**AIBMR Life Sciences, Inc.**

January 28, 2020

Susan Carlson, PhD
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5001 Campus Drive
College Park, MD 20740

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of *Société d'Exploitation de Produits pour les Industries Chimiques* (hereinafter called SEPPIC) (the notifier), the undersigned, Kayla Preece, submits, for FDA review, the enclosed notice that Ceramosides™ Powder Neutra is GRAS for use in foods.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or kayla@aibmr.com.

Sincerely,

Kayla Preece, ND (agent of the notifier)
Scientific and Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

#906

# Notice to US Food and Drug Administration of the Conclusion that the Intended Use of Ceramosides™ Powder Neutra is Generally Recognized as Safe


**Submitted by the Notifier:**

SEPPIC – Air Liquide Healthcare
Specialty Ingredients
50 boulevard National
CS 90020
92257 La Garenne Colombes Cedex, France
and
E.P.I. France
3 rue de Préaux,
02130 Villers-sur-Fère, France

**Prepared by the Agent of the Notifier:**

AIBMR Life Sciences, Inc
2800 E. Madison Street, Suite 202
Seattle WA 98112


**January 28, 2020**


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# Figures and Tables

# Part 1: Signed Statements and Certification

## 1.1 Submission of GRAS Notice

*Société d'Exploitation de Produits pour les Industries Chimiques* (hereinafter called SEPPIC) (the notifier) is submitting a new GRAS notice in accordance with 21 CFR Part 170, Subpart E, regarding the conclusion that Ceramosides™ Powder Neutra is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

## 1.2 Name and Address of the Notifier and Agent of the Notifier

### Notifier

Marie-Anne Milesi
Nutrition Regulatory Affairs Manager
Société d'Exploitation de Produits pour les Industries Chimiques (SEPPIC S.A.),
50 boulevard National, CS 90020, 92257 La Garenne Colombes Cedex, France,
exclusive distributor of E.P.I. France's products.
Tel: +33 1 42 91 41 34
marie-anne.milesi@airliquide.com

For E.P.I. France, 3 rue de Préaux, 02130 Villers-sur-Fère, France

### Agent of the Notifier

Kayla Preece, ND
Scientific and Regulatory Consultant
AIBMR Life Sciences, Inc.
2800 E. Madison Street, Suite 202
Seattle, WA 98112
Tel: (253) 286-2888
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## 1.3 Name of the Substance

Wheat polar lipids (Ceramosides™ Powder Neutra)

## 1.4 Intended Conditions of Use

Ceramosides™ Powder Neutra is intended to be used as an ingredient in various food categories detailed in Part 3 of this report. Addition levels will range from 0.1–

10 mg/g of Ceramosides™ Powder Neutra, depending upon the specific food category. Ceramosides™ Powder Neutra is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

## 1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of Ceramosides™ Powder Neutra for its intended conditions of use, stated in Part 1.4 of this notice, has been made based on scientific procedures.

## 1.6 Not Subject to Premarket approval

We have concluded that Ceramosides™ Powder Neutra is GRAS for its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of Ceramosides™ Powder Neutra is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

## 1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of *Société d'Exploitation de Produits pour les Industries Chimiques*, Paris La Défense - 50 boulevard National - CS 90020 - 92257 La Garenne Colombes Cedex – France, Telephone: +33 1 42 91 41 34, email: marie-anne.milesi@airliquide.com or will be sent to FDA upon request.

## 1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRAS notice are considered exempt from disclosure under the Freedom of Information Act (FOIA) as trade secret or commercial or financial information that is privileged or confidential.

## 1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of Ceramosides™ Powder Neutra.

2020-01-28

Marie-Anne Milesi
Nutrition Regulatory Affairs Manager
Notifier

Date

# Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

## 2.1 Identification

Ceramosides™ Powder Neutra is derived from the seed of wheat (*Triticum aestivum*). Taxonomic basonyms include *Triticum sativum* and *Triticum vulgare*. The product is made from purified wheat flour that is extracted with ethanol and acetone to isolate the specific fraction that is rich in polar lipids. The result of this process is a powder consisting of not less than 95% wheat lipids.

### 2.1.1 Source Material

Wheat lipids represent a minor fraction of the whole plant grain (from 1.5–2.5%).[1] The most abundant nonpolar lipids contained in flour are triacylglycerols (i.e. triglycerides or TAGs) and free fatty acids (FFAs), while the polar lipid fraction is composed of glycolipids, phospholipids and sphingolipids (see Figure 1). The major fatty acids present in wheat grain have been reported as: linoleic acid (56.3%), palmitic acid (24.5%), oleic acid (11.5%), linolenic acid (3.7%), and stearic acid (1.0%).[2] The glycolipids contained in wheat consist primarily of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) while the prominent phospholipids are *N*-acyl phosphatidylethanolamine (NAPE), phosphatidylethanolamine (PE), and phosphatidylcholine (PC). Other phospholipids present include phosphatidylinositol (PI) and phosphatidylserine (PS).[1] The sphingoid base, or amino alcohol base, is common to all sphingolipids, and replaces the glycerol backbone found commonly in other lipids.[3] Sphingolipids can be found as ceramides, glycolipids such as cerebrosides and gangliosides, and phospholipids such as sphingomyelin. Ceramides (the simplest sphingolipids) are composed of a sphingoid base coupled with a fatty acid by an amine link between the $NH_2$ functional group of the sphingoid base and the acyl group of fatty acids. Cerebrosides are simple glycolipids consisting of a ceramide plus a carbohydrate (sugar) moiety, while gangliosides are more complex sphingo-glycolipids. According to the origin (plant, animal, yeast or bacteria) of the sphingolipids, the sphingoid moiety can vary.[4 5]

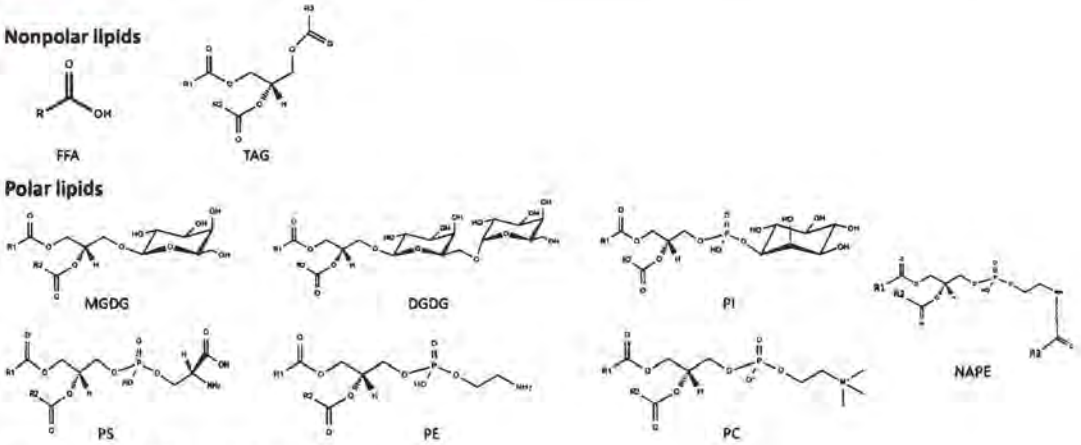


**Figure 1.** Wheat Lipids[1]

FFA: free fatty acids, TAG: triacylglycerol, MGDG: monogalactosyldiacylglycerol, DGDG: digalactosyldiacylglyerol, PI: Phosphatidylinositol, PS: phosphatidylserine, PE: phosphatidylethanolamine, PC: phosphatidylcholine, NAPE: n-acyl phosphatidylethanolamine.

### 2.1.2 Ceramosides™

Ceramosides™ Powder Neutra is a beige to light yellow fine powder with a characteristic cereal odor and is comprised of ≥ 90% wheat polar lipid fraction. In terms of total lipid content, the ingredient consists of ≥ 50% sphingolipids and ≥ 40% DGDG (see Table 1).

Qualitative analysis provided by SEPPIC demonstrates that other minor components of the polar lipid fractions of Ceramosides™ Powder Neutra are MGDG and glycosylated sterols. The predominant sphingolipids have been determined to be palmitoleoyl phytosphingosine, oleoyl sphingosine, and linoleoyl phytosphingosine while the sugar moiety in each case is allose (see Figure 2). A typical fatty acid composition of each ingredient is shown in Table 2 which is the mean analysis of four random batches of powder.

