corporation
2-1-3 Bunka
Sumida-ku, Tokyo

Morgan, Lewis & Bockius LLP
1111 Pennsylvania Ave, NW
Washington, DC
20004-2541, USA

1.2 Common Name of Notified Substance

The notified substance is *alpha*-linolenic acid diacylglycerol (ALA DAG) oil.

1.3 Conditions of Use

The ALA DAG oil is intended for the direct addition to finished food by consumers. The ingredient is not intended for use in infant formula or meat and poultry products under the jurisdiction of the United States Department of Agriculture. Similar to other oils, *e.g.*, olive oils, or dressings and margarines containing oil, ALA DAG oil can be added to vegetables, salads, or consumed with grain products (*e.g.*, bread and pasta) by the final consumer. The recommended daily intake of ALA DAG oil is 2.5 g, and the product will be labeled with that serving size information and recommended daily amount.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a) and (b) of the Code of Federal Regulations (CFR), the ALA DAG oil, as described herein, has been concluded to have GRAS status for the addition to finished food, on the basis of scientific procedures, as described herein.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the U.S. FDA for review and copying upon request during business hours at the offices of:

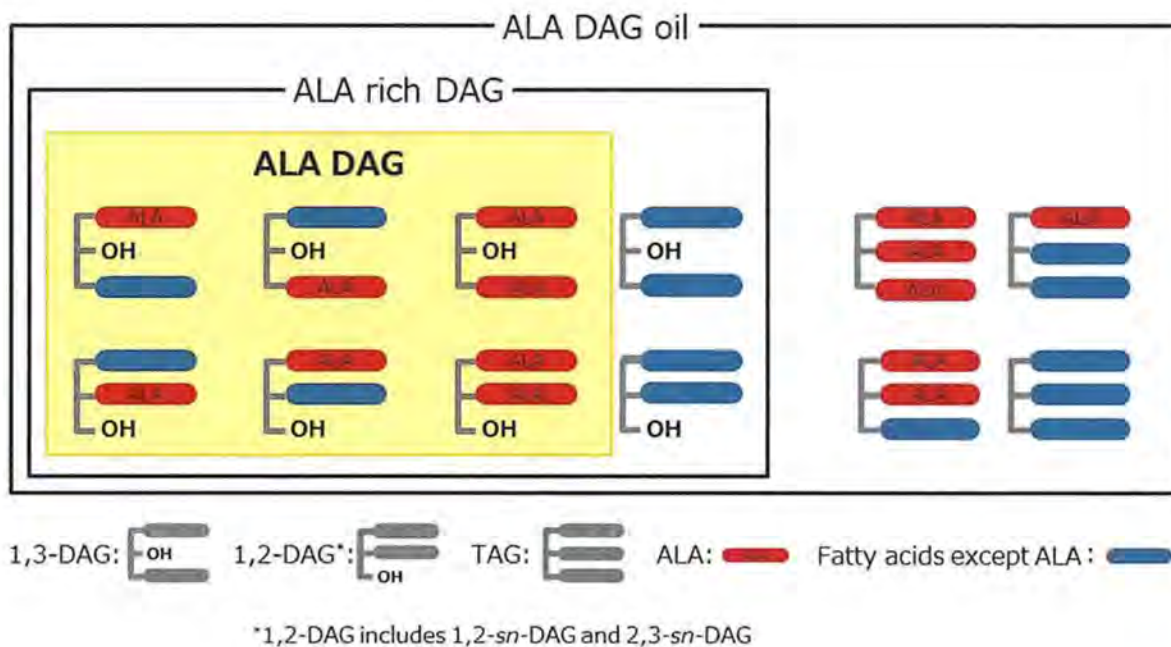
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Ave, NW
Washington, DC
20004-2541, USA

Part 2. Identity, Method of Manufacture, Specifications, and Physical or Technical Effect (21 CFR § 170.230)

2.1 Identity of the ALA DAG Oil

The ALA DAG oil is manufactured through enzymatic esterification of fatty acids derived from edible oil from flaxseed, with either monoacylglycerol or glycerol. The resulting product is composed primarily of diacylglycerol (DAG), with small quantities of monoacylglycerol (MAG) and triacylglycerol (TAG). The *alpha*-linolenic acid (ALA) is bound to the glycerol backbone at the 1-, 2-, or 3- positions in any combination as shown in Figure 2.1-1.

Figure 2.1-1 Chemical Structure of the ALA DAG Oil



2.2 Composition of the ALA DAG Oil

Compositional analysis of the ALA DAG oil was performed on 3 non-consecutive lots using an internal method based on capillary gas-liquid chromatography (GLC). The internal method was developed by Kao and validated. The results demonstrate that the ALA DAG oil is primarily composed of DAG (mean = 83%), and to a lesser extent, MAG (mean = 0.8%), TAG (mean = 14%), and free fatty acids (mean = 0.2%). The analytical results are summarized in Table 2.2-1 below.

Table 2.2-1 Compositional Analysis of 3 Non-Consecutive Lots of ALA DAG Oil

Ester Distribution (%)	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
Free fatty acids ^a	0.1	0.1	0.3
Monoacylglycerol	0.4	0.9	1.0
Diacylglycerol	82.4	85.9	80.4
Triacylglycerol	14.7	13.1	14.3
Total acylglycerols	97.6	100.0	96.0

^a Free fatty acids (%) is calculated from the acid values as C18:1.

The same lots were analyzed for the presence of phytosterols that may be carried over from the starting material (*i.e.*, flaxseed oil). The ALA DAG oil contains small quantities of sterols (see Table 2.2-2 below) and are consistent with the levels of phytosterols reported in a previous GRAS notice (GRN 256) for high linolenic acid flaxseed oil that received “no questions” from the U.S. FDA (U.S. FDA, 2009a). Furthermore, the levels of phytosterols are consistent with commercially available cold-pressed flaxseed oil (Tańska *et al.*, 2016).

Table 2.2-2 Identified Phytosterol Content of 3 Non-Consecutive Lots of ALA DAG Oil

Analysis Parameter	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
Campesterol (mg/100 g)	110	147	144
Stigmasterol (mg/100 g)	30	30	35
Sitosterol (mg/100 g)	204	284	288
Total (%)	0.344	0.461	0.467

The fatty acid composition of the DAG component was determined using capillary GLC. The results of the analysis are summarized in Table 2.2-3 below. *Alpha*-linolenic acid (ALA; C18:3) is the most abundant fatty acid in the ingredient, with lower levels of oleic acid, linoleic acid, stearic acid, and palmitic acid present.

Table 2.2-3 Fatty Acid Distribution of 3 Non-Consecutive Lots of ALA DAG Oil

Fatty Acid (%)	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
C16:0	3.3	2.5	2.4
C18:0	2.0	1.5	1.6
C18:1	23.1	26.1	23.2
C18:2	16.6	16.3	16.0

Table 2.2-3 Fatty Acid Distribution of 3 Non-Consecutive Lots of ALA DAG Oil

Fatty Acid (%)	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
C18:3	53.6	51.1	55.5
Others ^a	1.4	2.5	1.3
Total	100.0	100.0	100.0

C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2 = linoleic acid; C18:3 = *alpha*-linolenic acid.

^a “Others” includes fatty acids such as C16:2, C20:0, C20:1, *etc.*

2.3 Physical and Chemical Characteristics of the ALA DAG Oil

The physical and chemical properties of 3 non-consecutive lots of ALA DAG oil and flaxseed oil, the starting material, are summarized in Table 2.3-1 below. The smoke point, flash point, and ignition point of ALA DAG oil are similar to flaxseed oil. In comparison, the ALA DAG oil is slightly more viscous compared to flaxseed oil.

Table 2.3-1 Physical and Chemical Properties of 3 Non-Consecutive Lots of ALA DAG Oil and Flaxseed Oil

Parameter	Manufacturing Lot			
	Flaxseed Oil ^a	Lot No. B	Lot No. C	Lot No. D
Specific gravity (g/cm ³)	0.926 (20°C)	0.937 (20°C)	0.937 (20°C)	0.942 (20°C)
Viscosity (mPas)	44.8 (24.4°C)	53.4 (22.3°C)	54.3 (21.7°C)	49.8 (23.4°C)
Smoke point (°C)	214	209	202	196
Flash point (°C)	315	285	286	288
Ignition point (°C)	350	329	341	313

ALA DAG oil = *alpha*-linolenic acid diacylglycerol oil.

^a Sourced from the Nisshin Oillio group, Ltd.

2.4 Method of Manufacture

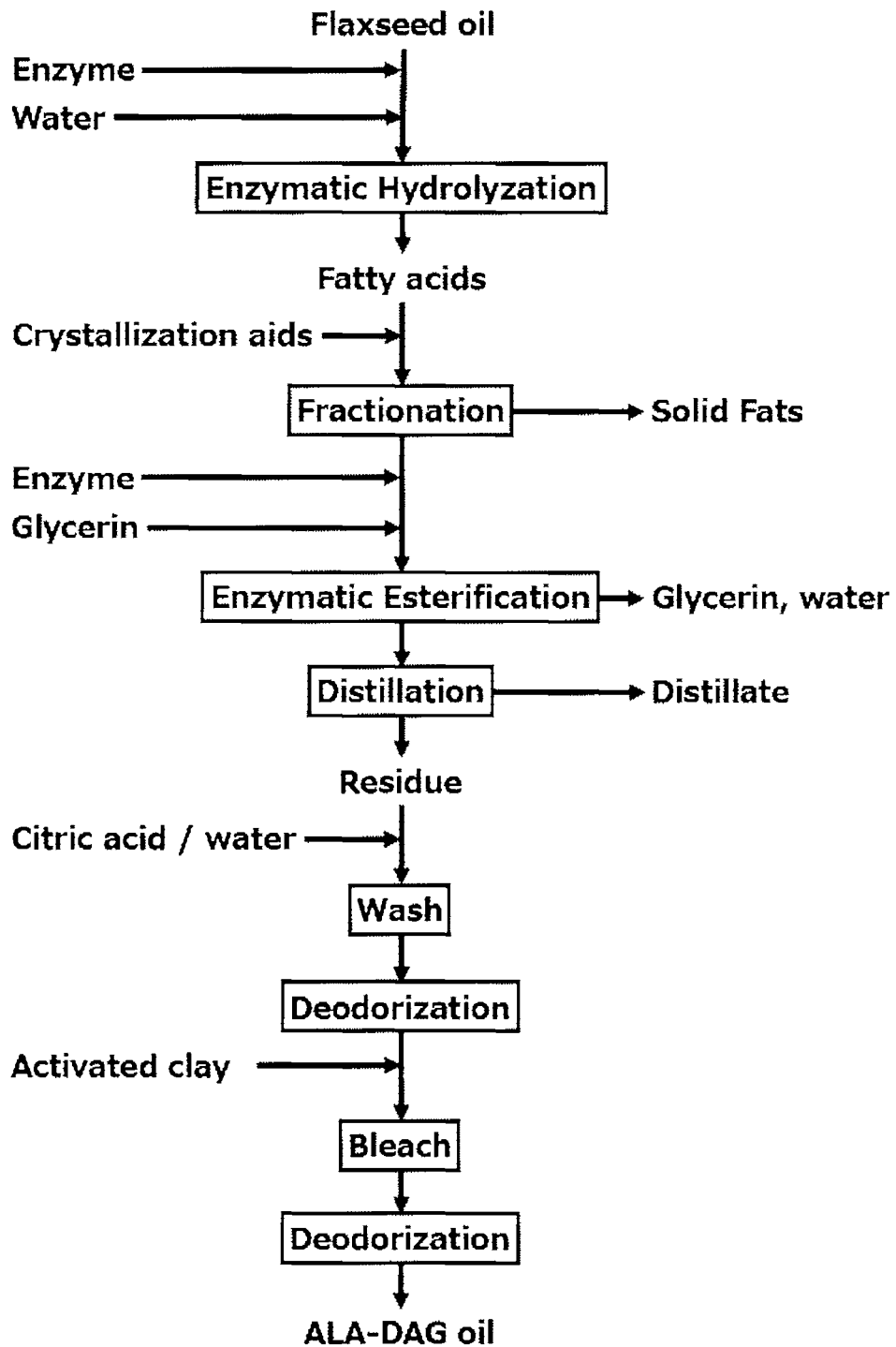
The ALA DAG oil is manufactured in a facility certified under ISO 14001. The ingredient will be manufactured in accordance with current good manufacturing practices (cGMP) as described under 21 CFR § 117 (U.S. FDA, 2017), and will include appropriate preventative controls in accordance with the Food Safety Modernization Act (FSMA).

The ALA DAG oil is manufactured using processes that are consistent with the production of other edible oils. These steps include enzymatic hydrolyzation, esterification, distillation, washing, deodorization, and bleaching, and are described briefly as follows. In the first step,

flaxseed oil is enzymatically hydrolyzed at mild temperatures under nitrogen to produce fatty acids. The fatty acids are then crystallized by polyglycerol fatty acid esters and fractionated at cool temperatures under nitrogen in a tank, where the solid portion is removed. Following the crystallization step, the solution containing the fatty acids and glycerin are passed through an ion exchange resin to enzymatically esterify the fatty acids, and then distilled. The resulting solution is a crude ALA DAG oil. The enzymes can be immobilized onto the ion exchange resin. Next, the crude solution is subject to a series of washing steps with citric acid and water under nitrogen in a centrifuge, and then subsequently steam deodorized and bleached with activated clay (bentonite). Finally, the solution is deodorized again to produce the ALA DAG oil. A schematic overview of the manufacturing process is shown in Figure 2.4-1.

All raw materials, processing aids, and additives used in the production of the ALA DAG oil are food-grade or equivalent (*e.g.*, Food Chemicals Codex (FCC), U.S. Pharmacopeia (USP), or European Pharmacopeia (EP)), and are used in accordance with an applicable FDA regulation (*e.g.*, 21 CFR), or have previously been determined to be GRAS. The enzyme utilized in the esterification and hydrolyzation steps is a commonly used enzyme in the hydrolyzation and/or esterification of fatty acids (*i.e.*, lipase). The production of ALA DAG oil utilizes lipase derived from *Candida cylindracea* or triacylglycerol lipase derived from a genetically modified strain of *Aspergillus oryzae* that have been previously determined to be GRAS (see GRN 81 and 103, respectively).

Figure 2.4-1 Schematic of the Manufacturing Process for the ALA DAG Oil



2.5 Product Specifications

Appropriate food-grade specifications have been established for the ALA DAG oil (Table 2.5-1). The final product contains over 36% ALA DAG (*i.e.*, ALA bound to DAG). Specification limits of 0.5 ppm have been established for lead and arsenic. Microbiological specification limits have not been established for the ALA DAG oil as it is unlikely that microbiological contamination would occur during the manufacturing process. The production process involves a series of steam deodorization and bleaching steps that are performed at high temperatures (upwards of 230°C) that are not conducive to microbiological growth, thus reducing the potential for microbial contamination in the final product. All methods of analysis are internationally recognized (*e.g.*, American Oil Chemists' Society (AOCS), Japan Oil Chemists' Society (JOCS)) or internal methods developed and validated by Kao.

Table 2.5-1 Product Specifications for the ALA DAG Oil

Specification Parameter	Specification
ALA DAG	≥36% by weight
Peroxide value	≤5 meq/kg of sample
Acid value	≤2 mg KOH/g of sample
Moisture	≤0.1% by weight
Lead	≤0.5 ppm
Arsenic	≤0.5 ppm

ALA DAG = *alpha*-linolenic acid diacylglycerol; KOH = potassium hydroxide; ppm = parts per million.

2.6 Batch Analyses

Three non-consecutive lots of the ALA DAG oil were analyzed to verify that the manufacturing process, as described in Section 2.4, produces a consistent product that meets the established product specifications. Lead and arsenic were below the detection limit of 0.05 and 0.1 ppm, respectively. A summary of the analytical results is presented in Table 2.6-1.

Table 2.6-1 Results of Batch Analysis of 3 Non-Consecutive Lots of the ALA DAG Oil

Specification Parameter	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
ALA DAG (%)	39	39	39
Peroxide value (meq/kg)	0.47	0.34	0.64
Acid value (mg KOH/g)	0.16	0.26	0.53
Moisture (%)	0.04	0.01	0.03

Table 2.6-1 Results of Batch Analysis of 3 Non-Consecutive Lots of the ALA DAG Oil

Specification Parameter	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
Lead (ppm)	<0.05	<0.05	<0.05
Arsenic (ppm)	<0.1	<0.1	<0.1

ALA DAG = *alpha*-linolenic acid diacylglycerol; KOH = potassium hydroxide; ppm = parts per million.

2.7 Additional Chemical Characterization

2.7.1 Glycidyl Esters

Three non-consecutive lots of ALA DAG oil were analyzed for glycidyl esters that can theoretically be generated during the deodorization step of oil processing (Craft *et al.*, 2012). Glycidyl esters were analyzed using the method described by DGF Standard Methods C-VI 18(10) and the results of the analysis are shown in Table 2.7.1-1. As demonstrated, the glycidyl ester content in 3 non-consecutive lots were less than the quantitation limit (0.1 mg/kg), indicating that the manufacturing process does not result in formation of glycidyl esters.

Table 2.7.1-1 Results of Analysis for Glycidyl Esters in 3 Non-Consecutive Lots of ALA DAG Oil

Specification Parameter	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
Glycidyl esters (mg/kg)	<0.1	<0.1	<0.1

ALA DAG oil = *alpha*-linolenic acid diacylglycerol oil.

2.7.2 Saponification Value and Unsaponifiable Matter

The saponification value and unsaponifiable matter of 3 non-consecutive lots of the ALA DAG oil were analyzed. Results of the analysis are shown in Table 2.7.2-1 below and demonstrate that the saponification value and unsaponifiable matter in all lots were similar.

Table 2.7.2-1 Results of Analysis for Saponification Value and Unsaponifiable Matter in 3 Non-Consecutive Lots of ALA DAG Oil

Specification Parameter	Method of Analysis	Manufacturing Lot		
		Lot No. B	Lot No. C	Lot No. D
Saponification value	JOCS 2.3.2.1-2013	185	184	184
Unsaponifiable matter (%)	JOCS 2.4.8-2013	0.84	1.23	1.23

ALA DAG = *alpha*-linolenic acid diacylglycerol; JOCS = Japan Oil Chemists' Society.

2.8 Stability of the ALA DAG Oil

As discussed in the Sections above, the ALA DAG oil has similar physical and chemical properties as other edible oils (e.g., flaxseed oil). ALA DAG oil is generally stable at room temperature and normal atmospheric pressure. The stability of ALA DAG oil was evaluated in a 1-month stability study at 30°C under nitrogen. The results of the study are summarized in Table 2.8-1 below. The results demonstrate that the product is stable for 1 month.

Table 2.8-1 Results of the 1-Month Stability Study on the ALA DAG Oil

Specification Parameter	Initial (0 month)	1 month
ALA DAG (%)	39	39
Peroxide value (meq/kg)	0.34	1.00
Acid value (mg KOH/g)	0.26	0.26
Moisture (%)	0.01	0.092
Lead (ppm)	<0.05	<0.05
Arsenic (ppm)	<0.1	<0.1

ALA DAG = *alpha*-linolenic acid diacylglycerol; KOH = potassium hydroxide; ppm = parts per million.

The shelf-life stability of the final ALA DAG product¹ was evaluated at room temperature (ranging from 10 to 35°C) and relative humidity of 20 to 60%. Samples were stored for 18 months after production and were tested at 0, 12, and 18 months. The results are summarized in Table 2.8-2 below. No appreciable changes in any of the established specification parameters were reported, indicating that the final ALA DAG product is stable for up to 18 months of storage.

¹ The final ALA DAG product is a formulation of ALA DAG oil with antioxidants and stabilizers that are permitted for use under U.S. federal regulations (e.g., 21 CFR) or have been determined to be GRAS.

Table 2.8-2 Results of the 18-Month Shelf-Life Stability Study on the Final ALA DAG Product^a

Specification Parameter	Initial (0 month)	12 months after production	18 months after production
ALA DAG (%)	39	39	39
Acid value (mg KOH/g)	0.7	0.7	0.8
Moisture (%)	0.02	0.04	0.05
Lead (ppm)	<0.05	<0.05	<0.05
Arsenic (ppm)	<0.1	<0.1	<0.1

ALA DAG = *alpha*-linolenic acid diacylglycerol; KOH = potassium hydroxide; ppm = parts per million.

^a The final product consists of the ALA DAG oil as described herein formulated with antioxidants.

Part 3. Dietary Exposure (21 CFR § 170.235)

3.1 Proposed Uses and Use Levels of ALA DAG Oil

The ALA DAG oil will be marketed in the U.S. to be directly added to finished food by consumers. In a similar manner to other oils, *e.g.*, olive oil, or dressings and margarines containing oils, ALA DAG oil can be added to vegetables, salads, or consumed with grain products such as bread and pasta. The recommended daily intake of ALA DAG oil is 2.5 g, and the product will be labeled with the serving size information and the recommended daily amount of ALA DAG oil.

3.2 Background Dietary Intakes of ALA DAG

ALA DAG is naturally occurring at low levels in flaxseed oil and rapeseed (canola) oil. Therefore, the U.S. population has had a limited background exposure to ALA DAG through consumption of these oils. The Food Commodity Intake Database (FCID), maintained by the U.S. Environmental Protection Agency (EPA), provides an estimation of the consumption levels of flaxseed, flaxseed oil, and rapeseed oil by the U.S. population. The background dietary intake of flaxseed and flaxseed oil were estimated using the FCID using 2-day average consumption levels (Table 3.2-1).