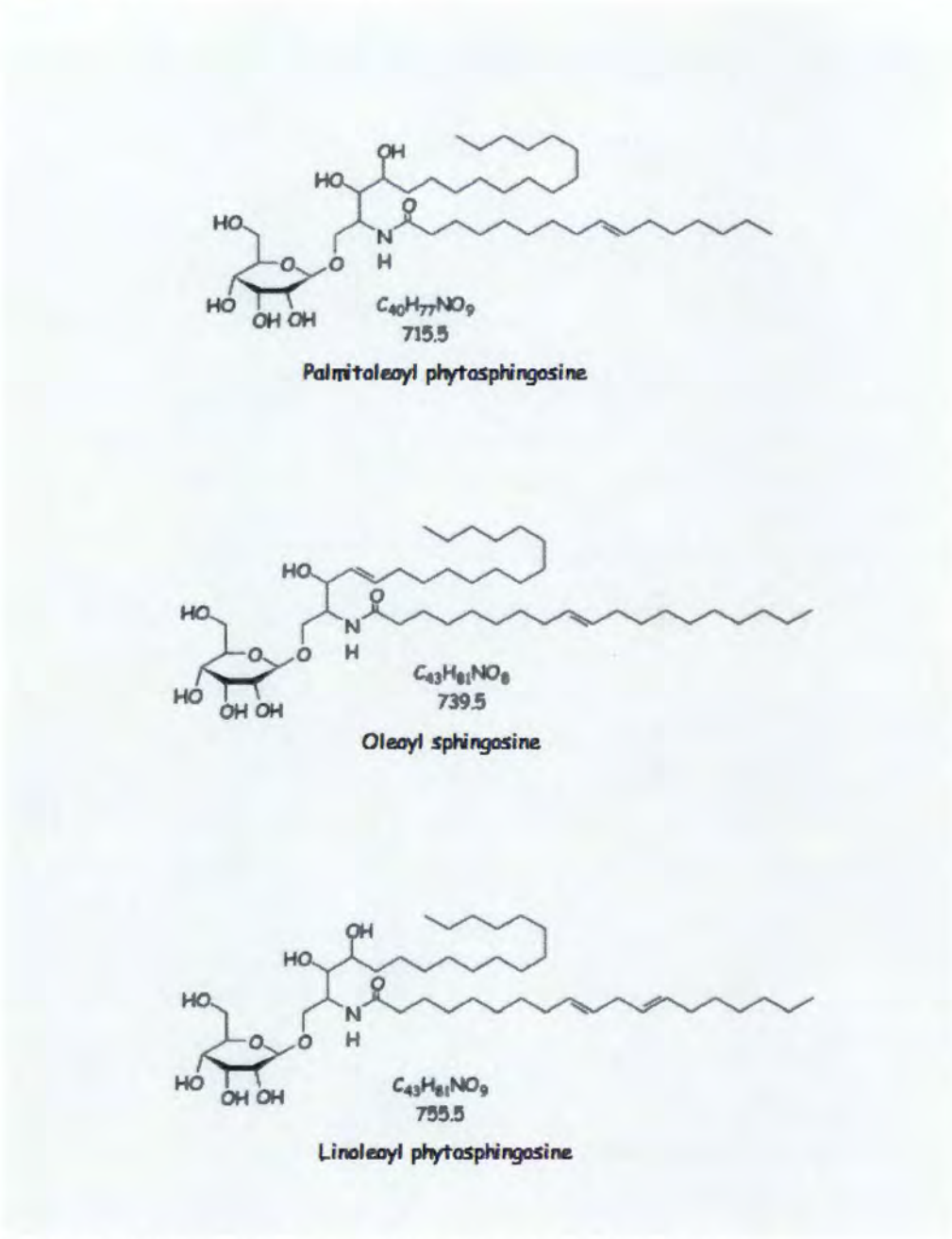


**Figure 2.** Ceramides Found in Ceramosides™ Powder Neutra

**Table 1**. Approximate Composition of Ceramosides™ Powder Neutra

| Ingredient | Content |
|---|---|
| Sphingolipids | 50 % |
| DGDG | 40 % |

| | |
|---|---|
| Triglycerides | 5 % |
| Water | 5 % |

**Table 2.** Approximate Fatty Acid Composition of Ceramosides™ Powder Neutra

| Common Name | Fatty Acids Lipid numbers + $\Delta^x$ | Ceramosides™ Powder Neutra (g/100 g of total fatty acids) |
|---|---|---|
| Myristic | C14:0 | 0.03 ± 0.06 |
| Pentadecanoic | C15:0 | 0.07 ± 0.06 |
| Palmitic | C16:0 | 19.17 ± 0.61 |
| Palmitoleic | C16:1, *cis*-$\Delta^9$ | 0.23 ± 0.06 |
| Margaric | C17:0 | 0.13 ± 0.06 |
| Heptadecenoic | C16:1, *cis*-$\Delta^{10}$ | 0.00 ± 0.00 |
| Stearic | C18:0 | 0.70 ± 0.00 |
| Oleic | C18:1, *cis*-$\Delta^9$ | 11.50 ± 0.26 |
| Linoleic | C18:2, all-*cis*-$\Delta^{9, 12}$ | 62.70 ± 0.89 |
| Alpha-linolenic | C18:3, all-*cis*-$\Delta^{9, 12, 15}$ | 3.90 ± 0.10 |
| Arachidic | C20:0 | 0.10 ± 0.00 |
| Gadoleic | C20:1, *cis*-$\Delta^9$ | 0.53 ± 0.06 |
| Eicosadienoic | C20:2, all-*cis*-$\Delta^{11, 14}$ | 0.1 ± 0.00 |
| Behenic | C22:0 | 0.10 ± 0.00 |
| Erucic | C22:1, *cis*-$\Delta^{13}$ | 0.10 ± 0.00 |
| Docosahexaenoic | C22:6, all-*cis*- $\Delta^{4, 7, 10, 13, 16, 19}$ | 0.13 ± 0.00 |
| Tricosanoic | C23:0 | 0.03 ± 0.06 |
| Lignoceric | C24:0 | 0.40 ± 0.44 |
| Nervonic | C24:1, *cis*-$\Delta^{15}$ | 0.10 ± 0.10 |

## 2.2 Manufacturing

### 2.2.1 Manufacturing Overview

**Wheat lipid extraction:** The wheat flour fraction is incorporated into ethanol under mechanical stirring in a stainless-steel tank, and the extraction is performed for a specified amount of time (see Figure 3).

**Filtration (solid/liquid separation):** Solid particles are separated from the liquid fraction by filtration under vacuum. The filtrate obtained contains the lipids and the solvent (ethanol).

**Concentration of wheat lipids:** The lipids contained in the filtrate are concentrated by the evaporation of ethanol and water under vacuum. The product obtained in this step is a wheat oil that is dispersed into acetone under mechanical stirring yielding

triglycerides and polar lipids. After decantation, the acetone and triglycerides are pumped, and the polar lipids are filtered under vacuum in a Büchner flask. After the filtration of polar lipids, they are recovered from the Büchner flask as a paste containing acetone. The paste is dried under vacuum to evaporate the acetone and produce a coarse powder. After drying, the powder is then crushed and sieved to make a thin powder.

**Packaging:** Ceramosides™ Powder Neutra is packaged in small and large quantities. Ceramosides™ Powder Neutra is conditioned into polyethylene terephthalate 15, Aluminum 9, polyethylene white 90 bags containing either 1.0 kg or 5.0 kg. The internal part of the bag is polyethylene white 90 which is compatible with food contact.

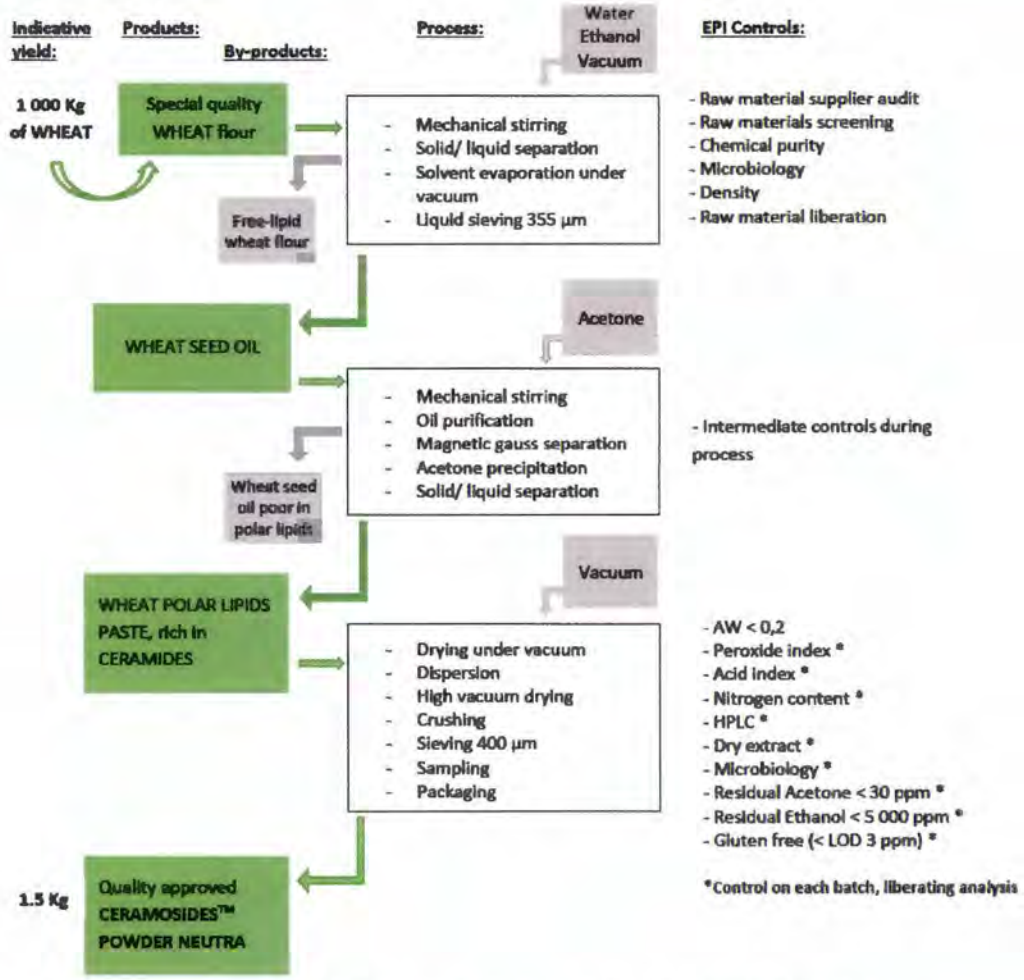


**Figure 3.** Manufacturing Flowchart Ceramosides™ Powder Neutra

### 2.2.2 Good Manufacturing Practice

Ceramosides™ Powder Neutra from SEPPIC is manufactured according to Good Manufacturing Practice (GMP) equivalent practices by EPI France, in an FDA registered facility (N° 19310227934) under a patented (France) Hazard Analysis Critical Control Point (HACCP) food grade process. SEPPIC requires suppliers to provide data, accreditations, certificates, and assays for purposes of verifying that their raw materials conform to currently enforceable EU food regulations for general food legislation, food hygiene, contaminants, and pesticides.

### 2.2.3 Raw Materials

Raw materials used in the production of Ceramosides™ Powder Neutra are of appropriate food grade.

## 2.3 Specifications

The specifications for the food-grade product Ceramosides™ Powder Neutra along with the specification methods, are listed in Table 3 below.

**Table 3.** Ceramosides™ Powder Neutra Specifications

| Test Items | Specification | Method |
|---|---|---|
| **Marker Compounds** | | |
| Total sphingolipids including glycosphingolipids | ≥ 50% | IM 06520-V3 (indirect[1]) |
| DGDG | ≥ 40% | IM 06522-V4 (RP-HPLC, ELSD) |
| Total Nitrogen linked with polar lipids | ≥ 0.6% | IM adapted from Dumas (combustion) NF EN ISO 16634-1-12/2008 |
| Polar Lipids Identification[2] | Identical to assay | IM 06223-V3 (TLC) |
| **Physical Characteristics** | | |
| Aspect | Thin powder | Visual and Olfactive |
| Color | Beige to light yellow | |
| Odor | Cereal characteristic | |
| **Chemical Data** | | |
| Acid Value | <15 mg KOH/g | NF EN ISO 660-09/2009 |
| Peroxide Value | <10 mEq/kg | NF EN ISO 3960-06/2010 |
| Humidity | < 5% | NF EN ISO 662-02/2001 |
| Dry Extract | > 95% | NF EN ISO 662-02/2001 |
| **Microbiological Tests** | | |
| Total aerobic plate count | ≤ 100 cfu/g | NF EN ISO 4833-1-10/2013 |
| Total Yeast & Mold | ≤ 100 cfu/g | NF V08-036-05/2003 |
| Enterobacteria | Absent/g | NF ISO 21528.1-12/2014 |

| | | |
|---|---|---|
| Staphylococci coagulase + (37°C) including *Staphylococcus aureus* | Absent/g | NF EN ISO 6888.3-06/2003 |
| *Escherichia coli* | Absent/g | Internal MA 104.4 adapted from ISO16649.3 |
| Salmonella | Absent/10 g | BRD 07/11-12/05 |
| *Listeria monocytogenes*[2] | Absent/25 g | ALOA-AES 10/3-09/00 |
| **Allergens** | | |
| Residual gluten | < 20 ppm | Internal Ridaquick Gliadine kit (R-Biopharm AG) test |
| Residual gluten[2] | < 20 ppm | AOAC-OMA 2012.01 |

*Abbreviations:* ALOA-AES, Proprietary Agar for testing Listeria developed by AES Chemunex; AOAC, Association of Official Agricultural Chemists; BRD, Bio-Rad; cfu, colony forming units; EN, European norm; ELSD, Evaporating light scattering detector; HS, Head Space; IM, Internal Method; ISO, International Organization for Standardization; MA, Adapted method; n/a, not applicable; NF, French norm; NF V, French norm for food industry; OMA, Official Method of Analysis; RP-HPLC, reverse phase high performance liquid chromatography; TLC, thin layer chromatography

[1] Calculation based upon total nitrogen content.