Consumption levels of flaxseed and flaxseed oil were reported to be approximately 27.7 g/capita/day or approximately 0.4 g/kg body weight/day for all ages. The background dietary intake of rapeseed (canola) oil was estimated to be approximately 60.3 g/capita/day or 1.6 g/kg body weight/day. Kao determined the ALA DAG content of flaxseed oil and rapeseed oil, and the mean levels reported were used to estimate the amount of ALA DAG consumed by the U.S. population from flaxseed oil and rapeseed oil (Table 3.2-1). Based on the levels of ALA DAG in these two edible plant oils, the background dietary intakes of ALA DAG is estimated to be approximately 0.49 g/day.

Table 3.2-1 Dietary Consumption of ALA DAG from Flaxseed, Flaxseed Oil, and Rapeseed Oil

	2-Day Average Consumption Level Based on FCID			
	Mean	90%	99%	Maximum
Flaxseed				
<i>Per capita</i>				
Flaxseed (g/day)	<0.005	0	0	0.5
ALA DAG ^a (g/day)	~0.000077	0	0	0.0077
Eaters only				
Flaxseed (g/day)	0.12	0.1	0.5	0.5
ALA DAG ^a (g/day)	0.0018	0.0015	0.0077	0.0077
Flaxseed Oil				
<i>Per capita</i>				
Flaxseed oil (g/day)	0.02	0	0	27.2
ALA DAG ^a (g/day)	0.00031	0	0	0.42
Eaters only				
Flaxseed oil (g/day)	2.78	6.2	17.2	27.2
ALA DAG ^a (g/day)	0.043	0.095	0.26	0.42
Rapeseed (Canola) Oil				
<i>Per capita</i>				
Rapeseed (canola) oil (g/day)	1.45	3.4	11.5	60.3
ALA DAG ^b (g/day)	0.0015	0.0034	0.011	0.060
Eaters only				
Rapeseed (canola) oil (g/day)	1.49	3.4	11.5	60.3
ALA DAG ^b (g/day)	0.0015	0.0034	0.011	0.060

ALA DAG = *alpha*-linolenic acid diacylglycerol; FCID = Food Commodity Intake Database.

^a Mean ALA DAG content of 1.53 g/100 g based on mean of 3 lots (160120SA, 161120NS, 161217NP) of flaxseed oil.

^b Mean ALA DAG content of 0.1 g/100 g based on mean of 3 lots (150629NS, 170510NS, 180923NS) of rapeseed (canola) oil.

3.3 Background Intakes of ALA

Polyunsaturated fatty acids, such as ALA, are components of cell membranes and are used in signal transduction pathways. In addition, in humans, ALA is used as a precursor for the synthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Synthesis of EPA and DHA involves the activity of desaturase enzymes ($\Delta 6$ and $\Delta 5$ desaturases). ALA is not endogenously synthesized in humans and is the parent compound of the n-3 polyunsaturated fatty acid (PUFA) group of essential fatty acids (Beare-Rogers *et al.*, 2001). Thus, ALA is an essential component of the diet. ALA is present at low levels in fish (<0.3%), and in high levels in various nuts and seeds and plant oils (up to 53.3% in flaxseed oil) (Table 3.3-1). ALA is a natural component of other plant oils, such as rapeseed and soyabean in which levels range from 5 to 13% and 4.5 to 11%, respectively (CODEX, 2015). Inadequate ALA consumption results in adverse clinical symptoms such as neural development and poor growth (IOM, 2005). The Institute of Medicine established a dietary reference intake and specifically an adequate intake (AI) of 1.6 g/day for males and 1.1

g/day for females based on an intake that supports normal growth and neural development (IOM, 2005). According to the Panel on Dietetic Products, Nutrition, and Allergies of the European Food Safety Authority (EFSA), ALA is the most abundant omega-3 fatty acid in food and the average intakes of ALA in adults in some European countries is 0.7 to 2.3 g/day (EFSA, 2009). In comparison, in the U.S. the average intake of ALA in males aged 20 to 59 years is 1.7 g/day or 1.3 g/day for females of the same age group (Morris, 2007). As shown in Table 3.3-1 below, ALA occurs naturally in flaxseed oil at levels of *ca.* 50%. The recommended serving of 2.5 g ALA DAG per day would provide an additional *ca.* 1.2 g ALA per day for the consumer (up to 2.9 g/day or 2.5 g/day of ALA for males and females, respectively). It should be noted that there is no tolerable upper limit for ALA and therefore the additional amount of ALA consumed *via* the ALA DAG oil would not pose a safety concern.

Table 3.3-1 ALA Content of Various Foods^a

Food Item	ALA Content (%)
<i>Fish</i>	
Catfish	0.1
Mackerel	0.2
Salmon	
Farmed	Trace
Wild	0.3
Canned	Trace
Swordfish	0.2
Tuna, White	
Canned in oil	0.2
Canned in water	Trace
<i>Nuts and Seeds</i>	
Butternuts	8.7
Flaxseed	18.1
Walnuts	9.1
<i>Plant Oils</i>	
Canola	9.3
Flaxseed	53.3

ALA = *alpha*-linolenic acid.

^a Adapted from DeFlippis and Sperling (2006).

3.4 Estimated Daily Intakes of the ALA DAG Oil Based on the Intended Food Uses

As discussed above, the ALA DAG oil is intended to be added directly to finished foods by consumers. The recommended daily intake of ALA DAG oils is 2.5 g per serving per day. The product label will state the consumption of 1 serving provides the recommended daily amount of ALA DAG oil.

The maximum daily background dietary consumption of ALA DAG was estimated to be approximately 0.49 g per person based on the levels of ALA DAG in flaxseed, flaxseed oil, and canola oil (see Section 3.2 for further details). Considering the intended uses of ALA DAG oil, this would result in an overall daily dietary consumption of up to *ca.* 1.39 g ALA DAG based on the ALA DAG content in the ingredient ($\geq 36\%$).

Part 4. Self-Limiting Levels of Use (21 CFR § 170.240)

No known self-limiting levels of use are associated with the ALA DAG oil.

Part 5. Experience Based on Common Use in Foods before 1958 (21 CFR § 170.245)

Not applicable as the ingredient has not been in use in foods before 1958.

Part 6. Narrative (21 CFR § 170.250)

The safety of the ALA DAG oil was established based on scientific procedures. As demonstrated in Section 2.2, the ingredient is comprised primarily of DAG (*ca.* 83%), and to a lesser extent, MAG (<1%), TAG (*ca.* 14%), and free fatty acids (<0.2%). Therefore, considering that the ingredient consists of primarily DAG, it is anticipated that the metabolic fate of the ALA DAG oil will follow the established metabolic pathway of DAG. ALA is the primary fatty acyl group bound to the glycerol backbone and accounts for *ca.* 50% of the fatty acid content; the other fatty acids that account for the remaining portion of the oil are oleic acid, linoleic acid, stearic acid, and palmitic acid. These fatty acids are all common components of the human diet and are expected to be metabolized by the body as such. The safety of the ALA DAG oil was evaluated in a standard toxicological battery, consisting of repeat-dose 14-day and 90-day oral toxicity studies, a bacterial reverse mutation assay, and *in vitro* and *in vivo* mammalian cell micronucleus tests. A prenatal developmental oral toxicity study was also conducted. All tests were performed in compliance with the Organisation of Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) (OECD, 1997a) and in accordance with each respective OECD Test

Guidelines for the Testing of Chemicals. In order to corroborate the product-specific studies, comprehensive and detailed searches of the published scientific literature were conducted through March 2019 to identify toxicology studies on ALA DAG oil. Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®, SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and Toxfile® served as the primary sources of published literature. Based on the results of the literature search, studies conducted with ALA DAG oil were not identified. Studies conducted with DAG oil were identified in the literature, however, as these studies did not specify the ALA content or the ALA content was low, they were not considered relevant to the safety of ALA DAG oil as they are not representative of Kao's ALA DAG oil, and therefore were not summarized below. Therefore, the basis for the safety of the ALA DAG oil was primarily based upon the history of safe consumption of DAG and ALA in the human diet, and is corroborated by the product-specific studies conducted with the ingredient as described herein.

6.1 Metabolic Fate of the ALA DAG Oil

MAG, DAG, and TAG respectively consist of 1, 2, or 3 esters of fatty acids (fatty acyl moieties) bound to a glycerol backbone. The ALA DAG oil is primarily composed of DAG that is manufactured through the enzymatic esterification of fatty acids derived from edible oils from plant sources. The ALA DAG oil also contains small levels of TAG and MAG. The distinguishing feature of ALA DAG oil is the predominance of ALA as the fatty acyl group bound to the glycerol backbone. The other fatty acids are oleic acid, linoleic acid, stearic acid, and palmitic acid which are all common components of the human diet. Thus, the overall metabolic pathway of ALA DAG oil is expected to be similar to the metabolic pathway of DAG oil reported in the published literature.

6.1.1 Absorption and Distribution

Following oral ingestion, both TAG and DAG are initially digested by gastric and pancreatic lipase in the stomach and small intestine, respectively (Yasukawa and Katsuragi, 2008). Lipase hydrolyzes the ester linkage between the fatty acyl moiety and the glycerol backbone converting TAG to DAG and DAG to MAG and free fatty acids (Yasukawa and Katsuragi, 2008). Lipase in the digestive tract preferentially cleaves the fatty acyl group at positions 1 and 3 of TAG and DAG (Yasukawa and Katsuragi, 2008). Thus, TAG is digested to 1,2-DAG (or 2,3-DAG), then to 2-MAG (fatty acyl moiety on the 2 position). Conversely, the product of 1,3-DAG digestion is 1-MAG (or 3-MAG). 1-MAG may be further digested by lipase to glycerol and free fatty acids according to results from 1 study in which radiolabeled 1,3-DAG was administered by intraduodenal infusion (Mansbach and Nevin, 1998; Kondo *et al.*, 2003). From a metabolic fate standpoint, the chemical structures of 1,2-DAG and 2,3-DAG can be considered equivalent and the chemical

structures of 1-MAG and 3-MAG can be considered equivalent and therefore for simplicity, the remainder of this discussion will use the format of 1,2-DAG and 1-MAG.

In an absorption study, groups of 8 male Sprague-Dawley rats housed individually in metabolism cages were administered diets containing TAG or DAG (20% by weight in the diet) for 13 to 15 days (Taguchi *et al.*, 2001). Feces were collected over the last 3 days of the study and feed intake, fat intake, fat excretion, fat content of dry feces, and fat absorption coefficients were evaluated. Both TAG and DAG were demonstrated to be absorbed from the intestinal lumen at similar rates following oral administration in the diets of rats. Similar findings were also reported by Meng *et al.* (2004) and Murase *et al.* (2001). Thus, following lipase digestion of TAG and DAG the products of hydrolysis (1-MAG, 3-MAG, free fatty acids, and glycerol) are completely absorbed from the digestive tract lumen into intestinal mucosal cells (Watanabe and Tokimitsu, 2008).

Inside the intestinal mucosal cells, 2-MAG is synthesized to 1,2-DAG *via* the enzyme monoacylglycerol acyltransferase (MGAT) using fatty acids as co-substrates (Yasukawa and Katsuragi, 2008). The enzyme diacylglycerol acyltransferase (DGAT) facilitates the acylation of 1,2-DAG to TAG (Kondo *et al.*, 2003; Yanagita *et al.*, 2004; Yasukawa and Katsuragi, 2008). TAG synthesized in the intestinal cells are incorporated into chylomicrons which passively diffuse out of the intestinal cell and enter the thoracic duct of the lymphatic system and eventually the general circulation (Friedman and Nylund, 1980; Ikeda and Yanagita, 2008).

Following absorption into intestinal cells, 1-MAG may be synthesized to 1,3-DAG and incorporated into the lipid bilayer of the cells or may be further hydrolyzed by lipase to glycerol and free fatty acids (Kondo *et al.*, 2003). In a study in rats that ingested 1,3-DAG, the concentration of free fatty acids, MAG, and DAG in lymph chylomicrons did not increase which indicates that 1,3-DAG is converted to 1,2-DAG (possibly through complete hydrolysis to glycerol and free fatty acids) then to TAG (Yanagita *et al.*, 2004). In a second study, radiolabeled 1,3-DAG and TAG (^{14}C was located on the fatty acyl groups) were administered intragastrically into rats with cannulated thoracic ducts in order to monitor the rate and composition of absorption of DAG and TAG (Ikeda and Yanagita, 2008). The study authors reported that after 24 hours there was a slight but significant difference in lymphatic recovery of radioactivity from 1,3-DAG and TAG (81.3 ± 1.0 versus $86.5 \pm 1.2\%$ of the administered dose, respectively) which indicates that 1,3-DAG is more slowly distributed into the body relative to TAG. However, in this study more than 90% of the recovered dose from both 1,3-DAG and TAG was in the triglyceride fraction in the lymph which indicates that DAG is slowly synthesized to a triglyceride *via* the glycerol-3-phosphate pathway and transported to the lymph.

6.1.2 Metabolism and Excretion

Chylomicrons containing TAG in the lymphatic system enter the general circulation and distribute to adipocytes where they undergo lipolysis to produce fatty acids and glycerol. The fatty acids are

then utilized in either energy production (*via beta-oxidation*) or are converted to other endogenously occurring products such as phospholipids or cholesterol, among others (Champe and Harvey, 1994). Glycerol is not metabolized by adipocytes and instead moves *via* the general circulation to the liver where it is metabolized by glycerol kinase to glycerol phosphate. Glycerol phosphate is then either used in the synthesis of TAG in the liver or metabolized to dihydroxyacetone phosphate *via* glycerol dehydrogenase. Dihydroxyacetone phosphate can then participate in either glycolysis or gluconeogenesis (Champe and Harvey, 1994). The metabolites of TAG and DAG are therefore incorporated into the body pool of fatty acids where they can be catabolized to produce energy and carbon dioxide which is eliminated in the respired air or are otherwise utilized by the body.

According to studies performed by Watanabe *et al.* (1997) and Murase *et al.* (2002), fatty acids from 1,3-DAG may be transported from the intestinal cells *via* the hepatic portal vein where they would be metabolized in the same manner as other fatty acids or may undergo *beta-oxidation* in the intestinal cells which may explain the difference in recovered radioactivity in the lymph in the study by Ikeda and Yanagita (2008).

Recently, a randomized, double-blind, placebo-controlled crossover study in humans was conducted to determine the effect of ALA DAG relative to a control TAG on fatty acid oxidation (Ando *et al.*, 2017a). In this study, subjects consumed a test meal containing ALA DAG or TAG and fatty acid oxidation was measured using a ¹³C-labelled triolein probe with CO₂ expiration measured every hour for 6 hours after the meal. The study authors reported that 17.1±4.0% and 14.8±4.3% of the ¹³C dose was recovered in expired air following ALA DAG ingestion and TAG ingestion, respectively, and this difference in recovery was reported to be statistically significant. Thus, the results of this study show that ALA DAG oil increased fatty acid oxidation following ingestion compared to TAG ingestion.

6.1.3 Interactions with Lipid-Soluble Vitamins

The European Food Safety Agency (EFSA) evaluated the safety of DAG oil in 2004 (EFSA, 2004). During this evaluation, the potential for DAG to influence the bioavailability of fat-soluble vitamins was considered. At the time of the evaluation, a single study was identified which compared blood concentrations of vitamins A, E, and D in male volunteers who were consuming 20 g DAG oil per day or 20 g TAG oil per day for 12 weeks (Watanabe *et al.*, 2001). There was no difference in fat-soluble vitamin concentrations in blood when samples were taken and analyzed on Weeks 4, 8, and 12. The EFSA Panel considered this study insufficient to make a complete assessment; however, the EFSA Panel did not expect that DAG oil would have an effect on the bioavailability of vitamins based on the mode of action of DAG.

Recently, a randomized, double-blind, controlled, parallel group study was performed wherein groups of 30 healthy subjects (33 males, 27 females; aged 20 to 64 years; BMI 23 to <30 kg/m²)

substituted 1 of their 3 daily meals with a shortbread containing 7.5 g of TAG or ALA DAG for 4 weeks (Yamanaka *et al.*, 2016). Subjects recorded their diet for 3 consecutive days before the measurements, and kept lifestyle records (intake of test meals, medication, exercise, etc). Subjects were restricted alcohol consumption and exercise before the measurements. At the start and end of this study, blood samples were obtained from the subjects and several parameters were measured including concentrations of fat soluble vitamins in the blood² following a 12 hour fast (additional study parameter details are provided in Table 7.3-1 below). With the exceptions of *alpha*-tocopherol, *delta*-tocopherol, and vitamin K1, there were no differences in the concentrations of these vitamins between the TAG and ALA DAG groups and there were no significant changes in the concentrations of these vitamins between the start and end of the study.

Yamanaka *et al.* (2016) reported that the change in *alpha*-tocopherol concentration from the start of the study to the end of the study was statistically significantly lower for subjects in the ALA DAG group relative to the TAG group; however, the absolute values of these parameters were generally similar at the start and end of the study and decreases in *alpha*-tocopherol concentrations were attributed to a decrease in total and low-density lipoprotein cholesterol levels in the blood. The change in *delta*-tocopherol concentration from the start of the study to the end of the study and the absolute *delta*-tocopherol concentration in the blood of subjects in the ALA DAG group were statistically significantly greater relative to subjects in the TAG group; however, the mean values of these parameters were within the normal range for humans and no deviations outside normal ranges were reported for any of the subjects. On Week 4 of the study, vitamin K1 concentrations in the blood of subjects in the ALA DAG group were significantly decreased relative to subjects in the TAG group; however, there was no statistically significant difference between the 2 groups for the change in vitamin K concentration from the start of the study to the end of the study. In addition, the concentration of vitamin K1 at the commencement of the study was lower in the ALA DAG group relative to the TAG group and although this difference was not statistically significant, the significant difference in vitamin K1 concentrations observed at Week 4 could be partly attributed to this initial difference.

Considering the similarity between ALA DAG and DAG and the recent findings from Yamanaka *et al.* (2016), no deleterious effect of ALA DAG on fat-soluble vitamin bioavailability is expected.

² The study authors measured blood concentrations of vitamin A, *alpha*-carotene, *beta*-carotene, lycopene, *alpha*-tocopherol, *delta*-tocopherol, *beta*,*gamma*-tocopherol, 25-OH vitamin D, vitamin K1, and vitamin K2. Changes in the concentration of each of these vitamins between the start and end of the study were calculated (Yamanaka *et al.*, 2016).

6.2 Toxicological Studies

6.2.1 Repeat-Dose Toxicity

6.2.1.1 14-Day Oral Toxicity

A 14-day dietary toxicity study was conducted in rats to investigate the maximum concentrations to be used in a 90-day toxicity study (Bushita *et al.*, 2018a). This study was performed in accordance with OECD Test Guideline No. 407 (OECD, 1995). Groups of Crl:CD(SD) rats (5/sex/group) were provided ALA DAG oil (Lot No. 150421) in the diets at concentrations of 0, 1.375, 2.75, or 5.5% for 14 days. All treatment diets were supplemented with rapeseed oil to a total concentration of 55,000 ppm (*i.e.*, the low-dose group was given a diet containing 1.375% ALA DAG oil and 4.125% rapeseed oil, and the mid-dose group was given a diet containing 2.75% of ALA DAG oil and rapeseed oil). The control group was provided a diet containing either 5.5% rapeseed oil or ALA TAG oil.

The authors reported “some” liver-related changes in biochemical parameters as compared to the rapeseed oil group (no further details provided). Furthermore, no changes in liver weight were reported. Based on the lack of effects observed in the 14-day study, the authors used similar doses in a 90-day oral toxicity study.

6.2.1.2 90-Day Oral Toxicity

The subchronic oral toxicity of ALA DAG in rats was assessed in a 90-day dietary study with a 28-day recovery period (Bushita *et al.*, 2018a). This study was performed in accordance with OECD Test Guideline No. 408 and OECD Principles of GLP (OECD, 1997a, 1998). Groups of 10 male and 10 female Crl:CD (SD) rats were provided ALA DAG oil (Lot No. 150803) in the diet at concentrations of 0, 1.375, 2.75, or 5.5% for 90 days. These concentrations were equivalent to an intake of 0, 738, 1,461, or 2,916 mg ALA DAG oil/kg body weight/day for males, respectively, and 0, 846, 1,703, or 3,326 mg ALA DAG oil/kg body weight/day for females, respectively. The concentrations were selected based on the results of a 14-day dietary toxicity study in which no statistically significant compound-related effects were observed in rats [Crl:CD (SD)] at concentrations of 1.375, 2.75, or 5.5% (see Section 7.2.1.1 above for further details). The test diets were prepared similar to the 14-day study. Control animals (10/sex/group) were provided either rapeseed oil or ALA TAG oil in the diet at concentrations of 5.5%. At the end of the study period, 5 animals/sex of the high-dose, ALA TAG oil, and rapeseed oil groups were carried onto a 28-day recovery period.