[2]Analysis guaranteed under statistical control: minimum once a year.

### 2.3.1 Batch Analysis

Production conformity and consistency of Ceramosides™ Powder Neutra is tested in production lots (see Table 4).

**Table 4.** Ceramosides™ Powder Neutra Batch Analyses

| Test Items | Specification | Lot# 21180709 Manufactured 07/09/2018 | Lot# 21180702 Manufactured 07/02/2018 | Lot# 21190307 Manufactured 03/07/2019 | Method |
|---|---|---|---|---|---|
| **Marker Compounds** | | | | | |
| Total sphingolipids including glycosphingolipids | ≥ 50% | 66.4% | 52.0% | 56.8% | IM 06520-V3 (indirect[1]) |
| DGDG | ≥ 40% | 41.3% | 40.8% | 41.3% | IM 06522-V4 (RP-HPLC, ELSD) |
| Total nitrogen linked to polar lipids | ≥0.6% | 0.8% | 0.9% | 0.8% | IM adapted from Dumas (combustion) NF EN ISO 16634-1 - 12/2008 |

| | | | | | |
|---|---|---|---|---|---|
| Polar Lipids Identification[2] | Identical to Assay | Conforms | Conforms | Conforms | IM 06223-V3 (TLC) |
| **Physical Characteristics** | | | | | |
| Aspect | Thin powder | Conforms | Conforms | Conforms | Visual and Olfactive |
| Color | Beige to light brown | Conforms | Conforms | Conforms | |
| Odor | Cereal characteristic | Conforms | Conforms | Conforms | |
| **Chemical Tests** | | | | | |
| Acid Value | <15 mg KOH/g | 5.1 mg KOH/g | 6.4 mg KOH/g | 6.75 mg KOH/g | NF EN ISO 660-09/2009 |
| Peroxide Value | <10 mEq/kg | < 0.1 mEq/kg | < 0.1 mEq/kg | <0.1 mEq/kg | NF EN ISO 3960-06/2010 |
| Humidity | < 5% | 1.0% | 0.8% | 0.7% | NF ISO 662-02/2001 |
| Dry Extract | > 95% | 99.0% | 99.2% | 99.3% | NF EN ISO 662-02/2001 |
| **Microbiological Tests** | | | | | |
| Total aerobic plate count | ≤ 100 cfu/g | < 10 cfu/g | < 10 cfu/g | < 10 cfu/g | NF EN ISO 4833-1-10/2013 |
| Total Yeast & Mold | ≤ 100 cfu/g | < 10 cfu/g | < 10 cfu/g | < 10 cfu/g | NF VO8-036-05/2003 |
| Enterobacteria | Absent/g | Absent/g | Absent/g | Absent/g | NF ISO 21528.1-12/2014 |
| Staphylococci coagulase + (37°C) including *Staphylococcus aureus* | Absent/g | Absent/g | Absent/g | Absent/g | NF EN ISO 6888.3-06/2003 |
| *Escherichia coli* | Absent/g | Absent/g | Absent/g | Absent/g | Internal MA.104.4 adapted from ISO16649.3 |
| Salmonella | Absent/10 g | Absent/10 g | Absent/10 g | Absent/10 g | BRD 07/11-12/05 |
| *Listeria monocytogenes*[2] | Absent/25 g | Absent/25 g | Absent/25 g | Absent/25 g | ALOA-AES 10/3-09/00 |

| Allergens | | | | | |
|---|---|---|---|---|---|
| Residual gluten | < 20 ppm | < 20 ppm | < 20 ppm | < 20 ppm | Internal Ridaquick Gliadine kit (R-Biopharm AG) test |
| Residual gluten[2] | < 20 ppm | < 20 ppm | < 20 ppm | < 20 ppm | AOAC-OMA 2012.01 |

*Abbreviations:* ALOA-AES, Proprietary Agar for testing Listeria developed by AES Chemunex; AOAC, Association of Official Agricultural Chemists; BRD, Bio-Rad; cfu, colony forming units; EN, European norm; ELSD, Evaporating light scattering detector; HS, Head Space; IM, Internal Method; ISO, International Organization for Standardization; MA, Adapted method; n/a, not applicable; NF, French norm; NF V, French norm for food industry; OMA, Official Method of Analysis; RP-HPLC, reverse phase high performance liquid chromatography; TLC, thin layer chromatography

[1] Calculation based upon total nitrogen content.

[2] Analysis guaranteed under statistical control: minimum once a year.

### 2.3.2 Heavy Metal Analysis

Heavy metals analysis is in compliance with European regulation (EC) N° 1881/2006 on the finished product and on the wheat raw material used for manufacturing. As EPI France has never received a positive test result in over 10 years, they schedule individual heavy metal frequency testing to occur once a year.

**Table 5.** Ceramosides™ Powder Neutra Heavy Metal Specifications

| Test Items | Specification | Method |
|---|---|---|
| Arsenic | ≤ 0.1 ppm | IM adapted from NF EN 15763 (ICP-MS) |
| Cadmium | ≤ 0.1 ppm | |
| Lead | ≤ 0.2 ppm | |
| Mercury | ≤ 0.1 ppm | |

*Abbreviation:* ICP-MS, Inductively coupled plasma mass spectrometry

### 2.3.3 Residual Solvent Analysis

Ethanol and acetone are class 3 solvents used in the manufacturing of Ceramosides™ Powder Neutra. Analyses are performed on each batch guaranteeing Ceramosides™ Powder Neutra contains <30 ppm of acetone and <5000 ppm of ethanol. Based on intended use and exposure estimates calculated in Part 3, solvent exposures meet International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH) guidelines.

### 2.3.4 Mycotoxins, Aflatoxins and Residual Pesticide Analysis

EPI France's plan for contaminant control is based on risk analysis (HACCP) and Food Safety Plan (FSMA US regulation). Based on this risk analysis, they have established that the raw material is tested once per year on one batch and finished product is tested once per year on one batch per grade respectively for mycotoxins, aflatoxins and residual pesticides.

### 2.3.5 Shelf–Life Stability

A two-year shelf-life from the time of manufacture has been recommended as an appropriate expiration period for Ceramosides™ Powder Neutra. This recommendation is based upon a 24-month long-term stability test of lot number 21101028. The long-term stability test was conducted at 15–25°C and 60–85% relative humidity under conditions of commercial packaging. Commercial packaging consists of aluminum bags with polyethylene covering and thermal seal.

The stability study demonstrates adequate stability along with an extended shelf-life of six months after recommendation. The ingredients remained in a thin powder form and all physical and chemical parameters were stable and all active components composed of wheat lipids including glycosyl ceramides and DGDG were preserved with no traces of peroxidation.

## 2.4 Physical or Technical Effect

Ceramosides™ Powder Neutra is not intended to produce any physical or other technical effects that are relevant to the safety of the ingredient.

# Part 3: Dietary Exposure

## 3.1 Intended Use

Ceramosides™ Powder Neutra is intended to be used as an ingredient in the food categories and at the addition levels shown in Table 5. Ceramosides™ Powder Neutra is not intended for use in infant formula, meat, poultry, egg, catfish, or any products that would require additional regulatory review by USDA.

**Table 6.** Ceramosides™ Powder Neutra Intended Uses

| Food Category | Maximum Intended Addition Level (mg/g) |
|---|---|
| | Powder Neutra |
| Bars | 1 |
| Chewing gums | 10 |
| Biscuits | 5 |
| Fruit juices, excluding citrus | 0.2 |
| Coffee | 0.1 |
| Tea | 0.1 |
| Fruit drinks | 0.2 |
| Beverage concentrates, dry, not reconstituted | 1 |
| Other functional beverages | 0.1 |
| Gelatin desserts or salads | 0.3 |
| Candies | 1 |
| Ready to eat cereals | 5 |
| Milk, fluid, imitation | 0.2 |
| Yogurt | 0.2 |
| Flavored milk and milk drinks, fluid | 0.2 |

## 3.2 Exposure Estimates

Exposure to Ceramosides™ Powder Neutra from the intended use categories was estimated for the U.S. population using food consumption data from the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). The NHANES 2013–2014 data were analyzed using Creme Food Safety software 3.6 (www.cremeglobal.com). These data were obtained from 7574 individuals who underwent two non-consecutive 24-hour dietary recall interviews (the first was collected in-person, the second by phone 3–10 days later).

WWEIA food codes that were considered most similar to the intended use categories were utilized in the assessment and were assigned the relevant intended use concentrations. Creme software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food groups and/or individual food ingredients. Creme Food Safety performs calculations using large-scale food consumption data sets. It bases the calculated estimates on each individual's body weight from the survey, as opposed to averaged body weights. Calculations also incorporated the NHANES assigned sample weights for each individual in the survey, which measure the number of people in the population represented by that specific subject and help to ensure that the results statistically represent the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories. The data are shown for "food consumers" (which includes only data from individuals who reported consuming one or more food/beverage categories intended to contain Ceramosides™ Powder Neutra over the two-day survey period, as opposed to the whole population). Results are given as both absolute exposure (mg/day), as well as exposure relative to body weight (mg/kg bw/day).

The relative standard error (RSE; calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100) is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population (the larger the RSE the less reliable the estimate).[6] RSE values greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[6, 7] For the purpose of this GRAS conclusion, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown for the 90$^{th}$ percentile values only, as the 90$^{th}$ percentile values are the most pertinent for the exposure estimates. All of the values were considered reasonably reliable using the 25% cut-off.

Data estimated directly from the NHANES short 2-day survey do not necessarily adequately represent individual usual long-term intake due to the large amount of random error. This is because it may not correctly capture infrequent consumers. It assumes that subjects who consumed a product on a survey day consume it every day of the year, and it does not adjust for potential day-to-day variation in intake (i.e., intra-individual variation over time is not accounted for). Thus estimation of "usual" or "lifetime" exposure was also added to the model based on methodologies developed by Nusser et al., 1996, at Iowa State University.[8] These lifetime data are considered the most relevant data, as GRAS exposure estimates should be based on expected regular exposure over the lifespan. The technique of estimating usual/lifetime intakes relies on the ability to transform the input daily average data

(from food consumers) into normality, which is tested using the Anderson-Darling test statistic within the Creme Global software.

The Ceramosides™ Powder Neutra exposure estimates from the intended use categories are shown for the total population (ages 2 years and older) below in Tables 7 and 8.

**Table 7.** Total (Aggregate) Absolute Exposure to Ceramosides™ Powder Neutra by Proposed Use Food Consumers Using NHANES 2013–14 Data (mg/day)

| Total Population (ages 2+) | N | % of total pop | Absolute Consumption Daily Average (mg/day) | | | | 90th% RSE Value | Lifetime 90th% Exposure Estimates (mg/day) |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | Mean std err | 90th% | 90th% std err | | |
| Powder Neutra | 6691 | 94.9 | 157.2 | 2.7 | 333.2 | 6.6 | 2.0 | 267.3* |

Creme runs #396
*Creme warning -2048, "Number of days per person should be constant for a Foods calculation" (data may still be used)

**Table 8.** Total (Aggregate) Exposure to Ceramosides™ Powder Neutra by Proposed Use Food Consumers Relative to Body Weight Using NHANES 2013–14 Data (mg/kg bw/day)

| Total Population (ages 2+) | N | % of total pop | Consumption Relative to Body Weight Daily Average (mg/kg bw/day) | | | | 90th% RSE Value | Lifetime 90th% Exposure Estimates (mg/kg bw/day) |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | Mean std err | 90th% | 90th% std err | | |
| Powder Neutra | 6691 | 94.9 | 2.67 | 0.05 | 5.99 | 0.13 | 2.2 | 5.24* |

Creme runs #396
*Creme warning -2048, "Number of days per person should be constant for a Foods calculation" (data may still be used)

According to the estimates above, approximately 95% of the U.S. total population (ages 2 and above) was identified as potential consumers of Ceramosides™ Powder Neutra from the proposed food uses. The lifetime 90th percentile estimated exposure to Ceramosides™ Powder Neutra was 267.3 mg/day (5.24 mg/kg bw/day). It should be noted that these estimates are considered extremely conservative, as they assume that 100% of the numerous intended use food products in the market will contain the maximum intended use levels of the SEPPIC product.

ICH guidelines state that exposure to class 3 solvents should be no more than 50 mg/day, or <5000 ppm (0.50%). As stated in part 2.3.2, the solvent specifications for the Powder Neutra product are <5000 ppm for ethanol and <30 ppm for acetone which are within this guideline, thus no further discussion of exposure is considered necessary.

## Part 4: Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

## Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for Ceramosides™ Powder Neutra is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information. To the best of our knowledge, Ceramosides™ Powder Neutra was not commonly used in foods prior to 1958.

# Part 6: Narrative

## 6.1 Absorption, distribution, metabolism, and excretion (ADME)

As previously discussed, Ceramosides™ Powder Neutra contains TAGs, sphingolipids, galactolipids (e.g. DGDG and MGDG), phospholipids, and other minor lipid components found in wheat. These lipids are all ubiquitously consumed daily through the diet. Ingested lipids are broken down into FFAs, 2-monoacyglycerol, unesterified cholesterol and other metabolites, and are made ready for absorption through enterocytes of the small intestine. Lipids are ultimately transported to the blood and peripheral tissues for utilization in the body, storage or excretion.[9]

Lipid digestion begins with enzyme activity from lingual lipase in the saliva and gastric lipase secreted from the gastric mucosa. As the dietary lipid moves through the digestive tract into the small intestine, emulsification occurs when bile salts and peristaltic mixing help to create a larger surface area on lipid droplets. This allows digestive enzymes from the pancreas to aid in further lipid breakdown.[9]

Products of lipid digestion form mixed micelles together with bile salts. These disc-shaped clusters are soluble in the aqueous environment of the small intestine because of their hydrophilic groups on the outer surface. Mixed micelles aid in absorption of longer chain fatty acid groups with the help of enterocytes at the brush border. Once lipids are absorbed by enterocytes, they migrate to the endoplasmic reticulum (ER) where biosynthesis of complex lipids take place.[9]

### 6.1.1 Fate of Triacylglycerol and Free Fatty Acids

Most triacylglycerol will undergo hydrolysis in the small intestine producing FFAs, mono- and diacylglycerols, and glycerol, with less than 10% remaining unhydrolyzed. After absorption, the FFAs, mono- and diacylglycerol, and glycerol are resynthesized as TAGs within the enterocytes, secreted into the lymph, packaged as chylomicrons, and transported to peripheral tissues of heart, muscle and adipose. TAGs in adipose tissue are under hormonal regulation. Hormones will initiate a phosphorylation signaling cascade resulting in the activation of hormone sensitive lipase and subsequent hydrolysis of a fatty acid from the glycerol backbone. This allows diacylglycerol lipase, followed by monoacylglycerol lipase, to act sequentially, resulting in complete hydrolysis of the TAG molecule and liberation of three FFAs and one glycerol per TAG. FFAs then move from the adipocyte to the blood stream via passive diffusion where they complex with albumin and are circulated to distant tissues for cellular uptake.

Some FFAs, in addition to their utilization as an energy substrate or resynthesis into TAGs, can contribute to the synthesis of phospholipids within local cell membranes

where they serve structural, regulatory, or transport roles.[10] Glycerol that is released from TAG is mainly used by the liver to produce glycerol 3-phosphate, which can enter glycolysis or gluconeogenesis metabolic pathways. Remnants of chylomicrons which include phospholipids, cholesterol esters, apolipoproteins and some TAG will bind to receptors in the liver where they are endocytosed and hydrolyzed at which point they may be recycled by the body.[9]

Oxidation of FFAs for energy production takes place within the mitochondria via the β-oxidation pathway. Metabolic activation of FFAs for β-oxidation requires the addition of coenzyme A (CoA) to the carboxylic acid head of the FFA which is catalyzed by a fatty acyl-CoA ligase, resulting in the formation of an acyl-thioester CoA conjugate. Short- or medium-chain fatty acids move freely through the mitochondrial membrane and interact with their specific ligases within the mitochondrial matrix. However, FFAs with 13 or more carbons in their hydrocarbon tail require a specific transport system due to the impermeability of the inner mitochondrial membrane to long-chain fatty acids and acyl CoAs. Long-chain fatty acid (LCFA) specific ligases are present in the outer mitochondrial membrane, where an acyl-CoA is formed. Acyl-CoA then enters the carnitine shuttle system in which carnitine acyltransferase I and carnitine acyltransferase II enzymes, respectively, replace CoA with carnitine on the outer membrane (forming an intermediate fatty acyl-carnitine) and carnitine with CoA on the inner membrane releasing the acyl-CoA within the matrix. Carnitine acyltransferase I also plays a role in the regulation of fatty acid metabolism as it is strongly inhibited by the first intermediate product in de novo fatty acid synthesis and can decrease β-oxidation of LCFA under conditions that favor synthesis.[10]

The ketogenic pathway is prominent during starvation or intentional prolonged fasting states. When dietary carbohydrates are limited or unavailable, the supply of citric acid cycle intermediates becomes limited, resulting in accumulation of acetyl-CoA derived from β-oxidation. When levels of acetyl-CoA rise, the β-ketothiolase reaction reverses, resulting in generation of acetoacetyl-CoA from two acetyl-CoAs. The β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase enzyme catalyzes the formation of HMG-CoA by the addition of a third acetyl-CoA to acetoacetyl-CoA, which, within the mitochondria, is split by HMG-CoA lyase to form acetoacetate and acetyl-CoA. Acetoacetate may be reduced to form D-β-hydroxybutyrate in an NADH-dependent reaction catalyzed by β-hydroxybutyrate dehydrogenase or, in small amounts, may spontaneously degrade to yield acetone. Collectively, acetoacetate, β-hydroxybutyrate, and acetone are referred to as ketone bodies. Ketogenesis occurs primarily in the liver and the ketone bodies are then transported to peripheral tissues where they can be utilized for energy production.[10]

### 6.1.2 Fate of sphingolipids

After ingestion, sphingolipids (e.g., ceramides and sphingosine) are hydrolyzed in the small and large intestine into metabolites. Sphingosine (amino alcohol + unsaturated hydrocarbon) and ceramides (sphingosine + N-acyl CoA) are rapidly taken up by intestinal cells and metabolized to FFAs.[11] A small amount of dietary sphingolipids are transported through the mucosa and appear in systemic circulation. Chylomicrons may be involved in the transport of sphingolipids as they are components of serum lipoproteins.[11]

There is no evidence that intake of sphingolipids through the diet is essential under normal conditions unless there is a defect in serine palmitoyl-transferase, the prominent enzyme in sphingolipid synthesis.[11] When there is abundant exogenous lipid intake, de novo synthesis is suppressed.[12] The regulation of sphingolipids is tightly controlled in the body and there is evidence that cells have mechanisms which sense and coordinate sphingolipid production. Enzymes and substrates particular to sphingolipid metabolism are embedded in and not diffusible through the plasma membrane. This allows for independent regulation of enzyme activity in subcellular locations corresponding to differences in sphingolipid quantities and function. Many reactions involved in sphingolipid metabolism are reversible, which allows for rapid interconversion of different metabolic intermediates and aides in the recycling of sphingolipids and the generation of "signaling" metabolites.[12]

The end products and biosynthetic intermediates of sphingolipid metabolism have biological activity when properly regulated and, if improperly regulated due to certain disease states such as Tay-Sachs, Niemann-Pick, Farber's Disease, Fabry Disease and Gaucher Disease, may result in cellular stress and pathological effects (e.g., inhibition of sphingolipid synthesis can block growth, while over production can trigger toxic effects[12]). These diseases are all relatively rare in occurrence, inherited, and are not caused by consumption of dietary sphingolipids.