All animals were observed once daily for clinical signs of toxicity, and individual body weights and food consumption were measured weekly. Ophthalmological examinations were conducted in all

animals prior to study initiation, on Week 13, and at the end of the 28-day recovery period. A functional observational battery, including motor activity, was performed on the last week of the study period. Urine samples were collected from fasted animals on Week 13 and at the end of the 28-day recovery period, and analyzed for the following parameters: pH, protein, glucose, ketone body, occult blood, urine sediments, volume, color, specific gravity, and sodium, potassium, and chloride concentrations. Blood samples were collected from the posterior vena cava at the scheduled necropsy and at the end of the 28-day recovery period from fasted animals for hematological and serum chemistry analyses. The following hematological parameters were evaluated: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte ratio and count, platelet count, and differential leukocyte ratio and count. The following serum chemistry parameters were measured: total protein, albumin, albumin/globulin (A/G) ratio, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), *gamma*-glutamyl transpeptidase (GGT), total bilirubin, total bile acids, total cholesterol, triglycerides, glucose, urea nitrogen, creatinine, and calcium, inorganic phosphorus, sodium, potassium, and chloride concentrations. Blood coagulation parameters, prothrombin time and activated partial thromboplastin time, were also measured. All animals were terminated following blood collection, and were subjected to a complete necropsy. Organs and tissues were removed, weighed, and prepared for macroscopic examination. Histopathological examination was performed in all control and high-dose animals.

One female of the ALA TAG oil group was found dead on Study Day 104. The study authors reported no abnormalities prior to death. No other mortalities were observed in the 90-day study period or 28-day recovery period. No compound-related clinical signs of toxicity or morbidity were observed in any animal. No significant changes in ophthalmological examination, functional observation battery (FOB), motor activity, body weight, food consumption, or macroscopic examination were noted in any ALA DAG oil group compared to the rapeseed or ALA TAG control groups.

In the urinalysis, a significant decrease in specific gravity was observed in females of the ALA DAG oil group compared to the rapeseed oil group at the end of the recovery period. However, as this finding was observed only in females, and no corresponding histopathological finding was noted, it was not considered toxicologically relevant.

Upon hematology, a number of significant changes were observed compared to the rapeseed oil control, including a significant decrease in WBC count in females of the high-dose group at the end of the recovery period; a significant increase in prothrombin time in males of the high-dose group at the end of the recovery period; and a significant increase in prothrombin time in females of the high-dose group at the end of the main study period. The decrease in WBC count was not considered

toxicologically-relevant as it was only observed in 1 sex. Moreover, the change in prothrombin time in females at the end of the study period was not judged to be treatment-related since the change was slight compared to that of the rapeseed oil group and no related changes such as hemorrhage were noted in the pathology. At the end of the main study period, a high eosinophil ratio was observed in females of the 2.75% ALA DAG oil group, and at the end of the recovery period, females of the 5.5% ALA DAG oil group showed decrease in WBC, lymphocyte, and large unstained cell counts. The authors reported that, as these changes were not observed during the main study period, they were not considered to be treatment-related.

The following significant changes compared to the rapeseed oil control were noted upon clinical chemistry examination: decrease in albumin, triglycerides, and calcium concentration in males of the high-dose group at the end of the main study period; decrease in glucose concentration in females of high-dose group at end of recovery period; and a decrease in total cholesterol in males of the mid-dose and high-dose groups at the end of the main study period. It should be noted that the change in albumin, calcium concentration, and glucose concentration were not observed in each respective group at the end of the recovery period. The decrease in total cholesterol and triglycerides was also observed at the end of the recovery period. However, as there were no corresponding changes in organ weight or histopathological examination, this change was not considered toxicologically relevant.

A significant decrease in relative-to-body submandibular gland weight was noted in females of the high-dose group compared to the rapeseed oil control. This effect was not judged to be treatment-related since the change was slight compared to that of the rapeseed oil group and no related changes such as hemorrhage were noted in the pathology. The authors reported a decrease in absolute and relative-to-body spleen weight in males of the 1.375% and 2.75% ALA DAG oil groups, and an increase in relative-to-body submandibular and adrenal gland weights in females of the 2.75% ALA DAG oil group at the end of the main study period. Further, at the end of the recovery period, females of the 5.5% ALA DAG oil group had an increase in absolute adrenal weight and relative-to-body submandibular gland weights, while a low absolute seminal vesicle weight was reported in males. No other changes compared to the rapeseed oil control in absolute or relative organ weights were observed in any animal. Furthermore, no dose-dependent effect of ALA DAG oil on organ weights were observed and were not associated with any macroscopic or microscopic changes that were suggestive of a treatment-related effect.

Upon histopathological examination, a number of findings were observed in animals of the rapeseed oil control and the high-dose ALA DAG oil group. Several changes were associated with the liver/hepatocytes, which were observed in the ALA DAG oil, ALA TAG oil, and rapeseed oil groups (*i.e.*, there was no clear pattern of adverse effect due to ALA DAG oil consumption). The incidence rates were similar between both groups, thus were not considered toxicologically

significant. Furthermore, the authors noted that the histopathological changes in the liver were observed in both sexes in all animals, and were “occasionally detected” in control rats, and were not dose-dependent, and therefore were considered to be spontaneous and/or incidental in nature.

Based on the results of this study, the study authors determined a no-observed-adverse-effect level (NOAEL) of 5.5% ALA DAG oil, the highest concentration tested, equivalent to 2,916 mg/kg body weight/day for males and 3,326 mg/kg body weight/day for females, respectively.

6.2.1.3 Protein Kinase C (PKC) Activation

The effects of dietary ALA DAG oil on protein kinase C (PKC) activation in the rat digestive tract and lingual mucosa was investigated in Wistar rats (Mori *et al.*, 2017). Groups of male Wistar rats (9/group) were provided *ad libitum* access to water and one of the following diets: 7.5% ALA DAG, 30% ALA DAG, 7.5% ALA TAG, 30% ALA DAG, or 30% rapeseed oil for 4 weeks. Food consumption was measured every 2 or 3 days and body weights were measured weekly throughout the study period. On the last day of the study period, animals were anesthetized with isoflurane and were killed by withdrawing blood from the abdominal aorta the next morning. The tongue, esophagus, stomach, small intestine (2 to 15 cm from the pylorus, including the duodenum and jejunum), and colon of the animals were dissected and mucosa removed. PKC activity in all tissues was measured.

PKC activity measurements were verified by a control experiment conducted using a direct PKC activator in the lingual mucosa, 1,2-tetradecanoylphorbol-13-acetate (TPA). Groups of male Wistar rats (10/group) were deprived of food for 12 hours prior to TPA treatment. TPA was dissolved in rapeseed oil and applied to the tongue of rats at concentrations of 30 or 100 μM in a 30 μL mixture twice in one day. One hour following the last administration, animals were killed, the tongue dissected, and mucosa removed. A significant increase in membrane PKC activity of lingual mucosa was observed in animals receiving 100 μM TPA, 1-hour after the second treatment. A dose-dependent increase in lingual PKC activity was observed. However, 19 hours after TPA treatment, no significant increase in membrane PKC activity was observed.

The final body weight, body weight gain, and food and energy intake was similar in all groups throughout the study period. The authors reported no significant differences in the cytosolic and membrane PKC activities of the lingua, esophagus, stomach, small intestine, and colon among all the animals. Based on the results of the study, the authors concluded that replacing common dietary oil (rapeseed oil) with ALA DAG oil would not result in an increased risk of carcinogenesis *via* PKC activation of the tongue and digestive tract mucosa (see Section 6.2.3 below for further details).

6.2.2 Developmental and Reproductive Toxicity

The developmental toxicity of ALA DAG oil was evaluated in a prenatal developmental toxicity study in pregnant Sprague-Dawley (CrI:CD) rats (Bushita *et al.*, 2018b). This study was performed in accordance with OECD GLP and OECD Test Guideline No. 414 (OECD, 1997a, 2001). Pregnant Sprague-Dawley (CrI:CD) rats (24/group) were administered ALA DAG oil (Lot No. 150803) at dose level of 0, 1.25, 2.5, or 5.0 mL/kg ALA DAG oil by gavage from Gestation Day (GD) 6 to 19. These concentrations were equivalent to doses of 0, 1,149, 2,325, or 4,715 mg/kg body weight/day, respectively. The control animals received either rapeseed oil or ALA TAG oil. The total doses each group received was 5.0 mL/kg.

All animals were observed twice daily for clinical signs of toxicity and mortality. Individual body weight and food consumption were measured on GD 0 and 1, respectively, and every 3 days thereafter. All animals were killed on GD 20 by exsanguination from the lateral iliac artery following a 30 mg/kg pentobarbital sodium injection. The thoracoabdominal organs and tissues were macroscopically examined after removal of the ovary and uterus. No gross lesions were observed on any organ or tissue. After the uterus weight was measured, the number of implantation, corpora lutea, early resorptions, late resorptions, dead fetuses, and live fetuses were counted. The placentas were macroscopically examined on GD 20. Skeletal and visceral examinations were conducted in all fetuses in the control and high-dose groups.

No mortalities or clinical signs of toxicity were observed throughout the study period. In addition, no significant differences in body weight, food consumption, or gravid uterus weight were observed between the control groups and the ALA DAG oil groups throughout the study period. The percent pre-implantation loss was significantly decreased in the high-dose group compared to the rapeseed oil control on GD 20. Upon skeletal examination, short supernumerary rib variation was observed in 17 fetuses from the high-dose group compared to the rapeseed control (n=7). Additionally, 7 lumbar vertebra, full supernumerary rib, and bipartite ossification of thoracic centrum were observed in 2, 1, and 2 fetuses, respectively, in the ALA DAG oil group. Upon visceral examination, dilatation of the ureter in 4 fetuses and thymic remnant in the neck in 2 fetuses were observed in the ALA DAG oil group. These findings were also observed at similar incidences in the control and were within the historical control ranges, and therefore, were not considered to be treatment-related. No other abnormalities, compound-related differences in C-section evaluation, or skeletal and visceral examination were observed in any animal.

Based on the results of this study, the study authors determined a NOAEL of 4,715 mg/kg body weight/day ALA DAG oil, the highest concentration tested, for maternal general toxicity, maternal reproductive function, and the embryo-fetal development.

6.2.3 Chronic Toxicity and Carcinogenicity

6.2.3.1 Carcinogenicity

The potential for ALA DAG oil to promote tumorigenesis in tongue and gastrointestinal tract tissues was investigated in a rat medium-term multi-organ carcinogenesis bioassay (Honda *et al.*, 2017). This study was conducted in accordance with GLP and Guidelines for Carcinogenicity Studies of Drugs 3.2 (*In Vivo* Additional Tests for Detection of Carcinogenicity). Five week old male F344/DuCrIj rats were initially treated with five genotoxic carcinogens, N-nitrosodiethylamine (DEN), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), 1,2-dimethylhydrazine dihydrochloride (DMH), and N-nitroso-N-methylurea (MNU) for 4 weeks, followed by a 1 week recovery period without treatment. Groups of 20 rats were fed a semi-synthetic diet (AIN-93G) containing ALA DAG oil at concentrations of 0, 13,750 (low-dose), 27,500 (mid-dose), and 55,000 (high-dose) ppm for 24 weeks. These concentrations were equivalent to an intake of 0, 616, 1,223, and 2,397 mg ALA DAG/kg body weight/day. Controls (20/group) were provided either standard basal diet (naïve control) or ALA TAG oil in the diet at a concentration of 55,000 ppm.

The general condition and mortality of animals were observed daily, and body weights measured weekly for 14 weeks, then bi-weekly until the end of the study for all surviving animals. Food and water consumption were measured for 2 consecutive days on a weekly basis. At the end of the study, the remaining surviving animals were fasted overnight and euthanized with isoflurane and animals were examined grossly. Organ weights were determined for the heart, spleen, thymus, pituitary, thyroid (and parathyroid), adrenal, liver, kidney, testis, prostate, epididymis, and brain. Histopathological examination was performed for the following tissues: tongue, esophagus, stomach (forestomach and glandular stomach), small intestine (duodenum, jejunum, and ileum), and large intestine (cecum, colon, and rectum).

The survival rates for the control and treatment groups were 75, 75, 80, and 60%, respectively. The survival rates for the naïve control and ALA TAG oil control were 80 and 85%, respectively. The clinical signs that were observed in all groups included emaciation, decrease in locomotor activity, forelimb paralysis, panting, anemia, nodule/mass in the skin/subcutis, eyeball opacity, abdominal distention, soiled perineal region, or loose stools. The incidence of these findings were consistent in all groups and were not considered to be treatment-related. No statistically significant differences in body weights were observed in the ALA DAG oil treatment groups and control animals. No dose-related differences was observed in food consumption in the treatment groups compared to the controls. A reduction in water consumption was observed in the ALA DAG groups compared to the naïve control, however, this was not significant and was attributed to differences in dietary composition. No treatment-related differences in organ weights were observed in ALA DAG and ALA TAG groups. Compared to the control, the authors observed the following changes in animals

consuming ALA DAG oil in the diet: decreased incidence of colon/rectum nodules and increased incidence of discolored spots in the stomach in the high-dose group; decreased incidence of spleen cysts in the mid-dose group; and increased liver discoloration spot in the high-dose group. The authors noted that the incidence of spleen cysts was not dose-dependent (no further information provided).

With the exception of a decreased incidence of adenocarcinoma in the large intestine (colon/rectum) in the mid-dose group compared to the control, there were no other histopathological findings in the tongue and gastrointestinal tract or other organs between all groups. The average number of adenomas in the large intestine (colon/rectum) was significantly higher in the low-dose group and significantly lower in the mid-dose group. Since the effects were independent of dose, they were not considered to be meaningful.

Based on the results of the study, the study authors concluded that ALA DAG oil does not promote tumor development in the digestive system and determined a NOAEL of 55,000 ppm, equivalent to 2,397 mg/kg body weight/day, the highest concentration tested.

6.2.4 Mutagenicity and Genotoxicity

6.2.4.1 Bacterial Reverse Mutation Test

The potential mutagenicity of ALA DAG oil was evaluated in a bacterial reverse mutation test performed according to OECD Test Guideline No. 471 (OECD, 1997b) (Honda *et al.*, 2016). A concentration-range finding test and a main test were conducted using the standard pre-incubation method in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA in the presence and absence of S9 metabolic activation. The negative control consisted of the vehicle (dimethyl sulfoxide). Appropriate positive controls were also included in the concentration-range finding test and the main test. In the absence of metabolic activation, the positive controls included 2-(2-furyl)-3-(5-nitro-2furyl)acrylamide, sodium azide, 9-aminoacridine hydrochloride, and in the presence of metabolic activation, the positive control used was 2-aminoanthracene. The concentration-range finding test was conducted in triplicate at final test article concentrations of 15, 50, 150, 500, 1,500, 5,000 µg/plate and the main test was conducted in triplicate at final concentrations of 313, 625, 1,250, 2,500, 5,000 µg/plate. A positive result was defined as a 2-fold or greater concentration-dependent increase in the number of revertant colonies observed compared to the negative control.

In the concentration-range finding test, test article precipitation was observed at 5,000 µg/plate upon addition and after 48-hour incubation, both in the presence and absence of metabolic activation. In addition, in the main test, upon addition of the test article, precipitation was observed at 2,500 µg/plate and greater in the absence of metabolic activation and at 5,000 µg/plate in the presence of

metabolic activation. After 48-hour incubation, precipitation was observed at 2,500 µg/plate and greater in both the absence and presence of metabolic activation. However, in both the concentration-range finding test and main test, no positive responses were observed. In contrast, positive controls displayed marked mutagenic activity. The test article was therefore considered to be non-mutagenic under the conditions of this study.

6.2.4.2 *In Vitro Mammalian Cell Micronucleus Test*

The genotoxic potential of ALA DAG oil was investigated in an *in vitro* mammalian micronucleus test conducted in the Chinese hamster lung fibroblast cell line CHL/IU in accordance with OECD Test Guideline No. 487 (OECD, 2014a), using both the short-term (3-hour) and continuous (24-hour) treatment methods (Honda *et al.*, 2016). The short-term assay was conducted in the presence and absence of S9 metabolic activation and the continuous assay was conducted in the absence of metabolic activation. The vehicle (dimethyl sulfoxide) served as the negative control. Mitomycin C and colchicine were used as the positive controls in assays conducted in the absence of metabolic activation and cyclophosphamide monohydrate was used as the positive control in the presence of metabolic activation. All control and treatments were conducted in duplicate. In the short-term assay conducted in the absence and presence of S9 metabolic activation, the fibroblasts were incubated with the test article at concentrations of 25, 500, 1,000, 2,000 µg/mL. In the 24-hour continuous assay conducted in the absence of S9 metabolic activation, the fibroblasts were incubated with the test article at concentrations of 50, 500, 1,000, 2,000 µg/mL. A positive result was defined as a 2-fold or greater concentration-dependent increase in the number of revertant colonies observed compared to the negative control. For the evaluation of the results, a statistically significant increase in the incidence of cells with micronucleus when compared to the negative control was considered to be a positive response, and all others considered a negative response.

Under all treatment conditions, test article precipitation was observed at 50 µg/mL and greater upon addition and 500 µg/mL and greater at the end of treatment, respectively. However, in both the short term and continuous treatments under all conditions, no positive responses were observed. In contrast, positive controls displayed marked mutagenic activity. Based on these findings, ALA DAG oil was non-genotoxic *in vitro* in the mammalian micronucleus test under the conditions of this study.

6.2.4.3 *In Vivo Mammalian Cell Micronucleus Test*

The genotoxic potential of ALA DAG oil was further investigated in an *in vivo* mammalian micronucleus test conducted in mice (Honda *et al.*, 2016). The study was conducted in accordance with OECD Test Guideline No. 474 (OECD, 2014b). Groups of 6 male Crl:CD1 (ICR) mice were orally administered ALA DAG oil at doses providing 500 (low-dose), 1,000 (mid-dose), or 2,000 (high-dose) mg /kg body weight/day for 2 consecutive days. A negative control group received 10

mL/kg body weight/day of the vehicle control (olive oil) and a positive control group (6 male mice) were administered 2 mg/kg body weight/day of mitomycin C intraperitoneally. Body weights were measured prior to the dosing period and before necropsy. Clinical signs of the animals in all groups were observed before dosing and approximately 1 and 4 hours after dosing. All mice were euthanized 48 hours after the first dose (24 hours after the first dose in the case of the positive control group), and their femurs were removed. Bone marrow samples were obtained from the femur of 5 rats per group to be assessed in the micronucleus assay.

No clinical signs of toxicity or adverse effects on body weight gain were observed in the test groups. There were no significant differences in the frequency of micronucleated cells in the test groups compared to the negative control group. In addition, no significant differences in the ratio of immature erythrocytes to the total number of analyzed erythrocytes were observed in the ALA DAG oil-administered groups compared to the negative control group. In contrast, marked increases in the incidence of micronucleated cells and a decrease in the ratio of immature erythrocytes to the total number of analyzed erythrocytes was observed in the mitomycin C positive control group compared to the negative control group. Based on these findings, ALA DAG oil was non-genotoxic *in vivo* in the mammalian erythrocyte micronucleus test.

6.3 Human Studies

A number of clinical studies conducted with ALA DAG oil were identified in the literature (Katsuragi *et al.*, 2001; Takei *et al.*, 2001; Ando *et al.*, 2016, 2017a,b; Saito *et al.*, 2016, 2017; Suzuki *et al.*, 2016; Yamanaka *et al.*, 2016). These studies were conducted in healthy subjects (Katsuragi *et al.*, 2001; Takei *et al.*, 2001; Ando *et al.*, 2016; Suzuki *et al.*, 2016; Yamanaka *et al.*, 2016) or obese and overweight subjects (Ando *et al.*, 2017a,b; Saito *et al.*, 2016, 2017) wherein ALA DAG oil was administered as an oil or in a shortbread, providing doses up to 12.5 g/day for up to 16 weeks. These studies are summarized in Table 6.3-1 below.

In the study by Yamanaka *et al.* (2016), healthy subjects were instructed to consume 180 g/day of a shortbread providing 7.5 g/day ALA DAG oil, approximately 3 times the effective dose (2.5 g/day). No significant changes in any hematological, clinical chemistry, or urinalysis parameter were observed compared to the control group. Suzuki *et al.* (2016) administered 12.5 g/day ALA DAG oil to 20 subjects for 4 weeks, and did not observe any significant difference in adverse events between the treatment and control groups. A significant increase in total protein and albumin concentration was noted at Study Week 2 compared to the control; however, this effect was not observed at the end of the study period. A significant decrease in change in phosphorus and magnesium level was observed at the end of the study period compared to the control. The change in levels of these minerals were within the biological control range reported by the study authors, and therefore, were not considered to be a treatment-related effect. It should be noted that the dose

of ALA DAG oil provided in this study (12.5 g/day) was 5 times the effective dose (2.5 g/day). Overall, ALA DAG oil was provided to a total number of 126 healthy subjects in shortbread or drink form, providing doses of 2.5 g/day for 14 days to 16 weeks (Takei *et al.*, 2001; Ando *et al.*, 2016, 2017a,b). Anthropometric parameters such as body weight, waist-hip circumference and ratio, and body mass index were measured with no significant changes in these parameters noted in any study subject.

In a randomized, double-blind, placebo-controlled study, 177 obese or overweight subjects (88 to 89/group) consumed a shortbread containing 2.5 g ALA DAG oil/day for 12 weeks (Saito *et al.*, 2016). In this study, anthropometric parameters, such as body weight, waist-hip circumference, and blood pressure, and clinical chemistry parameters including triglycerides, cholesterol, glucose, hemoglobin A1c, insulin, AST, ALT, ALP, GGT, lactate dehydrogenase (LDH), total protein, albumin, uric acid, creatinine, and blood urea nitrogen, were measured. In addition, the number of adverse events was also measured. No adverse events were reported, and no significant changes in any clinical chemistry parameter were reported in any study subject. A significant decrease in body weight and waist-hip circumference was observed in the ALA DAG oil group compared to the placebo.