Studies performed in mice show that not all sphingolipids taken in through diet are hydrolyzed and absorbed. Germ-free mice show reduced hydrolysis of sphingomyelin resulting in 43% of sphingolipids excreted in feces (25–60% ceramide and 40–70% intact molecule) while non-germ-free mice were found to have only 25% excreted in feces (80–90% ceramide, 3–6% free sphingosine and 10% intact molecule).[11] Along with gastrointestinal microbiota, other factors that will impact hydrolysis and absorption include the amount of sphingomyelin absorbed, the presence of bile salts, and the levels of enzymes present.[13]

Glycosphingolipids are a type of sphingolipid derived from ceramide that contain a carbohydrate moiety attached by an O-glycosidic bond. The type of glycosphingolipid will vary depending on the number and type of carbohydrate present. Glycosphingolipids are essential components of all cell membranes in the body and are found largely in nerve tissue.[3] Exogenously consumed

glycosphingolipids are broken down through hydrolysis in the small intestine where glycolipase activity is highest, leading to the formation of FFAs and galactolipids such as MGDG and DGDG.[15] [16]

### 6.1.3 Fate of galactolipids

After ingestion, galactolipids such as DGDG are hydrolyzed in the small intestine where bile salts and galactolipases are responsible for the release of FFAs, digalactosylmonoacylglycerol (DGMG), and water-soluble galactose containing compounds.[16] The hydrolysis of galactolipids is similar to that of phospholipid hydrolysis, whereby galactolipids are degraded into lysocompounds which allows for their absorption and use in mucosal cells.[16] Compounds released from galactolipid breakdown will then follow similar pathways as described in the fate of FFAs, where they will serve as energy substrates, be resynthesized into TAGs, or be synthesized into phospholipids serving structural, regulatory, or transport roles.[10] According to the Human Metabolome Database, the conversion of DGDG may also be irreversibly hydrolyzed to MGDG by alpha-galactosidase.[17]

### 6.1.4 Fate of phospholipids

Pancreatic enzymes facilitate phospholipid degradation. Phospholipase A2 is activated by trypsin and cleaves fatty acids from carbon two of a phospholipid, leaving a lysophospholipid. The remaining fatty acid at carbon one is removed by lysophospholipase, leaving a glyceryl phosphoryl base that can be excreted in the feces, further degraded or absorbed. Lysophospholipids are reacylated to form phospholipids by acyltranferases.[9] Phospholipids are important components of cell membranes while non membrane-bound phospholipids serve as components of lung surfactant and bile.[3]

### 6.1.5 Conclusion

In summary, Ceramosides™ Powder Neutra ingredients is extracted from wheat seed, and contains no less than 95% lipids including TAGs, sphingolipids, galactolipids and phospholipids. Humans have ubiquitously consumed these types of edible lipids through food intake (including from wheat seed) for millennia.[11] The metabolic pathways of these compounds have been studied and are well-described.

## 6.2 Toxicology Studies

No published studies investigating the potential toxicological effects of wheat lipid extracts were located during our literature searches. Likewise, no toxicological investigations of sphingolipids were located. This is likely due to the fact that both

wheat and its lipid constituents have been common components of the human diet for millennia without safety concerns, and the endogenous nature of sphingolipids, which are ubiquitously present (under homeostatic regulatory control) as components of all body cell membranes. Regardless, SEPPIC sponsored genotoxicity, acute, and subchronic studies on the Ceramosides™ Powder Neutra product. The studies are not published but serve as corroborative evidence of the safety of the product. Study descriptions and results are summarized below. All studies were Good Laboratory Practice (GLP) compliant.

### 6.2.1 Acute Toxicity Study

SEPPIC sponsored an unpublished, independent investigation, in accordance with OECD test guideline (TG) 423 (adopted 21 July 1997), of the acute oral toxicities of Ceramosides™ Powder Neutra ingredient.

In the toxicity study, three female Sprague Dawley rats were observed for a period of fourteen days. Each rat was administered by gavage a single dose of 5000 mg/kg bw of Ceramosides™ Powder Neutra on day zero of the study. Animals underwent daily systemic examinations to record any behavioral or toxic effects. Their weight was recorded on days zero, two, seven and fourteen of the study. On day fourteen, the animals were euthanized by sodium pentobarbital administration. The study reported no changes upon macroscopic evaluation of organs, body weight gain was comparable to historic control, and there were no observed adverse reactions. Twenty-seven hours after the dose of Ceramosides™ Powder Neutra was given, one rat was found deceased. After examination, the cause of death was determined to be spontaneous, as there were no gross necropsy findings and there were no signs of toxicity. In conclusion, the acute oral $LD_{50}$ of Ceramosides™ Powder Neutra was considered to be higher than 5000 mg/kg bw.

### 6.2.2 Bacterial Reverse Mutation Test

**Purpose:** In order to evaluate the mutagenic potential of Ceramosides™ Powder Neutra, a bacterial reverse mutation test was conducted in accordance with OECD TG 471 (adopted 21 July 1997).

**Methods:** Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2 *uvrA*) were used in the presence and absence of rat liver S9 metabolic activation with appropriate positive and negative controls. The concentrations of Ceramosides™ Powder Neutra used for the initial mutation test using a plate incorporation procedure and confirmatory mutation test using a pre-incubation procedure were: 5000, 1600, 500, 150, 50, and 16 µg/plate. Three replicates were conducted for each test concentration and control (untreated, vehicle, and positive reference).

**Results:** Spontaneous revertant colony numbers of the vehicle control were comparable with historical control data, and positive controls induced the expected responses. No biologically relevant increases were seen in revertant colony numbers in any of the five bacterial strains upon treatment with the test item at any of the concentration levels either in the presence or absence of the S9 activation system. All results were unequivocally negative according to the study criteria for both positive and biologically relevant responses.

**Conclusions:** Under the experimental conditions applied, Ceramosides™ Powder Neutra failed to induce gene mutations by base pair changes or frameshifts in the genome of the strains used at concentrations up to the maximum recommended test concentration of 5000 µg/plate.

### 6.2.3 In vitro Mammalian Chromosomal Aberration Test

**Purpose:** In order to evaluate the clastogenic potential of Ceramosides™ Powder Neutra, an in vitro mammalian chromosomal aberration assay was conducted in accordance with OECD TG 473 (adopted 29 July 2016).

**Methods:** Ceramosides™ Powder Neutra was suspended in Dulbecco's Modified Eagle's solution, and three concentrations (1250, 2500, and 5000 µg/mL) were chosen on the basis of preliminary cytotoxic investigations. The chromosomal aberration assays were conducted in two independent experiments (each in duplicate) using V79 (Chinese hamster lung) cells. The cells were exposed to the negative control or each test item concentration with and without metabolic activation using rat liver S9. Groups of cells were also exposed to the respective positive controls for use with or without S9-mix. Ethyl methanesulfonate was used as positive control without S9-mix and cyclophosphamide was used as the positive control with S9-mix. Exposure and sampling times were as follows:

- Experiment A: 3h treatment with and without S9-mix (1250, 2500, and 5000 µg/mL)/20h sampling time.
- Experiment B: 20h treatment without S9-mix (1250, 2500, and 5000 µg/mL)/20 and 28h sampling times.
- Experiment B: 3h treatment with S9-mix (1250, 2500, and 5000 µg/mL)/28h sampling time.

Following treatment and sampling, cells were exposed to selection agent, colchicine (0.2 µg/mL), 2.5 hours prior to harvesting and fixing for slide preparation. Chromosome aberration frequencies were then scored blind for at least 300 well-spread metaphase cells.

**Results:** In both A and B experiments, no statistically significant differences compared to concurrent and historical negative (solvent) controls in numbers of

chromatid or chromosome aberrations or in the rate of polyploid and endoreduplicated metaphases were observed after treatment with the different concentrations of Ceramosides™ Powder Neutra with or without metabolic activation. No dose-response relationships were noted.

**Conclusions:** Ceramosides™ Powder Neutra is not clastogenic in this test system.

### 6.2.4 In vivo Mammalian Micronucleus Test

**Purpose:** In order to evaluate the genotoxic potential of Ceramosides™ Powder Neutra, an in vivo mammalian micronucleus assay was conducted in accordance with OECD TG 474 (adopted 29 July 2016) under the permission of the Institutional Animal Care and Use Committee (IACUC) of Toxi-Coop Zrt.

**Methods:** Two doses of Ceramosides™ Powder Neutra were administered by gavage at 24-hour intervals to male Crl:NMRI BR mice at test concentrations of 0 (vehicle-control), 500, 1000, or 2000 mg/kg bw. The high dose is the limit dose for mammalian erythrocyte micronucleus tests. The negative control/vehicle was 1% aqueous methylcellulose. The positive control, cyclophosphamide, was administered by intraperitoneal injection at a concentration of 60 mg/kg bw. Each group consisted of five animals, and all treatments were administered at a uniform volume of 10 mL/kg bw. The micronucleus test was conducted at the doses described above in males only based on the results of a non-GLP, preliminary toxicity test that was conducted using a single gavage dose of Ceramosides™ Powder Neutra at a concentration of 2000 mg/kg bw in two animals/sex/group in order to determine the high-dose and assess sex differences. No mortality, signs of toxicity, or sex specific effects were observed in the preliminary test.

All animals were observed immediately following dosing and at regular intervals until sacrifice for mortality, signs of toxicity, or adverse reactions to treatment. Bone marrow smears were prepared in duplicate on standard microscope slides from samples obtained from the femurs of each animal from each dose group immediately following sacrifice 24 hours after the second treatment (after the single treatment in the positive controls). Four thousand polychromatic erythrocytes (PCE) per animal were scored for frequency of micronuclei with one slide from each animal being scored blind. The proportion of PCE to mature erythrocytes per animal was determined by the number of mature cells encountered while counting at least 500 PCE. Criteria for a positive response were according to the cited OECD test guideline.

**Results:** No mortality, clinical signs of toxicity, or adverse reactions to treatment were observed in any animals during the study. No significant differences were observed in frequency of micronucleated PCE or proportion of PCE to mature erythrocytes between the three dose groups compared to the negative control, and

all results were within the laboratory's historical control range. A slight non-significant decrease in PCE in the high-dose group compared to concurrent negative controls indicated exposure of the test item to bone marrow occurred.

**Conclusions:** Ceramosides™ Powder Neutra, at concentrations up to the limit dose of 2000 mg/kg bw, was negative for producing chromosomal damage in the bone marrow of mice under the experimental conditions.

### 6.2.5 Twenty-eight-day Repeated-Dose Oral Toxicity Study

**Purpose:** An unpublished 28-day study was conducted in accordance with OECD TG 407 (adopted 03 October 2008) as a preliminary evaluation of the potential health hazards, including identification of toxic effects and target organs, of repeated oral exposure to Ceramosides™ Powder Neutra. The study was conducted under the permission of the IACUC of Toxi-Coop Zrt and in compliance with the National Research Council Guide for Care and Use of Laboratory Animals[18] and the principles of the Hungarian Act 2011 CLVIII (modification of Hungarian Act 1998 XXVIII) regulating animal protection.

**Methods:** Ten SPF Hsd.Han Wistar rats/sex/group were administered Ceramosides™ Powder Neutra (formulated in a sunflower oil vehicle) at concentrations to provide for uniform administration by gavage of a dose volume of 10 mL/kg bw. Four groups were administered doses of 0 (vehicle-control), 500, 1000, and 2000 mg/kg bw/day for 28 days.

All tests and examinations were conducted according to study protocols and in compliance with above stated guideline.

**Results:** Sporadic increases in body weight were noted in the high-dose male group and in all female test groups. As the findings were transient in both sexes and additionally not dose-related in the female group, the finding was not considered test item related.

A statistically significant increase in total protein was noted in the male low- and mid-dose groups. A statistically significant decrease in calcium was seen in the male mid- and high-dose groups, and a statistically significant decrease in phosphorus was noted in the male mid-dose group compared to controls. Additionally, in the female groups, increased creatinine was observed in the low-dose group; the mid-dose group had an increase in alanine aminotransferase, and an increase in creatinine, a decrease in sodium, and an increase in albumin to globulin ratio; and the high-dose had an increase in cholesterol and decrease in calcium. All changes were statistically significant. These changes were considered normal physiologic variations and corresponded well with historical control (within or marginal to the historical reference range of the lab). Additionally, none of the changes were seen

in both sexes and many changes were noted only in low- or mid-dose groups. Therefore, the changes are not considered to be test item related.

Organ weights were normal for the low- and high-dose groups compared to the control. Brain weight was statistically significantly low for both absolute and relative to body weight and body weight was elevated relative to brain weight in the male mid-dose group. As the changes were not dose dependent, they were not considered toxicologically relevant.

Pyelectasis was reported in low incidence in all male test groups and in one female in the mid-dose. This is a common finding in this strain of rats and is not considered toxicologically relevant. Hernia diaphragmatica was seen in the control, low- and mid-dose males at low incidence. This structural change is common in the strain of rat studied and, due to its low- and non-dose-related incidence, is not considered test item related. Hydrometra was observed in similar incidence in all female test groups and, as it is a common neurohormonal response to the estrous cycle in female rats, is not considered toxicologically relevant. A thymus hemorrhage was observed in one female of the high-dose test group. This was considered a result of the exsanguination process and not test item related. A single male in the high-dose group had both an adhesion on the liver and a cyst on an adrenal gland. As it was in the same male, it was considered an individual change and not test item related. No inflammatory or pathologic lesions were noted in any of the examined organs.

Histopathologic examination was performed on the control and high-dose groups, as well as on any abnormal macroscopic organ observations. Examination revealed centrilobular vacuolation in control and high-dose males and females. This finding suggests possible hepatic lipidosis. Due to the fact it was seen in both control and test groups, it was not considered test item related. Acute emphysema was noted in one male at the high-dose group and thymic hemorrhage was noted in one female in the high-dose group. These findings are common with hypoxia during exsanguination and are not considered test item related. Interstitial fibrosis of the liver (focal, subcapsular) was noted in one control male and was attributed to irritation of Glisson's capsule due to hernia diaphragmatica. It is not considered test item related as it was in a control animal.

In summary, none of the following were affected by test article administration: mortality, body weight or body weight gain changes, changes in mean daily food consumption and feed efficiency, pathologic changes in the evaluated hematological or blood chemistry parameters, specific macroscopic changes in the gross pathology findings, ophthalmologic changes, changes in absolute or relative organ weights, or histopathological lesions.

**Conclusions:** Oral administration of Ceramosides™ Powder Neutra at doses up to 2000 mg/kg bw/day for 28 days did not cause signs of toxicity in male or female

SPF Hsd.Han Wistar rats. Based on these results, the no observed adverse effect level (NOAEL) was determined to be 2000 mg/kg bw/day; the highest dose tested.

### 6.2.6 Summary of Toxicity Study Results

As demonstrated in the study summaries above, Ceramosides™ Powder Neutra showed no mutagenic effect in the Ames or micronucleus assays. A subchronic toxicity study on Ceramosides™ Powder Neutra showed no effects up to 2000 mg/kg bw, the highest dose tested. While the information is unpublished, it helps support the safety narrative that Ceramosides™ Powder Neutra is not toxic.

## 6.3 Human Studies

A placebo-controlled, randomized, double-blind clinical study (n=60) comparing the effect of oral intake of Ceramosides™ Powder Neutra (30 mg/day x 60 days) to placebo on skin hydration and age-related symptoms (skin elasticity, smoothness, trans epidermal water loss, roughness and wrinkling) was published in 2017.[19] The authors reported no adverse effects and the test article was well tolerated throughout the 60-day study.

An unpublished pilot study was mentioned in the New Dietary Ingredient (NDI) notification for Ceramosides™ Powder Neutra to FDA in 2015, which was performed on 20 healthy women. This study investigated oral intake of Ceramosides™ Powder Neutra for two months at 20 mg/day and its effect on skin moisturization. No adverse events were reported. No additional human studies on wheat lipid extracts were found in the public domain.

## 6.4 Allergenicity

Ceramosides™ Powder Neutra has undergone extensive testing for wheat proteins, gluten and gliadin. Levels are confirmed to be < 3 ppm which is below the maximum limit for status of "gluten-free" under FDA standards. Ceramosides™ Powder Neutra raw material does not contain and is not exposed to any additional allergens through the manufacturing process. In addition, there is no allergenic potential of wheat lipids or sphingolipids mentioned in the public domain.

## 6.5 History of Consumption

Wheat consumption began in the Fertile Crescent of the Middle East approximately 10,000 years ago when humans transitioned from a hunting and gathering lifestyle to farming with the domestication of wheat. Today, approximately 430 million tons of domesticated wheat (known as bread wheat or common wheat) is produced

annually and estimated to provide about 1/5 of the calories consumed by humans worldwide.[21]

According to survey data extracted from NHANES 2014 WWEIA, the average lifetime consumption of fat in the United States was approximately 80 g/day, while the 90th percentile lifetime consumption was approximately 116 g/day.[22] As discussed in Part 3, lifetime 90th percentile exposure to Ceramosides™ Powder Neutra, which is > 95% lipids, is estimated at 0.267 g/day, respectively. As this product could be potentially additive with regard to fat intake in the diet of consumers, Ceramosides™ Powder Neutra would minimally increase the amount of fat consumed in the United States by up to approximately 0.3% (0.267 g/day of fat from Ceramosides™ Powder Neutra/116 g/day of fat from average American consumption). However, it is also possible that the ingredient will be substitutive for other oil ingredients in the intended use food categories.

Sphingolipids are constituents of many foods in relatively small quantities. As they are present in all cellular membranes, sphingolipids are found both in animal and vegetable foods. Sphingolipid concentrations can vary widely depending on the food type, with high fat dairy products like cream and cheese containing about 1692 μmol/kg and 1326 μmol/kg, respectively; eggs containing approximately 2250 μmol/kg; soybeans containing approximately 2410 μmol/kg; and wheat containing approximately 576 μmol/kg. Due to its high consumption per capita, wheat generally constitutes approximately 25% of the total annual sphingolipid consumption.[11] Average sphingolipid consumption varies widely as a function of dietary habits. In the USA, the average sphingolipid consumption per capita is estimated at 0.3 to 0.4 g/day.[11]

Glycolipids, such as DGDG, are major lipids in photosynthetic tissue of vegetable food but are also found in non-photosynthetic tissue such as potato tubers, apples, and seeds. Cereals such as barley, corn and wheat flour are rich in DGDG. Wheat flour has been reported to contain 158–230 mg DGDG per 100 g dry weight. The differences in values stem from the method of quantification and from natural variation in the raw material as plant glycolipid content can vary tremendously depending on the cultivar, growth conditions, stage of development and the day of harvest.[23] The Wheat Foods Council in 2004 estimated the daily intake of wheat flour to be about 165.3 g/day, which, based on the amount of DGDG in wheat flour described above, would approximate to a daily intake of DGDG from wheat flour of 260–380 mg/day for the average American.[24] In a 2012 Scientific Opinion on the substantiation of a health claim related to oral intake of Wheat Polar Lipid Extract (submitted by Extraction Purification Innovation France) and protection of the skin against dehydration, the European Food Safety Authority (EFSA) discussed the background consumption levels of ceramides and DGDG. EFSA's opinion was that the dose of 30 mg/day of the Wheat Polar Lipid Extract (providing 1.8 mg/day of

ceramides and 15 mg/day of DGDG), based on an unpublished clinical trial, would represent only a small fraction (approximately 10%) of the quantity that would be consumed in the diet by subjects eating a typical amount of wheat-derived foods.[20] Though 30 mg/day is below the anticipated exposure estimates outlined in Part 3 of this document, it serves as corroborative evidence of the safety of Ceramosides™ Powder Neutra.

## 6.6 Past Sales and Reported Adverse Events

No FDA letters regarding concern for safety to companies that market products containing ceramosides, wheat cerasomes, wheat ceramosides oil, wheat seed extracts, sphingolipids, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, or lysophosphatidylcholine were located.

Additionally, according the manufacturer E.P.I France, no adverse events have been reported with respect to the ingredient following sales of 4126 kg of Ceramosides™ Powder Neutra from 1998–2019 in various countries.

## 6.7 Similar Products in the Marketplace

A general Internet search as well as searches of the National Institutes of Health (NIH) Dietary Supplements Label Database and large distributors of dietary supplements resulted in a number of findings of wheat lipid extract(s) products, illustrating this type of ingredient is available in the United States. Examples of wheat lipid extracts are listed in Table 8:

**Table 9.** U.S. Products Containing wheat lipid extracts

| Company | Product Name | Serving Size(s) |
|---|---|---|
| Life Extension | Skin Restoring Ceramides<br>https://www.lifeextension.com/Vitamins-Supplements/item02096/Skin-Restoring-Ceramides | 350 mg daily<br>Ceratiq® wheat (*Triticum vulgare*) oil extract (providing glycolipids, phytoceramides and glycosylceramides) |
| Sports Research | Phytoceramides<br>https://sportsresearch.com/products/phytoceramides?variant=36600156272680 | 350 mg daily<br>Phytoceramides from non-GMO wheat (*T. vulgare*) |
| Healthy Choice Naturals | Phytoceramide Complex<br>http://shop.healthychoicenaturals.com/Phytoceramides-Skin-Rejuvenating-Complex-p/phyto.htm | 350 mg daily<br>Phytoceramides daily complex consisting of wheat germ oil and phytoceramides from (wheat extract) |
| Douglas Laboratories | Skin Nourish<br>https://www.douglaslabs.com/skin-nourish.html | 30 mg daily<br>Ceramosides™ Ceramide wheat seed extract (*T. vulgare*), (standardized to 50% glycosylceramides, 40% DGDG) |
| Reserveage | Collagen Hydra Booster with phytoceramides | 30 mg daily |

| | | |
|---|---|---|
| | https://reserveage.tlcchealth.com/product/collagen-hydra-booster-ceramides/ | Ceramosides™ Ceramide wheat extract (*T. vulgare*) (seed) standardized to 50% glycosylceramides (7.5 mg), 40% DGDG (6 mg) |
| ResVitale Collagen Hydraplump with Ceramides | Ceramosides™ Ceramide Wheat Extract http://www.resvitale.com/product/collagen-hydraplump-with-ceramides/ | 15 mg Ceramosides™ Ceramide Wheat Extract (*T. vulgare*) (seed) standardized to 50.0% glycosylceramides, 40.0% DGDG |

## 6.8 Current Regulatory Status

A thorough search for the current regulatory status of Ceramosides™ Powder Neutra, wheat seed extract, phospholipids, sphingolipids, ceramides, cerebrosides, and DGDG relevant to use in food in the United States was conducted. Searched databases included: List of NDI Notifications (NDIN[25]), FDA GRN (GRAS) inventory, 21 CFR, and a general internet search. A summary of the pertinent search results is shown below:

- An NDI notification to FDA (NDI 774)[26] was provided by the client and found in the FDA list of NDINs, for Ceramosides™ Powder Neutra. The NDI received a "no questions" letter from the Division of Dietary Supplement Programs, CFSAN, on November 6, 2012 for the use of Powder Neutra at a serving level of 30 mg/day for healthy adults.