In another randomized, double-blind, placebo-controlled study, 114 obese or overweight subjects (57/group) consumed 2.5 g/day ALA DAG oil or ALA TAG oil (control) for 12 weeks (Saito *et al.*, 2017). Treatment with ALA DAG or the control was ceased at the end of the 12-week study period. Study parameters were measured at 4 weeks before the start of treatment, at 0 (baseline), 4, 8, and 12 weeks. The authors measured body mass index (BMI), body weight, waist circumference, blood pressure, urinary parameters (glucose, protein, bilirubin, urobilinogen, ketone bodies, occult blood reaction, pH, and gravity), hematology parameters (WBC, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, and platelets), clinical chemistry parameters (total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, AST, ALT, ALP, gamma-glutamyl transferase, total protein, albumin, uric acid, creatinine, urea nitrogen, sodium, chlorine, calcium, phosphorus, and iron concentrations) and adverse events. A significant decrease in body weight, BMI, serum TAG concentration, total protein, and urea nitrogen was observed in the ALA DAG oil group compared to the control at Week 12, however no significant effect was observed after the 4-week recovery period. No significant changes in waist circumference, blood pressure, urinary parameters, hematology, or any other clinical chemistry parameter were reported. The authors reported an improvement in ALT concentration in the ALA DAG oil group compared to control at Week 12 and at the end of the 4-week recovery period, however no further details were provided. The authors noted no significant difference in the incidence of adverse events between the treatment and control groups.

Overall, the results of several human studies where a total number of 126 healthy subjects and 145 obese or overweight subjects consuming 2.5 g ALA DAG oil/day for up to 16 weeks indicate that ALA DAG oil consumption was well tolerated with no adverse events reported (Takei *et al.*, 2001; Ando *et al.*, 2016, 2017a,b; Saito *et al.*, 2016, 2017). Furthermore, in other clinical studies with healthy subjects where ALA DAG oil was provided at doses of 7.5 or 12.5 g/day for 4 weeks, no significant changes in safety-related parameters such as hematology, clinical chemistry, or urinalysis parameters, and no adverse events, were observed (Suzuki *et al.*, 2016; Yamanaka *et al.*, 2016). The results of these clinical studies support the safety of ALA DAG oil in humans when consumed at the estimated dietary level of 2.5 g ALA DAG oil/day, providing approximately 1.39 g ALA DAG/day (including the background dietary exposure and the ALA DAG content in ALA DAG oil).

Table 6.3-1 Summary of Clinical Studies on the ALA DAG Oil

Study Population	Duration (Study Design)	Test Material and Dose	Safety-Related Endpoints	Results	Reference
19 healthy men (mean age 40±8 y; BMI 23.0±2.6 kg/m ²)	14 days (randomized, double-blind, controlled, crossover) 14-day washout period	Control: 2.5 g/day TAG Treatment: 2.5 g/day ALA DAG oil product TAG and ALA DAG oil product provided as 60 g/day shortbread	Anthropometric parameters (bw, BMI, waist circumference)	<ul style="list-style-type: none"> NSD in anthropometric parameters 	Ando <i>et al.</i> (2016)
16 healthy subjects (11 M, 5 F; mean age 49±9 y)	14 days (randomized, double-blind, controlled, crossover) 21-day washout period	Control: 2.5 g/day TAG Treatment: 2.5 g/day ALA DAG oil product TAG and ALA DAG oil product provided as 60 g/day shortbread	Anthropometric parameters (bw, BMI, waist circumference)	<ul style="list-style-type: none"> NSD in anthropometric parameters 	Ando <i>et al.</i> (2017a)
17 healthy subjects (14 M, 3 F; mean age 47±7 y; BMI 25.7±2.0 kg/m ²)	4 weeks (randomized, double-blind, crossover) Intervention provided in two 4-week periods 4-week washout period	Control: 2.5 g/day TAG Treatment: 2.5 g/day ALA DAG oil ^a	Anthropometric parameters (bw, BMI, waist circumference), triglycerides, glucose, insulin, adverse events	<ul style="list-style-type: none"> NSD in adverse events NSD in anthropometric parameters, triglycerides, glucose, insulin 	Ando <i>et al.</i> (2017b)
114 obese or overweight subjects (90 M, 24 F;	12 weeks (randomized, double-	Control: 2.5 g/day TAG	Anthropometric parameters (bw, waist-	<ul style="list-style-type: none"> NSD in adverse events ↓ body weight, BMI, serum TAG concentration, total protein, and 	Saito <i>et al.</i> (2017)

Table 6.3-1 Summary of Clinical Studies on the ALA DAG Oil

Study Population	Duration (Study Design)	Test Material and Dose	Safety-Related Endpoints	Results	Reference
mean age 52±7 y in ALA DAG oil group and 51±7 y in ALA TAG oil group; BMI 25 to <30 kg/m ² n=57/group	blind, controlled, parallel), with 4-week recovery period	Treatment: 2.5 g/day ALA DAG oil ^a	hip circumference, blood pressure), adverse events, hematology ^b , clinical chemistry ^c , and urinalysis ^d	<ul style="list-style-type: none"> urea nitrogen at Week 12 (NSD after 4-week recovery period) NSD in waist circumference, blood pressure, urinalysis, hematology, or other clinical chemistry parameters “Improvement” in ALT concentration (no further details provided) 	
40 healthy subjects (16 M, 24 F; mean age 45±14 y) n=20/group	4 weeks (randomized, double-blind, controlled, parallel)	Control: TAG Treatment: 12.5 g/day ALA DAG oil product ^a	Anthropometric parameters (bw, waist-hip circumference, BMI, blood pressure), adverse events, and hematology ^b , clinical chemistry ^c , and urinalysis ^d	<ul style="list-style-type: none"> NSD in adverse events NSD in anthropometric parameters or hematology ↑ total protein and albumin at 2 weeks compared to control; effect not observed at 4 weeks ↓ change in phosphorus and magnesium levels at 4 weeks compared to control ↓ sodium levels at 2 weeks compared to control; effect not observed at 4 weeks 	Suzuki <i>et al.</i> (2016)
60 healthy individuals (33 M, 27 F, mean age 39±12 y) n=30/group	4 weeks (randomized, double-blind, controlled, parallel)	Control: 7.5 g/day TAG Treatment: 7.5 g/day ALA DAG oil product ^e TAG and ALA DAG oil product provided as 180 g/day shortbread	Anthropometric parameters (bw, waist-hip circumference, BMI, blood pressure), adverse events, and hematology, clinical chemistry, blood coagulation, blood endocrine factors,	<ul style="list-style-type: none"> NSD in adverse events NSD in anthropometric parameters, hematology, clinical chemistry, blood coagulation, blood endocrine factors, blood fat-soluble (pro) vitamins, urinalysis 	Yamanaka <i>et al.</i> (2016)

Table 6.3-1 Summary of Clinical Studies on the ALA DAG Oil

Study Population	Duration (Study Design)	Test Material and Dose	Safety-Related Endpoints	Results	Reference
			blood fat-soluble (pro) vitamins and urinalysis		
177 obese or overweight individuals (69 M, 108 F; mean age 50±8 y; BMI 27.1±1.5 kg/m ²) n=88 to 89/group	12 weeks (randomized, double-blind, controlled, parallel)	Control: 2.5 g/day TAG Treatment: 2.5 g/day ALA DAG oil product ^e TAG and ALA DAG oil product provided as 60 g/day shortbread	Anthropometric parameters (bw, waist-hip circumference, blood pressure), adverse events, and clinical chemistry ^f	<ul style="list-style-type: none"> • NSD in adverse events • ↓ bw and waist circumference in ALA DAG group compared to control • NSD in clinical chemistry parameters 	Saito <i>et al.</i> (2016)
66 healthy subjects (mean age 34±2, 37±2, 36±2 y, and BMI 23.4±0.6, 23.1±0.5, 23.4±0.5 kg/m ² for control, treatment 1 and 2, respectively) n=22/group	12 weeks (study design NR)	Control: Normal diet Treatment 1: 2.5 g/day ALA DAG oil product Treatment 2: 3.75 g/day ALA DAG oil product The ALA DAG oil product contained ~49% ALA	Anthropometric parameters (bw, waist and hip circumference and ratio, BMI)	<ul style="list-style-type: none"> • NSD in anthropometric parameters 	Takei <i>et al.</i> (2001) Japanese article – English abstract only
48 healthy subjects (mean age 38.3±1.6 and 37.9±1.5 y and BMI 24.3±0.5 and 24.4±0.4 kg/m ² for control and treatment, respectively)	16 weeks (study design NR)	Control: Normal diet Treatment: 2.5 g/day ALA DAG oil product	Anthropometric parameters (bw, waist and hip circumference and ratio, BMI)	<ul style="list-style-type: none"> • NSD in anthropometric parameters 	Takei <i>et al.</i> (2001) Japanese article – English

Table 6.3-1 Summary of Clinical Studies on the ALA DAG Oil

Study Population	Duration (Study Design)	Test Material and Dose	Safety-Related Endpoints	Results	Reference
n=16 (control) n=32 (treatment)		ALA DAG oil product provided in a drink			abstract only
30 healthy subjects (mean age 39.6±2.8 and 36.9±1.4 y and BMI 24.3±0.5 and 24.9±0.2 kg/m ² for control and treatment, respectively) n=10 (control) n=20 (treatment)	16 weeks (study design NR)	Control: Normal diet Treatment: 2.5 g/day ALA DAG oil product ALA DAG oil product provided in a drink	Anthropometric parameters (bw, waist and hip circumference and ratio, BMI)	<ul style="list-style-type: none"> NSD in anthropometric parameters 	Takei <i>et al.</i> (2001) Japanese article – English abstract only
Subjects (further details not provided)	6 weeks (study design NR)	Control: NR Treatment: 2 g/day ALA DAG oil product (% ALA NR)	GGT, AST, ALT	<ul style="list-style-type: none"> NSD in AST or GGT ↓ ALT compared to baseline 	Katsuragi <i>et al.</i> (2001) Japanese article – English abstract only

↑ = increase; ↓ = decrease; ALA DAG oil = *alpha*-linolenic acid diacylglycerol oil; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; BUN = blood urea nitrogen; bw = body weight; DAG = diacylglycerol; F = female; GGT = *gamma*-glutamyl transpeptidase; Kao = Kao Corporation; LDH = lactate dehydrogenase; M = male; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; n = number; NR = not reported; NSD = no significant difference; RBC = red blood cells; TAG = triacylglycerol; WBC = white blood cells; y = years.

^a The ALA DAG concentration was 37 g/100 g (37%)

^b Parameters evaluated include WBC, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, and platelets

Table 6.3-1 Summary of Clinical Studies on the ALA DAG Oil

Study Population	Duration (Study Design)	Test Material and Dose	Safety-Related Endpoints	Results	Reference
------------------	-------------------------	------------------------	--------------------------	---------	-----------

^c Parameters evaluated include ALT, AST, ALP, GGT, total protein, albumin, BUN, creatinine, uric acid, LDH, and sodium, potassium, chloride, calcium, magnesium, and phosphorus concentrations

^d Parameters evaluated include protein, glucose, pH, specific gravity, ketone bodies, blood, urobilinogen, bilirubin

^e The DAG bound ALA concentration was 35.3 g/100g (35.3%)

^f Parameters evaluated include triglycerides, cholesterol, glucose, hemoglobin A1c, insulin, AST, ALT, ALP, GGT, LDH, total protein, albumin, uric acid, creatinine, urea nitrogen

6.4 Allergenicity

As discussed in Section 2.4, the enzyme used in the esterification process is immobilized onto the ion exchange resin, and therefore is not expected to migrate into the ALA DAG oil. Three non-consecutive lots of ALA DAG oil were analyzed for protein using the method described by Japan Association for Inspection and Investigation of Foods including Fats and Oils (JIIFA) Fluorescence Method, with a detection limit of 1 ppm. The results of the analysis are shown in Table 6.4-1 below. In all lots, protein content was below the detection limit, demonstrating the absence of any potential residual protein in the oil. Therefore, due to the absence of protein in the ALA DAG oil, the potential for allergenicity of ALA DAG oil is low.

Table 6.4-1 Results of Analysis for Protein Content in 3 Non-Consecutive Lots of ALA DAG Oil

Specification Parameter	Manufacturing Lot		
	Lot No. B	Lot No. C	Lot No. D
Protein (ppm)	ND	ND	ND

ALA DAG = *alpha*-linolenic acid diacylglycerol; ND = not detected; ppm = parts per million.

6.5 Overall Conclusions Related to Safety

The safety of ALA DAG oil for use in the addition to finished food was evaluated in a number of preclinical toxicity studies, including a series of genotoxicity and mutagenicity tests, a 14-day and 90-day oral toxicity (by dietary) studies, prenatal developmental toxicity study, and clinical studies wherein ALA DAG oil product was provided to healthy study subjects at doses up to 5 times (12.5 g/day) the recommended serving size (2.5 g/day). A comprehensive literature search was performed to identify potentially relevant studies in which ALA content bound to the glycerol backbone or low ALA content was reported. No additional studies were identified that were representative of the ALA DAG oil. Therefore, the safety of ALA DAG oil was based on the available product-specific published and unpublished studies.

While the exact metabolic pathway of 1,3-DAG is unclear, available studies in humans and animals demonstrate that following oral ingestion, DAG and TAG are readily hydrolyzed by lipase in the small intestine and are absorbed as MAG, glycerol, or free fatty acids into intestinal cells. Once absorbed, these metabolites are resynthesized to TAG and distributed to adipocytes inside chylomicrons. The MAG, glycerol, and free fatty acids can also be further metabolized in the intestinal cells *via beta*-oxidation or are transported *via* the portal hepatic vein to the liver

where they undergo *beta*-oxidation. Once TAG reaches the adipocytes, they are converted to other endogenous fatty acid-based products or utilized for energy.

In the 90-day study, ALA DAG oil was provided in the diets of Crl:CD(SD) rats at concentrations up to 5.5%, providing doses of 2,916 or 3,326 mg/kg body weight/day for males and females, respectively. No mortalities or clinical signs of toxicity were observed in any animal of the ALA DAG oil group or control. In addition, no significant effects on ophthalmological examination, FOB, motor activity, body weight, food consumption, or macroscopic examination were observed in any group. A number of changes in urinalysis, hematology, clinical chemistry, organ weight, and histopathology were noted in various ALA DAG oil group compared to the rapeseed oil control. However, the observed changes were limited to 1 sex, did not show a dose-dependent relationship, or were not accompanied by a corresponding organ weight or histopathological finding. Therefore, the significant changes were not considered to be toxicologically relevant or compound-related. Based on the results of this study, a NOAEL of 5.5% ALA DAG oil, the highest concentration tested in the diet, equivalent to 2,916 mg/kg body weight/day for males and 3,326 mg/kg body weight/day for females, respectively, was determined. Based on the NOAEL and the daily ALA DAG oil intake of 2.5 g/day, a margin of safety of 82 to 93 exists. The safety of ALA DAG oil is further supported by a number of clinical studies conducted with ALA DAG oil. In these studies, ALA DAG oil product was provided to healthy subjects at doses of 2.5 g/day (Takei *et al.*, 2001; Ando *et al.*, 2016, 2017a,b; Saito *et al.*, 2016, 2017), 3.75 g/day (Takei *et al.*, 2001), 7.5 g/day (Yamanaka *et al.*, 2016), or 12.5 g/day (Suzuki *et al.*, 2016). Safety-related parameters such as anthropometric (body weight, BMI), adverse events, hematology, clinical chemistry, and urinalysis parameters were measured. Notably, in the study by Suzuki *et al.* (2016) where ALA DAG oil product was consumed in the diet at a dose of 12.5 g/day, equivalent to 5 times the effective intended use level, in 20 healthy subjects for 4 weeks, no adverse events or changes in anthropometric parameters and hematology were observed. Moreover, in the study by Yamanaka *et al.* (2016), ALA DAG oil product was provided to 30 healthy subjects at doses of 7.5 g/day, equivalent to 3 times the effective intended use level. Similar to the study by Suzuki *et al.* (2016), no adverse events or changes in anthropometric, hematology, clinical chemistry, blood coagulation, or urinalysis parameters were observed. Collectively, the results of several human studies where a total number of 126 healthy subjects and 145 obese or overweight subjects consuming 2.5 g ALA DAG oil/day for up to 16 weeks indicate that ALA DAG oil consumption was well tolerated with no adverse events reported (Takei *et al.*, 2001; Ando *et al.*, 2016, 2017b; Saito *et al.*, 2016, 2017). Furthermore, in other clinical studies in healthy subjects where ALA DAG oil was provided at doses of 7.5 or 12.5 g/day for 4 weeks, no significant changes in safety-related parameters such as hematology, clinical chemistry, or urinalysis parameters, and no adverse events, were observed (Suzuki *et al.*, 2016; Yamanaka *et al.*, 2016).

The results of these clinical studies support the safety of ALA DAG oil in humans when consumed at the estimated dietary level of 2.5 g ALA DAG oil/day, providing 1.39 g ALA DAG/day (including the background dietary exposure and the ALA DAG content in ALA DAG oil).

The scientific information and data as described herein were independently reviewed and evaluated by a GRAS Panel who unanimously concluded that the ALA DAG oil, meeting appropriate food-grade specifications, and manufactured as described herein, is GRAS for its intended use in the direct addition to finished food by final consumers. A summary of the conclusions of the GRAS Panel is provided in Appendix A.

Part 7. List of Supporting Data and Information (21 CFR § 170.255)

Ando Y, Saito S, Oishi S, Yamanaka N, Hibi M, Osaki N, et al. (2016). Alpha linolenic acid-enriched diacylglycerol enhances postprandial fat oxidation in healthy subjects: a randomized double-blind controlled trial. *J Oleo Sci* 65(8):685-691. DOI:10.5650/jos.ess16064.

Ando Y, Saito S, Yamanaka N, Suzuki C, Ono T, Osaki N, et al. (2017a). Alpha Linolenic Acid-enriched diacylglycerol consumption enhances dietary fat oxidation in healthy subjects: a randomized double-blind controlled trial. *J Oleo Sci* 66(2):181-185. DOI:10.5650/jos.ess16183.

Ando Y, Saito S, Mirua H, Osaki N, Katsuragi Y (2017b). Consumption of alpha-linolenic acid-enriched diacylglycerol induces increase in dietary fat oxidation compared with alpha-linolenic acid-enriched triacylglycerol: A randomized, double-blind trial. *Nutrition Research* 48:85-92. DOI:10.1016/j.nutres.2017.10.012.

Beare-Rogers J, Dieffenbacher A, Holm JV (2001). Lexicon of lipid nutrition (IUPAC Technical Report). *Pure Appl Chem* 73(4):685-744. DOI:10.1351/pac200173040685.

Bushita H, Ito Y, Saito T, Nukada Y, Ikeda N, Nakagiri H, et al. (2018a). A 90-day repeated-dose toxicity study of dietary alpha linolenic acid-enriched diacylglycerol oil in rats. *Regul Toxicol Pharmacol* 97:33-47. DOI:10.1016/j.yrtph.2018.05.017.

Bushita H, Liu S, Ohta T, Ito Y, Saito K, Nukada Y, et al. (2018b). Effects of dietary alpha-linolenic acid-enriched diacylglycerol oil on embryo/fetal development in rats. *Regul Toxicol Pharmacol* 98:108-114. DOI: 1016/j.yrtph.2018.07.007.

- Champ PC, Harvey RA (1994). Experimental dermatology. In: *Lippincott's Illustrated Reviews: Biochemistry, 2nd edition*. Philadelphia (PA): J.B. Lippincott Company, pp. 180-182.
- CODEX (2015). *Codex Standard for Named Vegetable Oils*. (CODEX STAN 210, Adopted in 1999. Revision: 2001, 2003, 2009. Amendment: 2005, 2011, 2013 and 2015). Rome, Italy: Codex Alimentarius Commission. Available at: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCODEX%2B210-1999%252FCXS_210e.pdf.
- Craft BD, Nagy K, Seefelder W, Dubois M, Destailats F (2012). Glycidyl esters in refined palm (*Elaeis guineensis*) oil and related fractions. Part II: practical recommendations for effective mitigation. *Food Chem* 132(1):73-79. DOI:10.1016/j.foodchem.2011.10.034.
- DeFilippis AP, Sperling LS (2006). Understanding omega-3's. *Am Heart J* 151(3):564-570. DOI:10.1016/j.ahj.2005.03.051.
- EFSA (2004). Opinion of the Scientific Panel on Dietetic products, nutrition and allergies [NDA] related to an application to market Enova oil as a novel food in the EU (Question no EFSA-Q-2004-089, adopted: 2 December 2004 by European Food Safety Authority). *EFSA J* 159:1-19. DOI:10.2903/j.efsa.2004.159. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/159>.
- EFSA (2009). Scientific Opinion of the Panel on Dietetic products, Nutrition and Allergies on a request from European Commission related to labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids (question no: EFSA-Q-2009-00548, adopted: 30 June 2009 by European Food Safety Authority) *EFSA J* 1176:1-11. DOI:10.2903/j.efsa.2009.1176. Available at: <https://www.efsa.europa.eu/en/efsajournal/pub/1176>.
- FASEB (1975). *Evaluation of the Health Aspects of Gelatin as a Food Ingredient*. (PB-254 527; FDABF-GRAS-360; SCOGS-58). Prepared by Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Sciences Research Office (LSRO), Select Committee on GRAS Substances (SCOGS) for Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Bureau of Food.
- FCC (2016). Linoleic acid. In: *Food Chemicals Codex, 10th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 764-765.

- Friedman HI, Nylund B (1980). Intestinal fat digestion absorption, and transport: a review. *Am J Clin Nutr* 33(5):1108-1139.
- Honda H, Fujita Y, Hayashi A, Ikeda N, Ito Y, Morita O (2016). Genotoxicity evaluation of *alpha*-linolenic-acid-diacylglycerol oil. *Toxicol Rep* 3:716-722.
- Honda H, Kawamoto T, Doi Y, Matsumura S, Ito Y, Imai N, et al. (2017). Alpha-linolenic acid-enriched diacylglycerol oil does not promote tumor development in tongue and gastrointestinal tract tissues in a medium-term multi-organ carcinogenesis bioassay using male F344 rat. *Food Chem Toxicol* 106(A):185-192. DOI:10.1016/j.fct.2017.04.040.
- Ikeda I, Yanagita T (2008). Fate of hydrolysis products originating from diacylglycerol and triacylglycerol in the intestine. In: Katsuragi Y, Yasukawa T, Matsuo N, Flickinger BD, Tokimitsu I, Matlock MG, editors. *Diacylglycerol Oil, 2nd edition*. Champaign (IL): AOCS Press (American Oil Chemists Society), pp. 67-73.
- IOM (2005). Dietary fats: total fat and fatty acids. In: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients)*. (National Academy of Sciences/NAS, Institute of Medicine/IOM, Food and Nutrition Board/FNB, Panel on Micronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes). Washington (DC): National Academy Press (NAP), pp. 422-541. Available at: http://www.nap.edu/openbook.php?record_id=10490&page=422.
- JEFCA (2006). General specifications and considerations for enzyme preparation used in food processing. In: *Combined Compendium of Food Additive Specifications [Online Edition]. General Specifications for Enzymes Analytical Methods, Volume 4: Analytical Methods, Test Procedures and Laboratory Solutions Used by and Referenced in the Food Specifications. 1st to 65th JECFA Meetings, 1965-2005*. (FAO JECFA Monograph 1). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available at: <http://www.fao.org/docrep/009/a0691e/A0691E00.htm#Contents>.
- Katsuragi Y, Takeda Y, Abe C, Mori K, Toi T, Takei A, et al. (2001). Effect of dietary α -linolenic acid-rich diacylglycerol on body fat in man (2): effects on resting metabolism and fat metabolism. *J Oleo Sci* 50(9):747-752.

- Kondo H, Hase T, Murase T, Tokimitsu I (2003). Digestion and assimilation features of dietary DAG in the rat small intestine *Lipids* 38(1):25-30 [published correction appears in *Lipids* 38(5):593].
- Mansbach CM II, Nevin P (1998). Intracellular movement of triacylglycerols in the intestine. *J Lipid Res* 39(5):963-968. Cited in: Watanabe and Tokimitsu, 2008.
- Meng X, Zou D, Shi Z, Duan Z, Mao Z (2004). Dietary diacylglycerol prevents high-fat diet-induced lipid accumulation in rat liver and abdominal adipose tissue. *Lipids* 39(1):37-41.
- Mori T, Honda H, Ishii S, Koga Y, Okahara F, Onishi S, et al. (2017). Effect of α -linolenic acid-rich diacylglycerol oil on protein kinase C activation in the rat digestive tract and lingual mucosa. *Food Chem Toxicol* 103:168-173. DOI:10.1016/j.fct.2017.02.033. Morris DH (2007). *Flax – A Health and Nutrition Primer, 4th edition*. Winnipeg (AB): Flax Council of Canada.
- Murase T, Mizuno T, Omachi T, Onizawa K, Komine Y, Kondo H, et al. (2001). Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice. *J Lipid Res* 42(3):372-378.
- Murase T, Aoki M, Wakisaka T, Hase T, Tokimitsu I (2002). Anti-obesity effect of dietary diacylglycerol in C57BL/6J mice: dietary diacylglycerol stimulates intestinal lipid metabolism. *J Lipid Res* 43(8):1312-1319.
- OECD (1995). Repeated dose 28-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 407) [Adopted: 27 July 1995]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: <http://www.oecd.org/chemicalsafety/risk-assessment/1948386.pdf> [New Version Adopted 3 October 2008].
- OECD (1997a). *OECD Principles of Good Laboratory Practice*. (Series on Principles of Good Laboratory Practice and Compliance Monitoring, no. 1 [ENV/MC/CHEM(98)17]). Paris, France: Organisation for Economic Co-Operation & Development (OECD), Environment Directorate, Chemicals Group and Management Committee, OECD Environmental Health and Safety Publications. Available at: http://www.oecd-ilibrary.org/environment/oecd-principles-on-good-laboratory-practice_9789264078536-en.

OECD (1997b). Bacterial reverse mutation test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 471) [Updated & Adopted: 21 July 1997]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=1ijgh8o34k0qd.delta.

OECD (1998). Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 408) [Updated & Adopted: 21 September 1998]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwsup322.delta.

OECD (2001). Prenatal developmental toxicity study. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 414) [Adopted: 22nd January 2001]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-414-prenatal-development-toxicity-study_9789264070820-en;jsessionid=nxmxloxwc054.epsilon.

OECD (2014a). In Vitro mammalian cell micronucleus test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 487) [Updated & Adopted: 26 September 2014]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264224438-en.

OECD (2014b). Mammalian erythrocyte micronucleus test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 474) [Updated & Adopted 26 September 2014]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-474-mammalian-erythrocyte-micronucleus-test_9789264224292-en.

Saito S, Fukuhara I, Osaki N, Nakamura H, Katsuragi Y (2016). Consumption of alpha-linolenic acid-enriched diacylglycerol reduces visceral fat area in overweight and obese subjects: a randomized, double-blind controlled, parallel-group designed trial. *J Oleo Sci* 65(7):603-611. DOI:10.5650/jos.ess16059.

Saito S, Mori A, Osaki N, Katsuragi Y (2017). Diacylglycerol enhances the effects of alpha-linolenic acid against visceral fat: a double-blind randomized controlled trial. *Obesity* 25(10):1667-1675. DOI:10.1002/oby.21938.

- Suzuki C, Saito S, Fukuhara I, Kozuma K, Osaki N, Tada N, et al. (2016). Safety evaluation of *alpha* linolenic acid-enriched diacylglycerol consumption at five times the effective dose against visceral obesity in healthy subjects — a randomized, double-blind controlled, parallel-group designed trial. *Jpn Pharmacol Ther* 44(12):1763-1770.
- Takei A, Katsuragi Y, Abe C, Mori K, Takeda Y, Seo Y, et al. (2001). Effects of dietary α -linolenic acid-rich diacylglycerol on body fat in man (1): Lowering effect on body fat. *J Oleo Sci* 50(9):735-746.
- Taguchi H, Nagao T, Watanabe H, Onizawa K, Matsuo N, Tokimitsu I, et al. (2001). Energy value and digestibility of dietary oil containing mainly 1,3-diacylglycerol are similar to those of triacylglycerol [published correction appears in *Lipids* (2003) 38(8):893]. *Lipids* 36(4):379-382.
- Tańska M, Roszkowska B, Skrajda M, Dąbrowski G (2016). Commercial cold pressed flaxseed oils quality and oxidative stability at the beginning and the end of their shelf life. *J Oleo Sci* 65(2):111-121. DOI:10.5650/jos.ess15243.
- U.S. EPA (2018). Commodity consumption calculator. In: *Food Commodity Intake Database: What We Eat in America [2005-2010]*. [FCID]. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Office of Pesticide Programs. Available at: <http://fcid.foodrisk.org/dbc/> ; <http://fcid.foodrisk.org/percentiles> [© University of Maryland 2012 - 2017].
- U.S. FDA (1964). Ion-exchange resins (21 CFR Part 121.1148). *Fed Regist (US)* 29(140):9708.
- U.S. FDA (2009a). *Agency Response Letter GRAS Notice No. GRN 000256 [High linolenic acid flaxseed oil, MB: Polar Foods, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=256> [Jan. 16, 2009].
- U.S. FDA (2002a). *Agency Response Letter GRAS Notice No. GRN 000081 [Lipase from *Candida rugosa*, Nagoya, Japan: Amano Enzyme, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: <https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=81> [Feb. 7, 2002].

U.S. FDA (2002b). *Agency Response Letter GRAS Notice No. GRN 000103 [Lipase enzyme preparation from Aspergillus oryzae carrying a gene constructed from a modified Thermomyces lanuginosus lipase gene and a portion of the Fusarium oxysporum lipase gene, Franklinton (NC): Novozymes North America, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=103> [Aug. 19, 2002].

U.S. FDA (2009b). *Agency Response Letter GRAS Notice No. GRN 000269 [Polyglycerol fatty acid esters with a degree of polymerization of 11 to 40 for the polyglycerol backbone, Yokkaichi, Japan: Taiyo-Kagaku Co., Ltd.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=269> [May 21, 2009].

U.S. FDA (2016). *Agency Response Letter GRAS Notice No. GRN 000652 [gamma-linolenic acid safflower oil, Arcadia Biosciences, Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: <https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm541962.htm> [December 16, 2016].

U.S. FDA (2017). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs*. (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at:
<http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR>.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	§	Section Title
110— Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food	110.5	Current good manufacturing practice
173— Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
	173.357	Materials used as fixing agents in the immobilization of enzyme preparations
182—Substances generally recognized as safe	182.1320	Glycerin
	182.3149	Ascorbyl palmitate
184—Direct food substances affirmed as generally recognized as safe	184.1033	Citric acid
	184.1155	Bentonite

Watanabe H, Onizawa K, Naito S, Taguchi H, Goto N, Nagao T, et al. (2001). Fat-soluble vitamin status is not affected by diacylglycerol consumption. *Ann Nutr Metab* 45(6):259-264.

Watanabe H, Onizawa K, Taguchi H, Fujimori N, Naito S, Gotoh N, et al. (1997). Effects of diacylglycerols on lipid metabolism in human. *J Jpn Oil Chem Soc* 46(3):309-314 [English translation].

Watanabe H, Tokimitsu I (2008). Digestion and absorption of diacylglycerols. In: Katsuragi Y, Yasukawa T, Matsuo N, Flickinger BD, Tokimitsu I, Matlock MG, editors. *Diacylglycerol Oil, 2nd edition*. Champaign (IL): AOCS Press (American Oil Chemists Society), pp. 31-47.

Yamanaka N, Yasunaga K, Nakamura H, Saito S, Tabata K, Katsuragi Y, et al. (2016). [Safety evaluation of excessive consumption of *alpha*-linolenic acid-enriched diacylglycerol in healthy subjects: A randomized, double-blind controlled, parallel-group designed trial] *Yakuri To Chiryō* [Basic Pharmacol Ther] 44(6):871-880 [Japanese].

Yanagita T, Ikeda I, Wang YM, Nakagiri H (2004). Comparison of the lymphatic transport of radiolabeled 1,3-dioleoylglycerol and trioleoylglycerol in rats. *Lipids* 39(9):827-832 [Erratum 2005, 40:53].

Yasukawa T, Katsuragi Y (2008). Chapter 1: Diacylglycerols. In: Katsuragi Y, Yasukawa T, Matsuo N, Flickinger BD, Tokimitsu I, Matlock MG, editors. *Diacylglycerol Oil, 2nd edition*. Champaign (IL): AOCS Press (American Oil Chemists Society), pp. 1-16.

APPENDIX A

EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of the use of Alpha-Linolenic Acid Diacylglycerol Oil (ALA DAG oil) added to finished food by the consumer. This GRAS conclusion for the intended use specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of ALA DAG oil has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of Alpha-Linolenic Acid Diacylglycerol Oil has been concluded to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. The ALA DAG oil is manufactured through enzymatic esterification of fatty acids derived from flaxseed oil with either monoacylglycerol or glycerol. The resulting product is composed of primarily diacylglycerol (DAG), with small quantities of triacylglycerol (TAG) and monoacylglycerol (MAG), with alpha-linolenic acid bound to the glycerol backbone.
2. The production of the ALA DAG oil is performed in a facility certified under ISO 14001. The ALA DAG oil will be manufactured in accordance with cGMP as described in 21 CFR § 117 (U.S. FDA, 2017) and will include appropriate preventative controls in accordance with the FSMA.
3. All raw materials, processing aids, additives, and food contact materials used in the production of the ALA DAG oil are food grade or equivalent [e.g., FCC, U.S. Pharmacopeia (USP), or European Pharmacopeia (EP)], and are used in accordance with an applicable FDA regulation (21 CFR), have previously been determined to be GRAS, or have been the subject of an accepted food contact notification.
4. Appropriate food-grade specifications and other quality testing has been established for the ALA DAG oil.
5. Batches were also analyzed for the presence of phytosterols as the ALA DAG oil is derived from flaxseed oil. The levels of these phytosterols are below the levels of total phytosterols reported in a previous GRAS notice (1 to 2% phytosterols,

including sterols and tocopherols) regarding a high linolenic acid flaxseed oil that received “no questions” from the U.S. FDA and are generally consistent with the phytosterol content of commercially available cold-pressed flaxseed oil

6. While the exact metabolic pathway of 1,3-DAG is unclear, available studies in animals demonstrate that following oral ingestion, both DAG and triacylglycerol (TAG) are readily hydrolyzed by lipase in the small intestine and are absorbed as monoacylglyceride (MAG), glycerol, or free fatty acids into intestinal cells. Once absorbed, these components are resynthesized to TAG and distributed to adipocytes inside chylomicrons or are further metabolized via beta oxidation in the intestinal cells or are transported via the portal hepatic vein to the liver where they undergo beta-oxidation. Once TAG reaches the adipocytes, they are converted to other endogenous fatty acid-based products or utilized for energy.
7. A clinical study compared blood concentrations of vitamins A, E, and D in male volunteers who were consuming 20 g DAG oil per day or 20 g TAG oil per day for 12 weeks. There was no difference in fat-soluble vitamin concentrations in blood when samples were taken and analyzed on Weeks 4, 8, and 12; it is unlikely that DAG oil would have an effect on the bioavailability of vitamins based on the mode of action of DAG.
8. The preclinical toxicity of ALA DAG oil was assessed in a standard toxicology battery, consisting of a repeated-dose 14-day and 90-day oral toxicity studies in rats, and a series of genotoxicity assays, including a bacterial reverse mutation test, an *in vitro* mammalian cell micronucleus test, and an *in vivo* mammalian cell micronucleus test in mice. In addition, a prenatal developmental oral toxicity study in rats was also conducted. All tests were performed in compliance with the Organisation of Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) and in accordance with the OECD Guidelines for the Testing of Chemicals. The 14-day range-finding study and the preliminary prenatal developmental toxicity study are not published, the results of these studies are corroborative evidence to support the safety of ALA DAG oil.
9. Based on the results of the 90-day toxicology study, a no-observed-adverse-effect level (NOAEL) of 5.5% ALA DAG oil, the highest concentration tested was determined. This is equivalent to 2,916 mg/kg body weight/day for males and 3,326 mg/kg body weight/day for females, respectively.


10. In the prenatal developmental toxicity study, a NOAEL of 4,715 mg/kg body weight/day ALA DAG oil, the highest concentration tested, for maternal general toxicity, maternal reproductive function, and the embryo-fetal development was determined.
11. ALA DAG oil was not mutagenic or genotoxic in a bacterial reverse mutation test, an *in vitro* mammalian cell micronucleus test, and an *in vivo* mammalian cell micronucleus test.
12. Clinical studies in healthy subjects and obese or overweight subjects consuming 2.5 g ALA DAG oil/day for up to 16 weeks suggest that ALA DAG oil consumption was well tolerated with no adverse events reported. Furthermore, in other clinical studies with healthy subjects where ALA DAG oil was provided at doses of 7.5 or 12.5 g/day for 4 weeks, no significant changes in safety-related parameters such as hematology, clinical chemistry, or urinalysis parameters, and no adverse events, were observed.
13. The ALA DAG oil will be marketed in aluminum packets containing 2.5 g of the oil, and the recommended number of servings will be 1 packet/day (or 2.5 g ALA DAG oil/serving). The product label will indicate to the consumer that 1 serving of ALA DAG oil provides the recommended daily amount (i.e., 2.5 g/day). At the estimated dietary intake (EDI) of 2.5 g ALA DAG oil/day, this will provide approximately 1.39 g ALA DAG/day (including the background dietary exposure and the ALA DAG content in ALA DAG oil).
14. The margin of safety between the NOAEL of 2,916 mg/kg body weight/day for males and 3,326 mg/kg body weight/day for females derived from the published 90-day study is adequate to support the EDI of 2.5 g/d. Additional published clinical studies of ALA DAG oil also support the safety of the EDI.
15. The safety of ALA DAG oil is corroborated by a prenatal developmental toxicity study.

Conclusion of the GRAS status of ALA DAG oil under the intended conditions of use has been made through the deliberations of Claire L. Kruger, PhD, DABT, CFS; Madhusudan G. Soni, PhD, FATS, FACN, and Henry N. Ginsberg, MD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of ALA DAG oil and the human exposure to ALA DAG oil resulting from its intended use from addition to finished food by the consumer:


There is no evidence in the available information on ALA DAG oil that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when ALA DAG oil is used at levels that might reasonably be expected from the proposed application of ALA DAG oil from addition to finished food by the consumer as proposed by Kao Corporation.

Therefore, ALA DAG oil is safe and GRAS at the proposed level of intake. ALA DAG oil is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.


Claire L. Kruger, PhD, DABT, CFS
GRAS Expert Panel Chairman
ChromaDex Spherix Consulting

Signature: 
Date: June 10, 2019

Madhusudan G. Soni, PhD, FATS, FACN
GRAS Expert Panel Member
Soni & Associates, Inc.

Signature: 
Date: June 10, 2019

Henry N. Ginsberg, MD
GRAS Expert Panel Member
Columbia University
Institute of Human Nutrition

Signature: 
Date: June 10, 2019

From: [Sanzo, Kathleen M.](#)
To: [Hall, Karen](#)
Subject: FW: Updated Kao Response regarding GRN 914
Date: Tuesday, September 29, 2020 9:26:21 AM
Attachments: [047724-01_116300988v2_Response_to_FDA.PDF](#)
[Attachment 1 - COA for 3 Production Batches of ALA DAG.pdf](#)
[Attachment 2 - COA for Stability Test.pdf](#)
[Attachment 3 - Full Publication of Yamanaka et al 2016.pdf](#)
[Attachment 4 - Literature Search Results.pdf](#)
[Table A-1.pdf](#)

Dear Ms. Hall,

Attached please find our response on behalf of Kao to the FDA additional questions concerning GRN 914. Please let me know if you have any questions concerning the response or the attachments.

Thank you

Kathy Sanzo

Kathleen M. Sanzo

Morgan, Lewis & Bockius LLP

1111 Pennsylvania Avenue, NW | Washington, DC 20004-2541

Direct: +1.202.739.5209 | Cell: +1.301.651.2901 | Main: +1.202.739.3000 | Fax: +1.202.739.3001

kathleen.sanzo@morganlewis.com | www.morganlewis.com

Assistant: Tricia M. Farringer | +1.202.739.5272 | tricia.farringer@morganlewis.com

DISCLAIMER

This e-mail message is intended only for the personal use of the recipient(s) named above. This message may be an attorney-client communication and as such privileged and confidential and/or it may include attorney work product. If you are not an intended recipient, you may not review, copy or distribute this message. If you have received this communication in error, please notify us immediately by e-mail and delete the original message.

Morgan Lewis

Kathleen M. Sanzo

Partner

+1.202.739.5209

kathleen.sanzo@morganlewis.com

September 28, 2020

VIA EMAIL

Karen Hall

Regulatory Review Scientist

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

5001 Campus Drive

College Park, MD 20740-3835

RE: GRAS Notice No. GRN 914 for Alpha-Linolenic Acid Diacylglycerol

Dear Ms. Hall:

Please find below responses to the United States (U.S.) Food and Drug Administration (FDA)'s follow-up questions on GRAS Notice (GRN) No. 914 pertaining to alpha-linolenic acid diacylglycerol (ALA DAG).

Question 1. Kao Corporation (Kao) indicates that the enzymes used in the manufacturing process can be immobilized on the ion-exchange resin. Please clarify if the enzymes are always immobilized on the ion-exchange resin or if they are only immobilized in certain cases. Also, please discuss if the ion-exchange resins comply with 21 CFR 173.25.

Response 1. The enzymes are always immobilized on the ion exchange resin using gelatin as an immobilizing agent. The resin is crosslinked phenol-formaldehyde polycondensate with tertiary amine functionality and complies with 21 CFR §173.25 and §173.357.

Morgan, Lewis & Bockius LLP

1111 Pennsylvania Avenue, NW
Washington, DC 20004
United States

T +1.202.739.3000
F +1.202.739.3001

Question 2. Kao states that an internal method was developed and validated for the analysis of *alpha*-linolenic acid diacylglycerol (ALA DAG). In addition, Kao Corporation indicates that all methods of analysis are internationally recognized or internal methods developed and validated by Kao. Please specify which methods are used to analyze for each parameter and provide a statement indicating that all analytical methods are validated for their intended purpose.