## 6.9 Basis for the GRAS Conclusion

The scientific procedures establishing the safety of Ceramosides™ Powder Neutra comprise the technical element of the GRAS standard. The common knowledge element is comprised of the general availability and general acceptance, throughout the scientific community of qualified experts, of the technical element. Together, the technical element and the common knowledge element form the basis for SEPPIC's conclusion of GRAS status of Ceramosides™ Powder Neutra for its intended use.

### 6.9.1 Data and Information that Establish Safety

Ceramosides™ Powder Neutra has been the subject of a thorough safety assessment as described above. The totality of evidence supporting the safety of Ceramosides™ Powder Neutra is comprised of data and information that establish the safety of Ceramosides™ Powder Neutra under the conditions of their intended use (the technical element) and data and information that is corroborative of safety. The

scientific data, information, and methods forming the technical element of this conclusion are:

- The establishment of identity, demonstrating composition of the ingredient as commonly consumed edible lipids;
- The method of manufacture and specifications, demonstrating safe production and high-quality control standards for Ceramosides™ Powder Neutra;
- ADME data demonstrating the well-studied pathways by which the body metabolizes the edible lipids that comprise Ceramosides™ Powder Neutra;
- The long history of daily high consumption of wheat, and hence wheat lipids, in the human diet, and the ubiquitous presence of sphingolipids, glycolipids (e.g. DGDG) and TAGs from other standard foods in the diet.

The Ceramosides™ Powder Neutra product contain no less than 95% of lipids from wheat, which have been consumed ubiquitously for millennia. The exposure estimates based on the ingredient's intended uses suggest a lifetime 90$^{th}$ percentile exposure of 267 mg/day of Ceramosides™ Powder Neutra. The 90$^{th}$ percentile lifetime exposure estimate is extremely conservative in that it assumes the ingredient is present in 100% of the food items in each of the intended use food categories, and it is not expected to result in any significant material increase in exposure to total fat beyond what is ingested through typical dietary exposure, as described in Part 6.5. The individual lipids found in the ingredient have well-described ADME pathways in the human body and are expected to be readily metabolized. As such, the totality of evidence supporting safety of the ingredient as described in this subpart supports a conclusion that the intended uses of Ceramosides™ Powder Neutra are reasonably certain to be safe.

### 6.9.2 Data and Information that is Corroborative of Safety

The safety of Ceramosides™ Powder Neutra is corroborated by unpublished genotoxicity studies and an unpublished twenty-eight-day repeated-dose oral toxicity study with a NOAEL of 2000 mg/kg bw/day, the highest dose tested, along with the lack of serious adverse events reported in a clinical trial using Ceramosides™ Powder Neutra at daily dosages up to 30 mg/day at durations up to 60 days, and the history of human consumption of approximately 4126 kg of Ceramosides™ Powder Neutra over a 20-year period with no serious adverse event reported. Additionally, an NDI notification for the product received a no questions filing by FDA at a dosage of 30 mg/day. While the exposure in the clinical trial and the NDI notice were at much lower exposures compared to those estimated for the intended uses in this GRAS conclusion, they add to general corroboration of the

safety of the ingredient at levels that may be typically found in a serving of food containing Ceramosides™ Powder Neutra.

### 6.9.3 General Recognition

The scientific data, information, and methods herein reported that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain aside from the acute, genotoxicity, and subchronic toxicity studies of Part 6.2 and the human clinical study of Part 6.3. Part 7 of this GRAS notice contains the bibliography for the published studies. This publicly available data and information fulfills the requirement for general availability of the scientific data, information, and methods relied on to establish the technical element of the GRAS standard. Additionally, the lack of Letters to the Editor or other dissenting opinions further provide reasonable certainty that consumption of Ceramosides™ Powder Neutra for its intended use is not harmful. The general availability and acceptance of this scientific data, information, and methods satisfies the common knowledge element of this GRAS conclusion.

## 6.10 Data and Information that is Inconsistent with the GRAS Conclusion

We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

## 6.11 Information that is Exempt from Disclosure under FOIA

There are no data or information in this report that are considered trade secret or commercial or financial information that is privileged or confidential.

# Part 7: Supporting Data and Information

Initial literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted through October 30, 2019.

## 7.1 Data and Information that are *not* Generally Available

Information described in this GRAS notice that is not generally available includes the unpublished studies described in sections 6.2 and the unpublished human pilot study in section 6.3. These studies are considered only corroborative as stated in 6.10.2. All pivotal information is published, as reviewed in 6.10.1.

## 7.2 References that *are* Generally Available


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| | |
|---|---|
| **From:** | John Endres |
| **To:** | Morissette, Rachel |
| **Cc:** | Kayla Preece; Amy Clewell; Jared Brodin |
| **Subject:** | Re: GRN 906 and 907: AIBMR Response and Follow-up Question |
| **Date:** | Tuesday, May 19, 2020 3:48:51 PM |
| **Attachments:** | image001.png<br>sugawara.pdf<br>Polocki 2016.pdf |

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Dear Dr. Morissette,

Thanks very much for all that you do with such limited resources. I feel like there must be some misunderstanding and I am very sorry if we did not make it clear that we are suggesting that the client severely restrict the exposure as follows:

1. Oil Nutra: 70 mg MAX EDI (10.5 mg sphingolipids (SL)., 10.5 mg DGDG)
2. Powder Nutra: 30 mg MAX EDI (15 mg spingolipids, 12 mg DGDG)

I am attaching (2) two papers that highlight the wide range of commonly consumed foods that humans eat that contain SL and DGDG.
For example (1) one cup of skimmed milk contains 15 mg of SL. Buttermilk contains even more and it is found in yogurt, cheese, whole milk, etc.

DGDG is also found fairly ubiquitously in the vegetable kingdom in grains and many fruits and vegetable in very appreciable amounts. For example 1/8 c of pumpkin purée (think pumpkin pie and muffins) has 12 mg DGDG. (see attached paper).

It seems that the 28-day study may be pivotal if published because the following statement seems not to be the case at this severely restricted exposure (per above); "A 28-day study cannot replace the need for a 90-day study on such an ingredient that has not been consumed at the proposed level."

We were thinking a 1000-fold uncertainty factor using the NOAEL from the 28-day study of 2000 mg/kg bw/day to be ultra conservative. (2000 mg/kg bw/day) / 1000 = 2 mg/kg bw/day in humans or 140 mg/day in a 70 kg human as the ADI (when published). Since the EDI would be 70 mg/day this would be an additional significant margin of safety.

As you probably know, we have been meeting with and submitting GRAS Notifications to OFAS for well over a decade with 100% success in getting the "FDA has no questions" letter. This is why I believe there has been some significant misunderstanding. Again, we apologize if we caused unnecessary confusion.

We very much appreciate your response and thank you for your valuable time (please see further comments below).

Best Regards,

**John R. Endres, ND**
*Chief Scientific Officer*

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On Tue, May 19, 2020 at 6:52 AM Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

> Dear Kayla,
>
> I spoke with our toxicologists about your proposal below and they provided the following:
>
> A 28-day study cannot replace a 90-day study for an ingredient that has not been used before at the proposed level. As discussed below, there are various reasons why a 90-day study is required, at a minimum:
>
> 1. Although the ingredient is derived from wheat, if you consider per capita wheat consumption, along with the fact that this ingredient is a minor fraction of the whole plant grain (1.5-2.5%), the normal exposure to this ingredient through daily consumption of wheat is really low (mg/person/day). Per above, this extends far beyond wheat in the daily diet with a very long (thousands of years) history of human exposure.
> 2. Because toxicity is a function of dose (exposure), something that is normally innocuous at a lower level of exposure may turn out to be not so innocuous when a whole lot of it is consumed. I think there was a misunderstanding as the suggested new exposure would be 70 mg/d for the oil and 30 mg/d for the powder, which doesn't seem like much at all compared to the typical daily diet.
> 3. A 28-day study cannot replace the need for a 90-day study on such an ingredient that has not been consumed at the proposed level. We understand this, but since there is such an extensive history of human exposure for thousands of years and a presumption of safety, with a 1000-fold uncertainty factory and such low EDis, it would seem like the 28-day study could now be considered pivotal for the ADI after publication in a peer-reviewed journal specializing in toxicology.
> 4. Based on the findings of the 28-day study, some of the effects that have been discounted as toxicologically not important may turn out to be toxicologically relevant. We will not know that without the longer study. Since there is such a long history of human exposure to a fairly significant amount of these substances and because they are widespread in the human diet, we don't anticipate any additional findings in a longer study. Quite similarly to GRN 773 that we submitted for a never before consumed algae based upon a 28-day study (NOAEL = 4000 mg/kg bw/day) allowing 2.8 g/day using a 100-fold

uncertainty factor. Received no objection to replace up to 100% of the protein in a typical human diet (i.e. 100 g) which is 250 g of the algae.

5. Because of the distribution of sphingolipids in the body, and because of the reports of sphingolipid-induced circulating factors associated with obesity (*e.g.*, saturated fatty acids, inflammatory cytokines), one would expect that the histopathological investigation in a 90-day study would, in addition to other organs, pay special attention to the brain and also determine the inflammatory cytokine status in the blood. If this were a problem at levels currently in the diet we would all be in trouble. Perhaps you were calculating based upon the original proposed exposure rather than the now severely restricted exposure?
6. Under such conditions, applying a higher safety factor is not logical because we do not know how much the alternative safety factor should be. Perhaps this could be reconsidered per the above toxicological perspectives and risk reduction.