Response 2. A summary of the methods of analysis used for each parameter of the ALA DAG ingredient is provided in Table 1. The specifications of ALA DAG have been updated to include limits for cadmium and mercury. All methods of analysis are internationally recognized [*e.g.*, American Oil Chemists Society (AOCS) or Japan Oil Chemists Society (JOCS)] or internally developed and validated by Kao. The internal method is based on solid phase extraction and gas chromatography-flame ion detection and validated according to Volume I - 5.4 Test Methods and Method Validation.

The certificates of analysis for 3 production batches showing conformance to the revised product specifications are provided in Attachment 1.

Table 1 Product Specifications for ALA DAG

Specification Parameter	Specification	Method of Analysis
ALA DAG	≥36% by weight	Internal method ^a
Peroxide value	≤5 meq/kg of sample	AOCS Cd 8b-90
Acid value	≤2 mg KOH/g of sample	AOCS Cd 3d-63
Moisture	≤0.1% by weight	AOCS Ca 2e-84
Lead	≤0.5 ppm	JOCS 2.6.3.2-2013
Arsenic	≤0.5 ppm	JOCS 2.6.3.7-2013
Cadmium	≤0.2 ppm	AOCS Ca 18-01 (modified)
Mercury	≤0.1 ppm	CV-AAS

ALA DAG = *alpha*-linolenic acid diacylglycerol; AOCS = American Oil Chemists' Society; CV-AAS = cold vapor atomic absorption spectroscopy; JOCS = Japan Oil Chemists' Society; KOH = potassium hydroxide; ppm = parts per million.

^a Internal method developed and validated by Kao.

Question 3. In Table 2.5-1, Kao provides specifications for ALA DAG, peroxide value, acid value, moisture, lead and arsenic. Since ALA DAG is derived from flaxseed, which is grown in soil, please provide specifications for mercury and cadmium, as well as data from the analysis of a minimum of three, but preferably five, non-consecutive lots to demonstrate conformance with the stated specifications.

Response 3. Four production batches of ALA DAG were analyzed for cadmium and mercury using atomic absorption spectrometry (AAS) and cold vapor AAS, respectively. The limit of quantitation (LOQ) was 0.01 ppm for each heavy metal. As shown in Table 2 below, levels of cadmium and mercury were below the LOQ in each batch. Kao has also set specifications for mercury and cadmium in ALA DAG of 0.1 and 0.2 ppm, respectively (see Table 1 for revised specifications).

Table 2 Cadmium and Mercury Analysis for ALA DAG

Heavy Metal	Production Lot No.			
	Lot No. A	Lot No. B	Lot No. C	Lot No. D
Cadmium (ppm) ^{a,b}	ND	ND	ND	ND
Mercury (ppm) ^{a,c}	ND	ND	ND	ND

^a Limit of quantitation: 0.01 ppm.

^b Method of analysis: Atomic absorption spectrometry.

^c Method of analysis: Cold vapor atomic absorption spectrometry.

Question 4. 3-Monochloropropane-1,2-diol esters (3-MCPDE) are chemical contaminants formed during the refining process of edible oils. Due to their toxicological properties, JECFA established a PMTDI for 3-MCPD and 3-MCPD esters of 4 µg/kg bw/d and EFSA derived a TDI of 2 µg/kg bw/d for 3-MCPD and its esters. Kao states that ALA DAG is produced from flaxseed oil via a process that is consistent with that of other edible oils. Therefore, given the stated toxicity concerns and recent efforts to reduce exposure to 3-MCPDE, please discuss (1) the potential presence of 3-MCPDE in ALA DAG, and (2) if present, please provide a narrative that supports the safe use of ALA DAG under the intended conditions of use. A discussion of mitigation strategies can be found in the Codex Code of Practice entitled “Reduction of 3-monochloropropane-1,2-diol esters (3-MCPDE) and glycidyl esters (GE) in Refined Oils and Food Products Made with Refined Oils” (adopted July 2019, 42nd session, Codex Alimentarius Commission).

Response 4. Three batches of ALA DAG on which this submission is based were analyzed for 3-MCPDE using DGF Standard Methods Section C-Fats C-VI 18(10), Assay A and Assay B. The results demonstrate levels of 3-MCPDE to be 0.2 mg/kg across all 3 batches. The potential intake of 3-MCPDE based on the intended uses of the ALA DAG oil of 2.5 g/day is approximately 0.00714 µg/kg bw/day for a 70-kg individual, which is well below the PMTDI of 4 µg/kg bw/day established by JECFA and TDI of 2 µg/kg bw/day established by EFSA. Therefore, the potential levels of 0.2 mg/kg in the ALA DAG product are not expected to pose any safety concerns. The production process of ALA DAG includes several bleaching and deodorization steps under conditions recommended by Codex¹ to reduce levels of GE and 3-MCPDE in the final product.

Question 5. In Table 2.8-2, Kao provides the results for all specifications at 0, 12, and 18 months after production, with the exception of peroxide value. Please provide the stability data for peroxide value for the 18-month stability study.

Response 5. The stability results, including the peroxide value, at 0, 12, and 18 months are provided in Table 3 below. The certificate of analysis is provided in Attachment 2.

¹ http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXC%2B79-2019%252FCXC_079e.pdf

Table 3 Results of the 18-Month Shelf-Life Stability Study on the Final ALA DAG Product^a

Specification Parameter	Initial (0 month)	12 months after production	18 months after production	Method of Analysis
ALA DAG (%)	39	39	39	Internal method ^b
Acid value (mg KOH/g)	0.7	0.7	0.8	AOCS Cd 3d-63
Peroxide value (meq/kg)	0	0	0	AOCS Cd 8b-90
Moisture (%)	0.02	0.04	0.05	AOCS Ca 2e-84
Lead (ppm)	<0.05	<0.05	<0.05	JOCS 2.6.3.2-2013
Arsenic (ppm)	<0.1	<0.1	<0.1	JOCS 2.6.3.7-2013

ALA DAG = *alpha*-linolenic acid diacylglycerol; KOH = potassium hydroxide; ppm = parts per million.

^aThe final product consists of the ALA DAG oil as described is formulated with antioxidants.

^bInternal and validated method.

Question 6. On page 7, Kao provides the results of batch analysis for levels of phytosterols contained in the ALA DAG oil. Please provide an updated literature search on the safety of phytosterols beginning from the submission of GRN 256 (i.e., 2008) and a narrative that discusses the safety of phytosterols at the notified levels in the context of exposure and relevant safety information.

Response 6. The levels of phytosterols, specifically campesterol, stigmasterol, and sitosterol across 3 lots of ALA DAG oil was 0.424%. Based on the intended use of 2.5 g/day of the ALA DAG oil, these levels would be equivalent to approximately 0.011 g/day or 0.15 mg/kg body weight/day for a 70-kg individual. The GRAS status of a number of phytosterols and phytosterols esters, including campesterol, and stigmasterol, have been notified to the FDA, all of which received no questions (Table 4). According to GRN 492 the most recently filed GRN pertaining to phytosterols and phytosterol esters, the dietary intakes of phytosterols are in the range of 6.6 g/day (mean) and 11 g/day (90th percentile) for the total U.S. population. In comparison, the levels of phytosterols in Kao's ALA DAG oil are low and the resultant intakes based on the intended uses of the ingredient are negligible compared to the food uses of other phytosterol/phytosterol ester ingredients. Hepburn *et al.* (1999)² reported a NOAEL of 4,200 mg/kg body weight/day in a 90-day subchronic oral toxicity study in rats. The NOAEL is approximately 30,000-fold greater than the intakes of phytosterols from ALA DAG oil. Likewise, in humans, consumption of 9 g/day of phytosterols for 2 months was well tolerated and did not produce any adverse clinical effects (Davidson *et al.*, 2001³), while consumption of 1.6 g/day for 1 year was also well tolerated and without adverse findings (Hendricks *et al.*, 2003⁴). The safety of phytosterols and phytosterol esters is generally recognized and no safety concerns from current food uses have been identified. A search of the PubMed databases for the phytosterols in the ALA DAG oil did not identify any recent studies since 2014 that would contradict the previous safety conclusions on phytosterols and phytosterol esters.

² Hepburn PA, Homer SA, Smith M (1999). Safety evaluation of phytosterol esters: Part 2. Subchronic 90-day oral toxicity study on phytosterol esters: a novel functional food. *Food Chem Toxicol* 37(5):521-532.

³ Davidson MH, Maki KC, Umporowicz DM, Ingram KA, Dicklin MR, Schaefer E et al. (2001). Safety and tolerability of esterified phytosterols administered in reduced-fat spread and salad dressing to healthy adult men and women. *J Am Coll Nutr* 20(4):307-319.

⁴ Hendriks HF, Brink EJ, Meijer GW, Princen HM, Ntanos FY (2003). Safety of long-term consumption of plant sterol esters-enriched spread. *Eur J Clin Nutr* 57(5):681-692.

Table 4 Summary of GRAS Notices Pertaining to Phytosterols and Phytosterol Esters Notified to the FDA

Substance	Intended Uses	Use Level	FDA Response	GRN No.
Phytosterols and phytosterol esters	As an ingredient in foods	750 mg/serving	No questions	492 (U.S. FDA, 2014)
Vegetable oil and tall oil derived phytosterol and phytosterol ester formulations	As an ingredient in baked goods and baking mixes; beverages and beverage bases; breakfast cereals; cheeses; coffee and tea; condiments and relishes; dairy product analogs; egg products; fats and oils; fish products; frozen dairy desserts and mixes; grain products and pastas; gravies and sauces; hard candy; herbs, seeds, spices, seasonings, blends, extracts, and flavorings; jams and jellies; whole and skim milk; milk products; nuts and nut products; processed fruits and fruit juices; processed vegetables and vegetable juices; snack foods; soft candy; and soups and soup mixes	0.5 or 1.0 grams per serving	No questions	398 (U.S. FDA, 2012)
Plant-derived esterified and non-esterified sterols and stanols (phytosterols)	As a food ingredient in baked goods and baking mixes; beverages and beverage bases; breakfast cereals; cheeses; coffee and tea (specialty coffee drinks and ready-to-drink tea beverages); dairy product analogs; fats and oils; frozen dairy; desserts; grain products and pastas; gravies and sauces; milk, whole and skim; milk products, including yogurt (including cultured yoghurts and cultured yoghurt-type products) as well as cultured dairy drinks; plant protein products; processed fruits and fruit juices; processed vegetables and vegetable juices; snack foods; soft candy; and soups and soup mixes.	2 g per serving; 1 g per serving for milk, fruit juice and vegetable juice	No questions	387 (U.S. FDA, 2011)
Pine tree phytosterol esters	Ingredient in multiple food categories including margarine and vegetable-based spreads, yogurt and yogurt-like products, milk-based juice beverages, ice cream and non-standardized ice cream products, cream cheese and cream cheese-like products, snack bars, salad dressing, standardized and non-standardized bread products, baked goods, beverages, dairy analogs, cheese and cream, breakfast cereal, mayonnaise, pasta and noodles, sauces, salty snacks, processed soups, puddings, confections, vegetarian meat analogs, fruit/vegetable juice, vegetable oils, egg products, including egg whites and substitute egg products	Levels providing a mean estimated intake of 5.5 to 7.3 g/person/day	No questions	335 (U.S. FDA, 2010)

Table 4 Summary of GRAS Notices Pertaining to Phytosterols and Phytosterol Esters Notified to the FDA

Substance	Intended Uses	Use Level	FDA Response	GRN No.
Plant sterols and stanols from pine trees	Ingredient in multiple food categories including margarine and vegetable based spreads, yogurt and yogurt-like products, milk-based juice beverages, ice cream and non-standardized ice cream products, cream cheese and cream cheese-like products, snack bars, salad dressings, standardized and non-standardized bread products, baked foods, beverages, dairy analogs, cheeses and cream, breakfast cereals, mayonnaise, pasta and noodles, sauces, salty snacks, processed soups, puddings, confections, vegetarian meat analogs, fruit/vegetable juices, vegetable oils, egg products, including egg whites and substitute egg products	Used as alternative source of phytosterols currently used as ingredients	No questions	250 (U.S. FDA, 2009)
Phytosterol esters and diglycerides resulting from transesterification of vegetable oils/fats with phytosterols	Ingredient in baked goods and baking mixes; fats and oils; frozen dairy desserts and mixes; gelatins, puddings, and fillings; grain products and pastas; gravies and sauces; hard candy; milk and milk products; soft candy, soups and soup mixes; and snack foods	0.65 gram phytosterol esters per serving	No questions	206 (U.S. FDA, 2006)
Phytosterols	Ingredient in egg products including egg whites and egg substitutes	1.1 g/serving	No questions	181 (U.S. FDA, 2006)
Plant sterol esters	Ingredient in ground coffee	1.0 gram per 8 ounce serving of brewed coffee	No questions	177 (U.S. FDA, 2005a)
Plant sterols and plant sterol esters from vegetable oils or sterols/stanols from tall oil	Ingredient in margarines and vegetable oil spreads, dressings for salads, beverages, snack bars, dairy analogs (including soy milk, ice cream and cream substitutes), cheese and cream, baked foods, ready-to-eat breakfast cereals, mayonnaise, pasta and noodles, sauces, salty snacks, processed soups, puddings, yogurt, confections, vegetarian meat analogs at a level up to 0.4 gram (g) sterol equivalents per serving; in fruit/vegetable juices at a level up to 1 g sterol equivalents per serving; and in edible vegetable oils, including diacylglycerol oil as a replacement, at a level up to 4 g/100g sterol equivalents per serving	Up to 4 g/100 g sterol equivalents per serving	No questions	176 (U.S. FDA, 2005b)
Phytosterols	Ingredient in vegetable spread, yogurt, milk-based juice beverages, ice cream, cream cheese, snack bars, salad dressings, and white bread products	0.75 g/serving (yogurt) 1.5g phytosterols/serving (all other food categories)	No questions	112 (U.S. FDA, 2003)

Table 4 Summary of GRAS Notices Pertaining to Phytosterols and Phytosterol Esters Notified to the FDA

Substance	Intended Uses	Use Level	FDA Response	GRN No.
Plant sterols/Plant sterol esters	Plant sterols as an ingredient in vegetable oil spreads, dressings for salad, health drinks, health bars, yogurt-type products at a level of 1 gram per serving; and as a raw material in the manufacture of plant sterol esters for use as an ingredient in the same foods at a level of 1.65 grams (i.e., 1 gram sterol equivalent) per serving	Up to 1.65 g/serving	No questions	61 (U.S. FDA, 2001)
Phytosterol esters	Ingredient in vegetable oil, at a level up to 13.3 percent by weight, for home use applications such as baking, frying, and salad dressings	13.3% by weight	No questions	53 (U.S. FDA, 2000a)
Vegetable oil phytosterol esters	Ingredient in vegetable oil spread, dressings for salad, bars, and yogurt	Not reported	No questions	48 (U.S. FDA, 2000b)
Tall oil phytosterols	Ingredient in vegetable oil spreads	Up to 12 percent free phytosterols	No questions	39 (U.S. FDA, 2000c)

Question 7. On page 16, Kao notes that ALA is used as a precursor for the synthesis to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Given some of the health risks associated with consumption of EPA and DHA such as increased bleeding time, please provide a reference for this statement and a narrative that discusses the efficiency of the conversion from ALA to EPA and DHA in humans (e.g., Kim et al., 2014). Please explain whether the estimated intake of ALA DAG oil would increase the internal exposure to EPA and DHA to a level that impacts the safety.

Response 7. The rates of conversion of ALA to EPA and DHA are reported to be less than 8% and 4%, respectively (Kim et al., 2014), while conversion rates up to 20% and 9% have been reported (Stark et al., 2008). Kao notes that EFSA reviewed the safety of supplemental intakes of EPA and DHA and concluded that intakes of up to 5 g/day, combined, do not pose a safety concern for the adult population (EFSA, 2012). In their review, EFSA evaluated 5 endpoints: (i) bleeding complications, bleeding time, and platelet function; (ii) glucose homeostasis; (iii) LDL-cholesterol concentrations; (iv) markers of lipid peroxidation; and (v) immune function. Based on the recommended use level of 2.5 g/day of ALA DAG and ALA content of approximately 53% across 3 tested batches, the highest potential daily exposure to EPA and DHA from the intended uses of ALA DAG are 0.267 g or 0.120 g, respectively. Therefore, Kao does not expect the uses of ALA DAG would significantly increase the current dietary exposures to EPA and DHA to levels that would pose a safety concern.

Question 8. There are multiple published studies that discuss the relationship between dietary intake of ALA and prostate cancer in humans (e.g., Hanson et al., 2020). Please review the relevant literature on this topic and provide a narrative that explains why the published literature does not contradict your GRAS conclusion.

Response 8. A search of PubMed using the terms “alpha-linolenic acid” and “prostate cancer” identified a number of meta-analyses and systematic reviews on the effect of ALA and prostate cancer in humans. The study by Hanson *et al.* (2020) indicated that ALA intake up to 5 g/day for up to 40 months was not associated with cancer death and may slightly increase the risk of prostate cancer (46 prostate cancer diagnoses in 4,010 male participants; relative risk = 1.30, 95% CI 0.72 to 2.32). The authors noted that data on any cancer diagnoses, including prostate cancer, were “too limited” to provide useful information, and therefore the effects are unclear. Furthermore, Hanson *et al.* noted that the slight increase in risk of prostate cancer diagnoses is based on low-quality evidence.

In addition to the study by Hanson *et al.*, a number of systematic reviews and meta-analyses were identified and revealed inconsistent results of studies on the effect of ALA on prostate cancer in humans (Attar-Bashi *et al.*, 2004; Brouwer *et al.*, 2004; Astorg, 2004, 2005; Brouwer, 2008; Simon *et al.*, 2009; Carayol *et al.*, 2010; Chua *et al.*, 2012; Carleton *et al.*, 2013; Kim *et al.*, 2014; Schwab *et al.*, 2014; Fu *et al.*, 2015; Dinwiddie *et al.*, 2016; Liu *et al.* 2020). These publications are summarized in Table A-1. These inconsistent results showed that consumption of ALA or n-3 omega fatty acids was inversely associated with increased risk of prostate cancer (Carayol *et al.*, 2010; Chua *et al.*, 2012; Schwab *et al.*, 2014; Fu *et al.*, 2015), showed no association with increased risk of prostate cancer and ALA intake (Simon *et al.*, 2009; Dinwiddie *et al.*, 2016; Liu *et al.*, 2020) or showed that evidence was not suitable to support a causal effect of ALA intake and prostate cancer (Attar-Bashi *et al.*, 2004; Kim *et al.*, 2014).

One study reported that a non-significant increase in prostate cancer risk was observed with ALA intake (RR=1.08), however, interpretation of this finding was complicated by heterogeneity of the data (Carleton *et al.*, 2013). Another study reported an increase risk of prostate cancer in males consuming “high levels” of ALA (levels were not quantified) (Brouwer *et al.*, 2004; Brouwer, 2008). Similarly, Astorg (2004, 2005) reported that ALA consumption was associated with an increase in prostate cancer risk in epidemiological studies but not animal or *in vitro* studies. A number of recent meta-analyses published in the past decade have demonstrated that there was either no association or consumption of 1.5 g/day of ALA was associated with a decrease in risk of prostate cancer. The Hanson *et al.* (2020) study reported a slight increase in risk of prostate cancer diagnosis, however, the study authors noted that the evidence was “low quality” and limited. As discussed in the GRAS notice, ALA is consumed in the background diet of the U.S. population at levels ranging between 1.3 and 1.7 g/day (Morris, 2007). The intended uses of Kao’s ALA DAG oil would provide approximately 1.2 g ALA/day, with total intakes up to 2.9 g/day of ALA; the combined intakes are well below the level associated with a slight increase in risk of prostate cancer (5 g/day) reported by Hanson *et al.* (2020). Thus, it is not expected that the intended uses of Kao’s ALA DAG would significantly increase the ALA consumption in the U.S. population that would pose a safety concern with respect to prostate cancer, and therefore does not contradict the GRAS conclusion on the ALA DAG oil under its intended conditions of use.

Question 9. The evidence in the published literature regarding increased dietary intake of ALA and the development of age-related macular degeneration is mixed (e.g., Heesterbeek *et al.*, 2020). Please review the literature on this topic and provide a narrative that explains why these publications do not contradict your GRAS conclusion.

Response 9. A search of PubMed using the terms “alpha-linolenic acid” and “macular degeneration” identified several studies on ALA intake and the development of age-related macular degeneration

(AMD) (Wu *et al.*, 2017; Heesterbeek *et al.*, 2020). Wu *et al.* (2017) evaluated the association of ALA intake and intermediate and advanced AMD as part of the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS). Extreme quintiles from the NHS and HPFS studies were pooled (Q1: 738 ± 64 and 840 ± 79 mg/day, respectively; Q5: $1,242 \pm 153$ and $1,445 \pm 195$ mg/day) and the results of the analysis showed that high intake levels of ALA were statistically significantly associated with intermediate AMD (p-value < 0.001); however, when the analysis was stratified by time period, comparing extreme quintiles pre- and post-2002, the results suggested that the association between high intake levels of ALA and intermediate AMD was only statistically significant before 2002 (p-value = 0.008 and 0.21, respectively). No statistically significant associations between ALA intake at any quintile were reported for advanced AMD. The authors concluded that the lack of association between ALA intake and AMD after 2002 strongly suggests that ALA is not the primary causal factor for AMD development. In a more recent review, Heesterbeek *et al.* (2020) identified three longitudinal population studies showing no association between higher dietary intake of ALA and three prospective studies with inconsistent findings. Based on the findings from Wu *et al.* and Heesterbeek *et al.* the intakes of ALA from the intended uses of ALA DAG would not reasonably be expected to pose a safety concern with respect to AMD, and would not change the GRAS conclusion on the ALA DAG oil under its intended conditions of use.


Question 10. In Section 6.1.3 "Interactions with Lipid-Soluble Vitamins," Kao cites a clinical study by Yamanaka *et al.*, 2016 which per the notifier is written in Japanese. Given that this study is discussed in detail in this section as well as cited throughout the notice, please provide an English translation of this manuscript. Please note that the translation should be proper and accurate.

Response 10. The study by Yamanaka *et al.* (2016) is provided in Attachment 3.

Question 11. On page 19, Kao states that a search of the published literature was conducted through March 2019. Please provide the results of an updated literature search, through at least July 2020, for studies relevant to the safety of ALA DAG oil.

Response 11. An updated search of the scientific literature in the following databases was conducted from March 2019 through to September 2020: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®, SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and Toxfile®. The results are provided in Attachment 4. No new studies relevant to the safety of ALA DAG oil were identified.

Sincerely,


Kathleen M. Sanzo
Counsel for Kao Corporation

Enclosures: Attachment 1 – Certificates of Analysis for 3 Production Batches of ALA DAG
Attachment 2 – Certificate of Analysis for ALA DAG in the Stability Study
Attachment 3 – Full publication of Yamanaka *et al.* (2016)
Attachment 4 – Literature Search Results
Table A-1

Certificate of Analysis

Kao Corporation

Sample name: ALA DAG Lot A (150619), B (151116), C (161220)

Analysis date: April 5, 2017

Test results:

Specification Parameter	Specification	Lot No. 150619	Lot No. 151116	Lot No. 161220	Method of Analysis
Alpha-linolenic acid diacylglycerol [%]	≥36% by weight	36	39	39	Internal method
Acid value [mg KOH/g]	≤2 mg KOH/g of sample	0.16	0.16	0.26	AOCS Cd 3d-63
Peroxide value [meq/kg]	≤5 MEQ/kg of sample	0.86	0.47	0.34	AOCS Cd 8b-90
Moisture [%]	≤0.1% by weight	0.03	0.04	0.01	AOCS Ca 2e-84
Lead [ppm]	≤0.5 ppm	Not detected	Not detected	Not detected	JOCS 2.6.3.2-2013
Arsenic (as As) [ppm]	≤0.5 ppm	Not detected	Not detected	Not detected	JOCS 2.6.3.7-2013

This is to certify that the following results have been obtained from our analysis on the above-mentioned samples.

Signatures: _____

Date: Sep 28, 2020

CERTIFICATE OF ANALYSIS

Client: Kao Corporation
2-1-3 Bunka, Sumida-ku, Tokyo 131-8501, Japan

Sample name: A-Oil (Lot No. A)

Received date: September 07, 2020

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm	1	
Mercury	Not detected	0.01 ppm	2	

QL: Quantitation limit N: Notes M: Method


Method

1: Atomic absorption spectrometry

2: Cold vapor atomic absorption spectrometry



Signed for and on behalf of JFRL


Takeko Arai
Section of Analysis Documentation

Sep. 14, 2020
Date

CERTIFICATE OF ANALYSIS

Client: Kao Corporation
2-1-3 Bunka, Sumida-ku, Tokyo 131-8501, Japan

Sample name: A-oil (Lot No. B)

Received date: September 07, 2020

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm	1	
Mercury	Not detected	0.01 ppm	2	

QL: Quantitation limit N: Notes M: Method

Method

1: Atomic absorption spectrometry

2: Cold vapor atomic absorption spectrometry



Signed for and on behalf of JFRL

Takeko Arai

Section of Analysis Documentation

Sep. 14, 2020

Date

CERTIFICATE OF ANALYSIS

Client: Kao Corporation
2-1-3 Bunka, Sumida-ku, Tokyo 131-8501, Japan

Sample name: A-Oil (Lot No. C)

Received date: September 07, 2020

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm	1	
Mercury	Not detected	0.01 ppm	2	

QL: Quantitation limit N: Notes M: Method


Method

1: Atomic absorption spectrometry

2: Cold vapor atomic absorption spectrometry



Signed for and on behalf of JFRL



Takeko Arai
Section of Analysis Documentation

Sep. 14, 2020

Date

CERTIFICATE OF ANALYSIS

Client: Kao Corporation
2-1-3 Bunka, Sumida-ku, Tokyo 131-8501, Japan

Sample name: A-Oil (Lot No. D)

Received date: September 07, 2020

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Mercury	Not detected	0.01 ppm		2

QL: Quantitation limit N: Notes M: Method


Method

1: Atomic absorption spectrometry

2: Cold vapor atomic absorption spectrometry



Signed for and on behalf of JFRL



Takeko Arai
Section of Analysis Documentation

Date

Sep. 14, 2020

Certificate of analysis

Kao Corporation

Sample: ALA DAG oil formulated with antioxidants; rosemary extract, tocopherols, ascorbyl palmitate (Lot No. P0767)

Study Period: April 4, 2017~Oct 4, 2018

Methods: Samples were stored at room temperature for 18 months after production, and were analyzed at 0, 12, and 18 months.

Results:

Specification Parameter	Specification	Initial (0 month)	12 months after production	18 months after production	Method of Analysis
Alpha-linolenic acid diacylglycerol [%]	≥36% by weight	39	39	39	Internal method
Acid value [mg KOH/g]	≤2 mg KOH/g of sample	0.7	0.7	0.8	AOCS Cd 3d-63
Peroxide value [meq/kg]	≤5 meq/kg of sample	<0.1	<0.1	<0.1	AOCS Cd 8b-90
Moisture [%]	≤0.1% by weight	0.02	0.04	0.05	AOCS Ca 2e-84
Lead [ppm]	≤0.5 ppm	*1 Not detected	*1 Not detected	*1 Not detected	JOCS 2.6.3.2-2013
Arsenic (as As) [ppm]	≤0.5 ppm	*2 Not detected	*2 Not detected	*2 Not detected	JOCS 2.6.3.7-2013

*1 Limit of quantitation; 0.05 ppm

*2 Limit of quantitation; 0.1 ppm

No appreciable changes in any of the established specification parameters were reported, indicating that the ALA DAG oil formulated with antioxidants is stable for up to 18 months of storage.

Signatures: _____

Date: Sep 28, 2020.

Yamanaka, “Safety Evalttation of Excessive Consumption of Alpha—linolenic Acid—enriched Diacyiglycerolin Healthy Subjects”, Japanese Pharmacology & Therapeutics (2016). (11 pages)

Search Strategy

Databases: AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, ToxFile®

Set#	Searched for	Results
S2	("alpha-linolenic acid" AND "diacylglycerol") and (pd(>20190301))	7°
S1	"alpha-linolenic acid" AND "diacylglycerol"	100°

° Duplicates are removed from the search and from the result count.

Document 1 of 7

Co-immobilization of bi-lipases on magnetic nanoparticles as an efficient catalyst for synthesis of functional oil rich in diacylglycerols, phytosterol esters and alpha-linolenic acid

Author: Yao, Guihong; Wang, Xiujuan; Yang, Minli; Chen, Fengming; Ling, Yun; Liu, Tong; Xing, Shige; Yao, Meiyi; Zhang, Feng

Publication info: LWT - Food Science and Technology 129 (Jul 2020): Article No.: 109522.

[ProQuest document link](#)

Databases: BIOSIS Previews® (1926 - current)

Document 2 of 7

A transferase interactome that may facilitate channeling of polyunsaturated fatty acid moieties from phosphatidylcholine to triacylglycerol

Author: Xu, Yang 1 ; Caldo, Kristian Mark P 1 ; Jayawardhane, Kethmi 1 ; Ozga, Jocelyn A 1 ; Weselake, Randall J 1 ; Chen, Guanqun 1 1 Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada, Canada gc24@ualberta.ca

Publication info: The Journal of biological chemistry 294.41 (Oct 11, 2019): 14838-14844.

[ProQuest document link](#)

Databases: MEDLINE® (1946 - current)

Document 3 of 7

In vitro digestion of galactolipids from chloroplast-rich fraction (CRF) of postharvest, pea vine field residue (haulm) and spinach leaves

Author: Wattanakul, Jutarat 1 ; Sahaka, Moulay; Amara, Sawsan; Mansor, Syamila; Gontero, Brigitte; Carrière, Frédéric; Gray, David 1 Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK, UK david.gray@nottingham.ac.uk

Publication info: Food & function 10.12 (Dec 11, 2019): 7806-7817.

[ProQuest document link](#)

Databases: MEDLINE® (1946 - current)

Document 4 of 7

Prolongation of secondary drying step of phospholipid lyophilization greatly improves acidolysis reactions catalyzed by immobilized lecithase ultra

Author: Verdasco-Martín, Carlos M 1 ; Corchado-Lopo, Carlos 1 ; Fernández-Lafuente, Roberto 1 ; Otero, Cristina 1 1 Department of Biocatalysis, Institute of Catalysis and Petroleochemistry, CSIC, C/ Marie Curie 2 L10, Madrid 28049, Spain, Spain coterol@icp.csic.es

Publication info: Enzyme and microbial technology 132 (Jan 2020): 109388.

[ProQuest document link](#)

Databases: MEDLINE® (1946 - current)

Document 5 of 7

Maternal High Linoleic Acid Alters Placental Fatty Acid Composition

Author: Shrestha, Nirajan 1 ; Holland, Olivia J 2 ; Kent, Nykola L 3 ; Perkins, Anthony V 1 ; McAinch, Andrew J 4 ; Cuffe, James S M 3 ; Hryciw, Deanne H 5 1 School of Medical Science, Griffith University, Southport, QLD 4222, Australia, Australia 2 School of Medical Science, Griffith University, Southport, QLD 4222, Australia, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD 4000, Australia, Australia 3 School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4067, Australia, Australia 4 Institute for Health and Sport, Victoria University, Melbourne, VIC 3000, Australia, Australian Institute for Musculoskeletal Science (AIMSS), Victoria University, St. Albans, VIC 3021, Australia, Australia 5 Institute for Health and Sport, Victoria University, Melbourne, VIC 3000, Australia, School of Environment and Science, Griffith University, Nathan, QLD 4111, Australia, Environmental Futures Research Institute, Griffith University, Nathan, QLD 4111, Australia, Australia

Publication info: Nutrients 12.8 (Jul 23, 2020).

[ProQuest document link](#)

Databases: MEDLINE® (1946 - current)

Document 6 of 7

Emblica officinalis extract standardized to diacyl glycerol of fatty acids

Publication info: Antony, B. WO 2020026104. WO.

[ProQuest document link](#)

Databases: FSTA® (1969 - current)

Document 7 of 7

**Fatty acid composition and oil content of seeds from perilla (*Perilla frutescens* (L.) var. *frutescens*)
germplasm of Republic of Korea**

Author: Kim, Hyun Uk; Lee, Kyeong-Ryeol; Jeon, Inhwa; Jung, Ha Eun; Heo, Jae Bok; Kim, Tae-Yun; Chen, Grace Q.

Publication info: Genetic Resources and Crop Evolution 66.7 (Oct 2019): 1615-1624.

[ProQuest document link](#)

Databases: BIOSIS Previews® (1926 - current)

Contact ProQuest

Copyright © 2020 ProQuest LLC. All rights reserved. - **Terms and Conditions**

Table A-1 Summary of Reviews and Meta-Analyses on the Effect of ALA Consumption and Prostate Cancer

Study details	Reference
<p>Dietary Intake of N-3 and N-6 Polyunsaturated Fatty Acids and Risk of Cancer: Meta-Analysis of Data from 32 Studies</p> <p>Author: Liu J, Li X, Hou J, Sun J, Guo N, Wang Z</p> <p>Publication Info: Nutr Cancer. 2020;1-13.</p> <p>Abstract: Background: Large epidemiological studies have yielded conflicting results regarding the relationship between polyunsaturated fatty acids (PUFAs) and cancers. Here, we performed a meta-analysis to examine the link between dietary intake of n-3 and n-6 PUFAs and cancer risk. Materials and methods: We performed a search on PubMed, EMBASE, and the Cochrane Library. Studies that reported adjusted relative risk (RR) estimates with 95% confidence intervals (CI) for the associations of interest were included. Results: Thirty-two studies involving 1,445,732 participants were included. Colorectal, breast and prostate cancer had been analyzed in our study. Specifically, for colorectal cancer, total n-3 PUFAs, marine n-3 PUFAs, α-linolenic acids (ALA) and n-6 PUFAs were not associated with the risk of it (RR 1.04, 95% CI 0.85-1.28; RR 0.99, 95% CI 0.89-1.09; RR 1.05, 95% CI 0.93-1.19; RR 1.02, 95% CI 0.94-1.11, respectively). For breast cancer, only marine n-3 PUFAs, but not total n-3 PUFAs, ALA, and n-6 PUFAs, was associated with a lower risk of it (RR 0.70, 95% CI 0.55-0.91). For prostate cancer, ALA and n-6 PUFAs also have no association with the risk of it. Conclusions: Most subtypes of PUFAs are probably not related to cancers. However, additional high-quality trials are warranted to corroborate the findings of this meta-analysis.</p>	<p>Liu <i>et al.</i> (2020)</p>
<p>Omega-3 Fatty Acid Consumption and Prostate Cancer: A Review of Exposure Measures and Results of Epidemiological Studies</p> <p>Author: Dinwiddie MT, Terry PD, Whelan J, Patzer RE</p> <p>Publication info: J Am Coll Nutr. 2016 Jul;35(5):452-68. Epub 2015 Nov 23.</p> <p>Abstract: Animal studies have shown that dietary omega-3 polyunsaturated fatty acids (n-3) may play a role in the development of prostate cancer, but the results of epidemiologic studies have been equivocal. Associations in humans may vary depending on study design, measurement methodology of fatty acid intake, intake ranges, and stage of cancer development. To address this, we identified 36 published studies through PubMed (Medline) from 1993 through 2013 on long-chain n-3s and prostate cancer. Exposure measurements included dietary assessment and biomarker levels. Associations for total, early, and late stage prostate cancer were examined by subgroup of study design and exposure measure type and by using forest plots to illustrate the relative strength of associations within each subgroup. We also tested for potential threshold effects by considering studies that included measurement cut-points that met intake levels recommended by the American Heart Association. We found no consistent evidence supporting a role of n-3s in either the causation or prevention of prostate cancer at any stage or grade. Results did not vary appreciably by study design, exposure measurement, intake level, or stage of cancer development.</p>	<p>Dinwiddie <i>et al.</i> (2016)</p>
<p>Effect of individual omega-3 fatty acids on the risk of prostate cancer: a systematic review and dose-response meta-analysis of prospective cohort studies</p> <p>Author: Fu YQ, Zheng JS, Yang B, Li D</p> <p>Publication Info: J Epidemiol. 2015;25(4):261-274.</p> <p>Abstract: Epidemiological studies have suggested inconsistent associations between omega-3 polyunsaturated fatty acids (n-3 PUFAs) and prostate cancer (PCa) risk. We performed a dose-response meta-analysis of prospective observational studies investigating both dietary intake and circulating n-3 PUFAs and PCa risk. PubMed and EMBASE prior to February 2014 were searched, and 16 publications were eligible. Blood concentration of docosahexaenoic acid, but not alpha-linolenic acid or eicosapentaenoic acid, showed marginal positive association with PCa risk (relative risk for 1% increase in blood docosahexaenoic acid concentration: 1.02; 95% confidence interval, 1.00-1.05; I(2) = 26%; P = 0.05 for linear trend), while dietary docosahexaenoic acid intake showed a non-linear positive association with PCa risk (P < 0.01). Dietary alpha-linolenic acid was inversely associated with PCa risk (relative risk for 0.5 g/day increase in alpha-linolenic acid intake: 0.99; 95% confidence interval, 0.98-1.00; I(2) = 0%; P = 0.04 for linear trend), which was dominated by a single study. Subgroup analyses indicated that blood eicosapentaenoic acid concentration and blood docosahexaenoic</p>	<p>Fu <i>et al.</i> (2015)</p>

acid concentration were positively associated with aggressive PCa risk and nonaggressive PCa risk, respectively. Among studies with nested case-control study designs, a 0.2% increase in blood docosapentaenoic acid concentration was associated with a 3% reduced risk of PCa (relative risk 0.97; 95% confidence interval, 0.94-1.00; I(2) = 44%; P = 0.05 for linear trend). In conclusion, different individual n-3 PUFA exposures may exhibit different or even opposite associations with PCa risk, and more prospective studies, especially those examining dietary n-3 PUFAs and PCa risk stratified by severity of cancer, are needed to confirm the results.

Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: a systematic review

Schwab *et al.*
(2014)

Author: Schwab U, Lauritzen L, Tholstrup T, *et al.*

Publication Info: Food Nutr Res. 2014;58:10.3402/fnr.v58.25145. Published 2014 Jul 10.

Abstract: The effects of both the amount and quality of dietary fat have been studied intensively during the past decades. Previously, low-fat diets were recommended without much attention to the quality of fat, whereas there is general emphasis on the quality of fat in current guidelines. The objective of this systematic review (SR) was to assess the evidence of an effect of the amount and type of dietary fat on body weight (BW), risk factors, and risk of non-communicable diseases, that is, type 2 diabetes (T2DM), cardiovascular diseases (CVD), and cancer in healthy subjects or subjects at risk for these diseases. This work was performed in the process of updating the fourth edition of the Nordic Nutrition Recommendations from 2004. The literature search was performed in October 2010 covering articles published since January 2000. A complementary search was done in February 2012 covering literature until December 2011. Two authors independently selected articles for inclusion from a total of about 16,000 abstracts according to predefined criteria. Randomized controlled trials (RCT) and prospective cohort studies (PCS) were included as well as nested case-control studies. A few retrospective case-control studies were also included when limited or no data were available from other study types. Altogether 607 articles were quality graded and the observed effects in these papers were summarized. Convincing evidence was found that partial replacement of saturated fat (SFA) with polyunsaturated fat (PUFA) or monounsaturated fat (MUFA) lowers fasting serum/plasma total and LDL cholesterol concentrations. The evidence was probable for a decreasing effect of fish oil on concentration of serum/plasma total triglycerides as compared with MUFA. Beneficial effect of MUFA both on insulin sensitivity and fasting plasma/serum insulin concentration was considered as probable in comparisons of MUFA and carbohydrates versus SFA, whereas no effect was found on fasting glucose concentration in these comparisons. There was probable evidence for a moderate direct association between total fat intake and BW. Furthermore, there was convincing evidence that partial replacement of SFA with PUFA decreases the risk of CVD, especially in men. This finding was supported by an association with biomarkers of PUFA intake; the evidence of a beneficial effect of dietary total PUFA, n-6 PUFA, and linoleic acid (LA) on CVD mortality was limited suggestive. Evidence for a direct association between total fat intake and risk of T2DM was inconclusive, whereas there was limited-suggestive evidence from biomarker studies that LA is inversely associated with the risk of T2DM. However, there was limited-suggestive evidence in biomarker studies that odd-chain SFA found in milk fat and fish may be inversely related to T2DM, but these associations have not been supported by controlled studies. The evidence for an association between dietary n-3 PUFA and T2DM was inconclusive. **Evidence for effects of fat on major types of cancer was inconclusive regarding both the amount and quality of dietary fat, except for prostate cancer where there was limited-suggestive evidence for an inverse association with intake of ALA** and for ovarian cancer for which there was limited-suggestive evidence for a positive association with intake of SFA. This SR reviewed a large number of studies focusing on several different health outcomes. The time period covered by the search may not have allowed obtaining the full picture of the evidence in all areas covered by this SR. However, several SRs and meta-analyses that covered studies published before year 2000 were evaluated, which adds confidence to the results. Many of the investigated questions remain unresolved, mainly because of few studies on certain outcomes, conflicting results from studies, and lack of high quality-controlled studies. There is thus an evident need of highly controlled RCT and PCS with sufficient number of subjects and long enough duration, specifically regarding the effects of the amount and quality of dietary fat on insulin sensitivity, T2DM, low-grade inflammation, and blood pressure. New metabolic and other potential risk markers and utilization of new methodology in the area of lipid metabolism may provide new insight.

α -Linolenic acid: nutraceutical, pharmacological and toxicological evaluation

Kim *et al.* (2014)

Author: Kim KB, Nam YA, Kim HS, Hayes AW, Lee BM

Publication Info: Food Chem Toxicol. 2014;70:163-178.

Abstract: α -Linolenic acid (ALA), a carboxylic acid with 18 carbons and three cis double bonds, is an essential fatty acid needed for human health and can be acquired via regular dietary intake of foods that contain ALA or dietary supplementation of foods high in ALA, for example flaxseed. ALA has been reported to have cardiovascular-protective, anti-cancer, neuro-protective, anti-osteoporotic, anti-inflammatory, and antioxidative effects. ALA is the precursor of longer chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), but its beneficial effects on risk factors for cardiovascular diseases are still inconclusive. The recommended intake of ALA for cardiovascular health is reported to be 1.1-2.2g/day. Although there are limited toxicological data for ALA, no serious adverse effects have been reported. **The evidence on an increased prostate cancer risk in association with dietary ALA is not conclusive.** Based on the limited data currently available, it may be concluded that ALA may be beneficial as a nutraceutical/pharmaceutical candidate and is safe for use as a food ingredient.