I hope this information if helpful.

Best regards,

*Rachel*

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**Rachel Morissette, Ph.D.**
*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**
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

**From:** Kayla Preece <kayla@aibmr.com>
**Sent:** Monday, May 18, 2020 3:52 PM
**To:** Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
**Cc:** Amy Clewell <amy@aibmr.com>; John Endres <john@aibmr.com>
**Subject:** GRN 906 and 907: AIBMR Response and Follow-up Question

Dear Dr. Morissette,

Thank you very much for your email and the opinion that lowering the exposure will not be useful at this point. We are thinking that the conference call on the 27th will likely not be necessary now, but we do have one last question for you that will help us make our final decision. As the 28-day repeated dose study in rats has already been performed (but is unpublished at the moment), we would appreciate your opinion about one additional strategy. What if Seppic were to publish the 28-day study, and then, as discussed by phone, use a higher uncertainty factor (higher than 100) to determine an ADI? This would of course be in addition to lowering the exposure such that the EDI would be less than the ADI.

We truly appreciate your time and consideration in this matter, and look forward to hearing your thoughts.

--

Best regards,
Kayla
---
**Kayla Preece, ND**

*Scientific and Regulatory Consultant*

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| | |
|---|---|
| **From:** | Kayla Preece |
| **To:** | Morissette, Rachel |
| **Cc:** | West-Barnette, Shayla; Honigfort, Mical; John Endres; Amy Clewell; Jared Brodin |
| **Subject:** | FDA GRN 906 and 907-Response to FDA questions |
| **Date:** | Tuesday, July 14, 2020 2:03:45 PM |
| **Attachments:** | Response to FDA Round II GRN 906 and 907 questions FINAL 2020-07-14 comments.docx |

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Hello Dr.Morissette-

We received confirmation from our client sooner than we anticipated and have the responses to the FDA's questions regarding GRNs 906 and 907 now ready. Please find the attachment below. Thank you for your assistance through this process.

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Best regards,
Kayla
---
**Kayla Preece, ND**

*Scientific and Regulatory Consultant*

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Dear Dr. Morissette,
Please find our responses to FDA Round II GRN 906 and 907 questions (2020-06-30) in red below:

In response to Question 2:

- SEPPIC provided references for updated versions of EN ISO 3960-06/2010, NF EN ISO 662-02/2001, and NF ISO 21528.1-12/2014 but not for NF EN ISO 660-09/2009. Please provide a reference for an updated version of NF EN ISO 660-09/2009 or explain why a method that has been withdrawn was used.

Seppic utilizes the NF EN ISO standard listed on the French Agency for Normalization (AFNOR) (https://www.boutique.afnor.org/norme/nf-en-iso-660/corps-gras-d-origines-animale-et-vegetale-determination-de-l-indice-d-acide-et-de-l-acidite/article/687231/fa158625) which has not updated NF EN ISO 660-09/2009 and therefore it remains the utilized method of Seppic.

In response to Questions 7 and 8:

- SEPPIC provided estimates of dietary exposure at the 90$^{th}$ percentile based on lifetime exposure. Please provide estimates at the 90$^{th}$ percentile that are not based on lifetime exposure.

The daily average exposure estimate assessments performed using Creme Global software with regard to the new conditions of intended use are as follows:

For GRN 906, the 90$^{th}$ percentile daily average exposure estimate from use in functional beverages is 39.8 mg/day (0.79 mg/kg bw/day).

For GRN 907, the 90$^{th}$ percentile daily average exposure estimate from use in bars is 100 mg/day (1.7 mg/kg bw/day).

These daily average exposure estimates are greater than the lifetime/usual estimates of 30 mg/day for GRN 906 and 70 mg/day for GRN 907 that were given in the last response to FDA, which is not unusual. However, lifetime/usual exposure estimates are widely accepted, and are considered to represent a more realistic daily intake estimate over the lifetime as opposed to the daily average estimates above.[1-3]

- SEPPIC provided food codes used to estimate dietary exposure to wheat seed powder from its uses in functional beverages. Based on the selected food codes, we assume that the intended use is limited to functional beverages that are fruit/vegetable juice drinks and that are either considered to be energy drinks or contain high levels of added vitamin C. Please confirm that this is a correct assumption. In addition, please clarify if wheat seed oil is intended for use in all bars or in a subset of bars.

The wheat seed oil is intended for use in “functional bars”, and as there are no specific food codes in NHANES data for such bars, all NHANES food codes for bars were used in the exposure estimate assessment in order to get a broad estimate of potential exposure from the ingredient in bars.

The wheat seed powder is intended for use in “functional beverages”, and similarly there are few specific food codes in NHANES data for such beverages. Thus, various beverage codes for drinks that contain a “functional ingredient” (e.g. vitamin C) were chosen for the exposure estimate to represent consumption of this type of beverage. The wheat seed powder will be limited to use in beverages that are generally fruit/vegetable juices, but not specifically to those that contain vitamin C or are considered energy drinks. Again, as there are no current perfect food code choices in NHANES for drinks that contain “wheat seed powder”, the food codes chosen for the estimates merely represent surrogate

beverages that contain functional ingredients, and aren't necessarily considered identical to the functional beverages that wheat seed powder will be added to.

## References


1. Hoffmann K, Boeing H, et al. Estimating the distribution of usual dietary intake by short-term measurements. *Eur J Clin Nutr*. 2002;56 Suppl 2:S53-62
2. Food And Nutrition Board and Institute of Medicine. Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. 1997. 1-454.
3. National Center for Health Statistics (NCHS). NHANES Dietary Web Tutorial. Modeling Usual Intake Using Dietary Recall Data. Task 2: Describing Statistical Methods that Have Been Used to Estimate the Distribution of Usual Intake with a Few Days of 24-hour Recalls. Key Concepts about Statistical Methods that have been used to Estimate the Distribution of Usual Intake with a Few Days of 24-hour Recalls. [2011] from https://www.cdc.gov/nchs/tutorials/dietary/Advanced/ModelUsualIntake/Info2.htm.

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|---|---|
| **From:** | Kayla Preece |
| **To:** | Morissette, Rachel |
| **Cc:** | West-Barnette, Shayla; Honigfort, Mical; John Endres; Amy Clewell; Jared Brodin |
| **Subject:** | FDA GRN 906 and 907-Response to FDA questions |
| **Date:** | Tuesday, July 14, 2020 2:03:45 PM |
| **Attachments:** | Response to FDA Round II GRN 906 and 907 questions FINAL 2020-07-14 comments.docx |

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Best regards,
Kayla
---
**Kayla Preece, ND**

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beverages that contain functional ingredients, and aren't necessarily considered identical to the functional beverages that wheat seed powder will be added to.

## References


1. Hoffmann K, Boeing H, et al. Estimating the distribution of usual dietary intake by short-term measurements. *Eur J Clin Nutr*. 2002;56 Suppl 2:S53-62
2. Food And Nutrition Board and Institute of Medicine. Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. 1997. 1-454.
3. National Center for Health Statistics (NCHS). NHANES Dietary Web Tutorial. Modeling Usual Intake Using Dietary Recall Data. Task 2: Describing Statistical Methods that Have Been Used to Estimate the Distribution of Usual Intake with a Few Days of 24-hour Recalls. Key Concepts about Statistical Methods that have been used to Estimate the Distribution of Usual Intake with a Few Days of 24-hour Recalls. [2011] from https://www.cdc.gov/nchs/tutorials/dietary/Advanced/ModelUsualIntake/Info2.htm.

| | |
|---|---|
| **From:** | Kayla Preece |
| **To:** | Morissette, Rachel |
| **Cc:** | John Endres; Amy Clewell; Jared Brodin |
| **Subject:** | Seppic: GRN 906: additional question for GRN 906 |
| **Date:** | Thursday, August 20, 2020 6:38:47 PM |
| **Attachments:** | image001.png<br>Response to FDA Round III GRN 906 questions FINAL 2020-08-20 comments.pdf |

Hello Dr. Morissette-

Please find the attached document addressing your question.

Best regards,
Kayla
---
**Kayla Preece, ND**

*Scientific and Regulatory Consultant*

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On Thu, Aug 20, 2020 at 8:32 AM Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

> Dear Dr. Preece,
>
> In your amendment dated July 14, 2020, you stated that the intended use of wheat seed polar lipids is limited to “functional beverages” and explained that these beverages are generally fruit/vegetable juices containing a “functional ingredient.” We note that some of the eight food codes chosen to estimate dietary exposure represent fruit/vegetable juice drinks, but none of them represents a fruit/vegetable juice. Please confirm that the ingredient is not intended for use in fruit/vegetable juices. We note that if the ingredient is intended for use in juices, the dietary exposure to the ingredient needs to be reassessed to include fruit/vegetable juices. In addition, please provide a statement that the ingredient will not be used in juices for which a standard of identity may preclude its use.
>
> Best regards,
>
> Rachel

---

**Rachel Morissette, Ph.D.**
*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**
rachel.morissette@fda.hhs.gov

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Best regards,
Kayla
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**Kayla Preece, ND**

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August 20, 2020

Dear Dr. Morissette,
Please find our responses to FDA Round III GRN 906 questions in red below:

FDA's question:

In your amendment dated July 14, 2020, you stated that the intended use of wheat seed polar lipids is limited to "functional beverages" and explained that these beverages are generally fruit/vegetable juices containing a "functional ingredient." We note that some of the eight food codes chosen to estimate dietary exposure represent fruit/vegetable juice drinks, but none of them represents a fruit/vegetable juice. Please confirm that the ingredient is not intended for use in fruit/vegetable juices. We note that if the ingredient is intended for use in juices, the dietary exposure to the ingredient needs to be reassessed to include fruit/vegetable juices. In addition, please provide a statement that the ingredient will not be used in juices for which a standard of identity may preclude its use.

Thank you for catching this--indeed FDA is correct in their assumption. We should have stated that the intended use is limited to beverages that are generally fruit/vegetable juice **drinks** that contain a functional ingredient, NOT fruit/vegetable juices that contain a functional ingredient. The ingredient will not be used specifically in fruit/vegetable juices, or any other food, for which a standard of identity may preclude its use.

Please don't hesitate to let us know if you have any other questions.

Two publications have been removed in accordance with copyright laws. The removed reference citations are

S. POTOČKI: Potential health benefits of sphingolipids in milk and dairy products, *Mljekarstvo 66* (4), 251-261 (2016) 251

Sugawara, T., Miyazawa, T. Separation and determination of glycolipids from edible plant sources by high-performance liquid chromatography and evaporative light-scattering detection. *Lipids* **34,** 1231 (1999). https://doi.org/10.1007/s11745-999-0476-3