Case-control and prospective studies of dietary α -linolenic acid intake and prostate cancer risk: a meta-analysis

Carleton *et al.*
(2013)

Author: Carleton AJ, Sievenpiper JL, de Souza R, McKeown-Eyssen G, Jenkins DJ

Publication Info: BMJ Open. 2013;3(5):e002280. Published 2013 May 14.

Abstract: Objective: α -Linolenic acid (ALA) is considered to be a cardioprotective nutrient; however, some epidemiological studies have suggested that dietary ALA intake increases the risk of prostate cancer. The main objective was to conduct a systematic review and meta-analysis of case-control and prospective studies investigating the association between dietary ALA intake and prostate cancer risk.

Design: A systematic review and meta-analysis were conducted by searching MEDLINE and EMBASE for relevant prospective and case-control studies.

Included studies: We included all prospective cohort, case-control, nested case-cohort and nested case-control studies that investigated the effect of dietary ALA intake on the incidence (or diagnosis) of prostate cancer and provided relative risk (RR), HR or OR estimates.

Primary outcome measure: Data were pooled using the generic inverse variance method with a random effects model from studies that compared the highest ALA quantile with the lowest ALA quantile. Risk estimates were expressed as RR with 95% CIs. Heterogeneity was assessed by χ^2 and quantified by I².

Results: Data from five prospective and seven case-control studies were pooled. The overall RR estimate showed ALA intake to be positively but non-significantly associated with prostate cancer risk (1.08 (0.90 to 1.29), p=0.40; I²=85%), but the interpretation was complicated by evidence of heterogeneity not explained by study design. A weak, non-significant protective effect of ALA intake on prostate cancer risk in the prospective studies became significant (0.91 (0.83 to 0.99), p=0.02) without evidence of heterogeneity (I²=8%, p=0.35) on removal of one study during sensitivity analyses.

Conclusions: This analysis failed to confirm an association between dietary ALA intake and prostate cancer risk. Larger and longer observational and interventional studies are needed to define the role of ALA and prostate cancer.

Relationship of dietary intake of omega-3 and omega-6 Fatty acids with risk of prostate cancer development: a meta-analysis of prospective studies and review of literature

Chua *et al.* (2012)

Author: Chua ME, Sio MC, Sorongon MC, Dy JS

Publication Info: Prostate Cancer. 2012;2012:826254.

Abstract: Objective. To determine the relationship between dietary omega-3 fatty acids (n-3 PUFA) and omega-6 fatty acids (n-6 PUFA) with prostate cancer risk from meta-analysis of prospective studies. Design. The literature retrieved from electronic biomedical databases up to June 2011 was critically appraised. General variance-based method was used to pool the effect estimates at 95% confidence interval. Heterogeneity was assessed by Chi² and quantified by I². Results. Eight cohort studies were included for meta-analysis. n-3 PUFA, n-6 PUFA, and their derivatives were not significantly associated with risk of prostate cancer in general. **A significant negative association between high dietary intake of alpha-linolenic acid (ALA) and prostate cancer risk (pooled RR: 0.915; 95% CI: 0.849, 0.985; P = 0.019) was noted.** Likewise, a slightly positive association was noted on dietary long-chain n-3 PUFA, composed of eicosapentaenoic acid (EPA) and docosahexaenoic

acid (DHA) with prostate cancer risk (pooled RR: 1.135; 95% CI: 1.008, 1.278; P = 0.036); however, when two other cohort studies with data of EPA and DHA, both analyzed separately, were included into the pool, the association became not significant (RR: 1.034; 95% CI: 0.973, 1.096; P = 0.2780).
Conclusion. Intake of n-3 PUFA and n-6 PUFA does not significantly affect risk of prostate cancer.
High intake of ALA may reduce risk of prostate cancer, while intake of long-chain omega-3 fatty acids does not have a significant effect.

Prospective studies of dietary alpha-linolenic acid intake and prostate cancer risk: a meta-analysis

Carayol *et al.*
(2010)

Author: Carayol M, Grosclaude P, Delpierre C

Publication Info: Cancer Causes Control. 2010;21(3):347-355

Abstract: Individual-based studies that investigated the relation between dietary alpha-linolenic acid (ALA) intake and prostate cancer risk have shown inconsistent results. We carried out a meta-analysis of prospective studies to examine this association. We systematically searched studies published up to December 2008. Log relative risks (RRs) were weighted by the inverse of their variances to obtain a pooled estimate with its 95% confidence interval (CI). We identified five prospective studies that met our inclusion criteria and reported risk estimates by categories of ALA intake. **Comparing the highest to the lowest ALA intake category, the pooled RR was 0.97 (95% CI:0.86-1.10) but the association was heterogeneous. Using the reported numbers of cases and non-cases in each category of ALA intake, we found that subjects who consumed more than 1.5 g/day of ALA compared with subjects who consumed less than 1.5 g/day had a significant decreased risk of prostate cancer: RR = 0.95 (95% CI:0.91-0.99).** Divergences in results could partly be explained by differences in sample sizes and adjustment but they also highlight limits in dietary ALA assessment in such prospective studies. **Our findings support a weak protective association between dietary ALA intake and prostate cancer risk** but further research is needed to conclude on this question.

The relation of alpha-linolenic acid to the risk of prostate cancer: a systematic review and meta-analysis

Simon *et al.*
(2009)

Author: Simon JA, Chen YH, Bent S

Publication Info: Am J Clin Nutr. 2009;89(5):1558S-1564S.

Abstract: Background: alpha-Linolenic acid (ALA; 18:3n-3) has been associated inconsistently with an increased risk of prostate cancer. Additional studies have become available since the publication of 2 previous meta-analyses.

Objective: The objective was to review the published data on the relation between ALA and prostate cancer.

Design: We conducted a systematic review to identify studies that included data on ALA and risk of prostate cancer. Data were pooled from studies that compared the highest ALA quantile with the lowest ALA quantile, and risk estimates were combined by using a random-effects model.

Results: The relation between ALA and prostate cancer is inconsistent across studies. We pooled data from 8 case-control and 8 prospective studies. The summary estimate revealed that **high ALA dietary intakes or tissue concentrations are weakly associated with prostate cancer risk (relative risk [RR]: 1.20; 95% CI: 1.01, 1.43).** When examined by study type (ie, retrospective compared with prospective or dietary ALA compared with tissue concentration) or by decade of publication, **only the 6 studies examining blood or tissue ALA concentrations revealed a statistically significant association. With the exception of these studies, there was significant heterogeneity and evidence of publication bias. After adjustment for publication bias, there was no association between ALA and prostate cancer (RR: 0.96; 95% CI: 0.79, 1.17).**

Conclusions: Studies examining the relation between ALA and prostate cancer have produced inconsistent findings. High ALA intakes or high blood and adipose tissue concentrations of ALA may be associated with a small increased risk of prostate cancer. However, these conclusions are qualified because of the heterogeneity across studies and the likelihood of publication bias.

Omega-3 PUFA: good or bad for prostate cancer?

Brouwer (2008)

Author: Brouwer IA

Publication Info: Prostaglandins Leukot Essent Fatty Acids. 2008;79(3-5):97-99.

Abstract: Introduction: The objective of this meta-analysis was to estimate quantitatively the associations between intake or status of omega-3 polyunsaturated (omega-3 PUFA) fatty acids and occurrence of prostate cancer in observational studies in humans.

Methods: We combined risk estimates across studies using random-effects models.

Results: The combined estimate showed an increased risk of prostate cancer in men with a high intake or blood level of alpha-linolenic acid (ALA) (combined relative risk (RR) 1.36; 95% CI 1.08-1.70). The association is stronger in the case-control studies (RR 1.84; 95% CI 1.04-3.25) than in the prospective studies (RR 1.10; 0.91-1.32). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were not significantly associated with prostate cancer.

Discussion: The association between high intake of ALA and prostate cancer is of concern and needs further study. However, the fact that the prospective studies do not show a clear association makes a true effect of intake of ALA on prostate cancer less likely.

[Dietary fatty acids and colorectal and prostate cancers: epidemiological studies]

Astorg (2005)

Author: Astorg P

Publication Info: Bull Cancer. 2005;92(7):670-684.

Abstract: Objective: This study reviews epidemiological works having studied the associations of dietary fatty acids, especially of n-6 or n-3 polyunsaturated fatty acids (PUFA), with the risks of colorectal and prostate cancers.

Methods: The epidemiological studies reviewed were those having tested the association of colorectal and prostate cancer risk with the dietary intake or the blood or adipose tissue levels of fatty acids, especially of n-6 and n-3 PUFA, and with the dietary intake of fish and seafood.

Results: Most studies based on a dietary questionnaire did not find any association of the risk of colorectal cancer with the consumption of either total fatty acids or any particular fatty acid, after adjustment for total energy intake had been made. A few studies suggest that trans fatty acid consumption could increase colorectal cancer risk. Most studies based either on a dietary questionnaire or on biomarkers, did not find any association of total, saturated or monounsaturated fatty acid, as well as of linoleic or arachidonic acids, with prostate cancer risk, after adjustment for total energy intake. Most studies failed to find an association of prostate cancer risk with fish or long-chain n-3 PUFA intake, but recent cohort studies did find an inverse association of fish consumption with the risk of the latest stages of prostate cancer. In contrast, **alpha-linolenic acid intake was associated with an increase of prostate cancer risk in a majority of epidemiological studies, but other studies did not find this association.** This latter point might be of concern, and needs to be clarified by other results, especially those of ongoing prospective studies.

Dietary N-6 and N-3 polyunsaturated fatty acids and prostate cancer risk: a review of epidemiological and experimental evidence

Astorg (2004)

Author: Astorg P

Publication Info: Cancer Causes Control. 2004;15(4):367-386.

Abstract: Objective: This study reviews epidemiological and experimental works dealing with the effects of dietary n -6 or n -3 polyunsaturated fatty acids (PUFA) on prostate cancer (PCa) development and PCa risk.

Methods: Systematic literature searches were made using Medline. The epidemiological studies reviewed (ecological, case-control, cohorts, and nested case-control) were those having tested the association of PCa risk with the dietary intake or the blood or adipose tissue levels of PUFA (n -6 PUFA, n -3 PUFA, long-chain n -3 PUFA, linoleic acid, alpha -linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid), and with the dietary intake of fish and seafood. Experimental studies dealing with the effects of PUFA on PCa development in animal models or with PCa cell growth in vitro were also reviewed, as well as studies on the mechanisms of the effects of PUFA on PCa.

Results: There is no or little evidence of an association of linoleic or arachidonic acids with PCa

risk. Most epidemiological studies failed to find an association of PCa risk with fish or long-chain n -3 PUFA intake, but two recent cohort studies did find an inverse association of fish consumption with the risk of the latest stages of PCa. **alpha -linolenic acid intake was associated with an increase of PCa risk in a majority of epidemiological studies, but other studies did not find this association.**

Experimental work in vitro and in vivo, as well as mechanistic studies, support a protective effect of long-chain n -3 PUFA on PCa, but data on the effects of linoleic and alpha -linolenic acids are scarce.

Conclusions: Long-chain n -3 PUFA from fish are possible promising nutrients for the dietary prevention of PCa, but to-date with little epidemiological support. In contrast, studies suggest that alpha -linolenic acid intake might be a risk factor. New work, both epidemiological and experimental, is awaited to clarify these results.

Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis

Brouwer *et al*
(2004)

Author: Brouwer IA, Katan MB, Zock PL

Publication Info: J Nutr. 2004;134(4):919-922.

Abstract: The objective of this meta-analysis was to estimate quantitatively the associations between intake of alpha-linolenic acid [ALA, the (n-3) fatty acid in vegetable oils], mortality from heart disease, and the occurrence of prostate cancer in observational studies. We identified 5 prospective cohort studies that reported intake of ALA and mortality from heart disease. We also reviewed data from 3 clinical trials on ALA intake and heart disease. In addition, we identified 9 cohort and case-control studies that reported on the association between ALA intake or blood levels and incidence or prevalence of prostate cancer. We combined risk estimates across studies using a random-effects model. High ALA intake was associated with reduced risk of fatal heart disease in prospective cohort studies (combined relative risk 0.79, 95% CI 0.60-1.04). Three open-label trials also indicated that ALA may protect against heart disease. However, **epidemiologic studies also showed an increased risk of prostate cancer in men with a high intake or blood level of ALA (combined relative risk 1.70; 95% CI 1.12-2.58).** This meta-analysis shows that consumption of ALA might reduce heart disease mortality. However, the association between high intake of ALA and prostate cancer is of concern and warrants further study.

Alpha-linolenic acid and the risk of prostate cancer. What is the evidence?

Attar-Bashi *et al.*
(2004)

Author: Attar-Bashi NM, Frauman AG, Sinclair AJ

Publication Info: J Urol. 2004;171(4):1402-1407.

Abstract: Purpose: Several studies have examined the association between polyunsaturated fatty acids and prostate cancer risk. We evaluated the evidence on the association between the essential polyunsaturated fatty acid, known as alpha-linolenic acid, and the risk of prostate cancer in humans.

Materials and methods: We comprehensively reviewed published studies on the association between alpha-linolenic acid and the risk of prostate cancer using MEDLINE.

Results: A number of studies have shown a positive association between dietary, plasma or red blood cell levels of alpha-linolenic acid and prostate cancer. Other studies have demonstrated either dietary or plasma alpha-linolenic acid levels are positively associated with prostate tissue alpha-linolenic acid levels, and measurement errors of dietary, plasma and red blood cell alpha-linolenic acid levels.

Conclusions: More research is needed in this area before it can be concluded that there is an association between alpha-linolenic acid and prostate cancer.

From: [Sanzo, Kathleen M.](#)
To: [Hall, Karen](#)
Subject: [EXTERNAL] RE: Regarding GRAS Notice GRN 000914--Kao Response
Date: Friday, February 19, 2021 9:42:40 AM

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear Ms. Hall,

In response to your email of February 8, 2021, concerning further questions on GRN 000914, Kao provides the below responses. Please let me know if there are any further questions. Thank you.

Regards,
Kathy Sanzo

1. The intended use of the *alpha*-linolenic acid diacylglycerol (ALA DAG) oil is not clear.
 - Please clarify if the ALA DAG oil is intended to be used as a replacement for other edible oils, such as olive oil.
 - If the ALA DAG oil is intended to be used as a replacement for other edible oils, please describe why the serving size for the ALA DAG oil (2.5 g/serving) is less than the amount used for the same applications for other edible oils.
 - If the ALA DAG oil is not intended to be used as a replacement for other edible oils, please provide a description of how the oil will be used and explain why such a small serving size is used.

Kao Response: ALA DAG Oil is intended to be used as a partial, lighter replacement for edible salad oils and other dressings which are lightly sprayed onto salads and other vegetables to provide sufficient taste and light fat mouth feel but to avoid excessive use. Only a small amount of oil (2.5 g/serving) is necessary to provide the attributes of ALA DAG oil. There are multiple olive oil products on the market that are delivered in a small serving size via sprayer or other package design and which describe their serving size as 1-3 second spray and a very small serving size (e.g., Mantova Oil, La Tourangelle Olive Oil Spray, Avola Olive Oil, Sussed Olive Oil Spray). These products are labeled as being applied directly to salads and vegetables and other foods. KAO expects its product to be used similarly in terms of a smaller serving size.

2. The notifier describes two stability studies. The first study evaluates the stability of ALA DAG oil for one month at 30 °C under nitrogen. The second study evaluates the stability of an ALA DAG product that has been formulated to contain antioxidants and stabilizers for 18 months at room temperature (10 to 35 °C) and a relative humidity of 20 to 60%. We believe that the first stability study is conducted on an ALA DAG oil that has not been formulated to contain any antioxidants and stabilizers. Please confirm that our understanding is correct. In addition, if our understanding is correct and there are two different ALA DAG oil products, please clarify which product is the subject of this GRAS notice.

KAO Response: The FDA's understanding is correct. The first study relates to the stability of ALA-DAG oil without any antioxidants and stabilizers, and this is the substance that is the subject of this GRAS notice. Kao only provided the second study results for stability over 18 months as an as an example of a possible finished formulation.

3. We have the following questions on the exposure estimate:

- The notifier indicates that the recommended daily intake of ALA DAG oil is 2.5 g/d. Please provide a source for this recommendation.

KAO Response: As noted in response to question 1, the recommended daily intake of ALA DAG oil is 2.5g/d and the safety of this amount is supported by the safety data and literature in the submission and confirmed by the expert panel determination.

- We presume that the serving size of 2.5 g/d is for the ALA DAG oil product containing 36% ALA DAG and not 2.5 g/d of ALA DAG. Please confirm that our understanding is correct.

KAO Response: Yes, correct.

- The notifier indicates that 1 serving of the ALA DAG oil will provide the recommended daily intake of ALA DAG oil of 2.5 per serving per day. Presuming that the final ALA DAG oil product would contain a minimum of 36% ALA DAG, the notifier estimates an exposure to ALA DAG from intended use to be 0.9 g/d. This value is then added to the background exposure to ALA DAG of 0.49 g/p/d to obtain a cumulative exposure to ALA DAG of 1.39 g/p/d. Please confirm that our understanding of the exposure estimate is correct.

KAO Response: Yes, correct.

- We note that the exposure was based on the minimum specification for ALA DAG. Please indicate if there is a maximum level of ALA DAG that would be expected in the ALA DAG oil so that an upper bound exposure to ALA DAG could be estimated.

KAO Response: As noted in GRN 000914, the minimum level of ALA DAG expected in the oil is at least 0.9 (36%), and, as noted in the batch analysis in Table 2.6-1, routinely standardizes at 39%. Therefore, the expected level of ALA DAG oil will be between 0.9 and 1.0. There is no maximum level, but Kao's

manufacturing process consistently produces product with about 39% ALA DAG.

Kathleen M. Sanzo

Morgan, Lewis & Bockius LLP

1111 Pennsylvania Avenue, NW | Washington, DC 20004-2541

Direct: +1.202.739.5209 | Cell: +1.301.651.2901 | Main: +1.202.739.3000 | Fax: +1.202.739.3001

kathleen.sanzo@morganlewis.com | www.morganlewis.com

Assistant: Tricia M. Farringer | +1.202.739.5272 | tricia.farringer@morganlewis.com



From: Hall, Karen <Karen.Hall@fda.hhs.gov>

Sent: Monday, February 08, 2021 9:30 AM

To: Sanzo, Kathleen M. <kathleen.sanzo@morganlewis.com>

Subject: Regarding GRAS Notice GRN 000914

[EXTERNAL EMAIL]

Good Morning Kathy,

After reviewing Kao Corporation's GRAS Notice 000914 for the intended use of ALA DAG oil, we noted some additional concerns that need to be addressed.

1. The intended use of the *alpha*-linolenic acid diacylglycerol (ALA DAG) oil is not clear.
 - Please clarify if the ALA DAG oil is intended to be used as a replacement for other edible oils, such as olive oil.
 - If the ALA DAG oil is intended to be used as a replacement for other edible oils, please describe why the serving size for the ALA DAG oil (2.5 g/serving) is less than the amount used for the same applications for other edible oils.
 - If the ALA DAG oil is not intended to be used as a replacement for other edible oils, please provide a description of how the oil will be used and explain why such a small serving size is used.

2. The notifier describes two stability studies. The first study evaluates the stability of ALA DAG oil for one month at 30 °C under nitrogen. The second study evaluates the stability of an ALA DAG product that has been formulated to contain antioxidants and stabilizers for 18 months at room temperature (10 to 35 °C) and a relative humidity of 20 to 60%. We believe that the first stability study is conducted on an ALA DAG oil that has not been formulated to contain any antioxidants and stabilizers. Please confirm that our understanding is correct. In addition, if our understanding is correct and there are two different ALA DAG oil products, please clarify which product is the subject of this GRAS notice.

3. We have the following questions on the exposure estimate:
 - The notifier indicates that the recommended daily intake of ALA DAG oil is 2.5 g/d. Please provide a source for this recommendation.

We presume that the serving size of 2.5 g/d is for the ALA DAG oil product containing 36% ALA DAG and not 2.5 g/d of ALA DAG. Please confirm that our understanding is correct.

- The notifier indicates that 1 serving of the ALA DAG oil will provide the recommended daily intake of ALA DAG oil of 2.5 per serving per day. Presuming that the final ALA DAG oil product would contain a minimum of 36% ALA DAG, the notifier estimates an exposure to ALA DAG from intended use to be 0.9 g/d. This value is then added to the background exposure to ALA DAG of 0.49 g/p/d to obtain a cumulative exposure to ALA DAG of 1.39 g/p/d. Please confirm that our understanding of the exposure estimate is correct.
- We note that the exposure was based on the minimum specification for ALA DAG. Please indicate if there is a maximum level of ALA DAG that would be expected in the ALA DAG oil so that an upper bound exposure to ALA DAG could be estimated.

Responses may be sent in an email or in a separate document. Please do not send a revised copy of the notice. **We respectfully request a response within 5 business days.** If you are unable to complete the response within that time frame or have questions, please contact me to discuss further options at 240-402-9195 or via email.

Karen Hall

Regulatory Review Scientist

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

Karen.Hall@fda.hhs.gov

DISCLAIMER

This e-mail message is intended only for the personal use of the recipient(s) named above. This message may be an attorney-client communication and as such privileged and confidential and/or it may include attorney work product. If you are not an intended recipient, you may not review, copy or distribute this message. If you have received this communication in error, please notify us immediately by e-mail and delete the original message.