PHARMANUTRA

24 November 2021

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

Dear Dr. Gaynor:

Re: GRAS Notice for Cetylated Fatty Acids

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Pharmanutra S.p.A, as the notifier, is submitting one hard copy and one electronic copy (on CD) of all data and information supporting the company's conclusion that cetylated fatty acids is GRAS on the basis of scientific procedures, for use in in various conventional food and beverage products; these food uses of cetylated fatty acids are therefore not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act. Information setting forth the basis for Pharmanutra's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Germano Tarantino
Chief Scientific Officer
Pharmanutra S.p.A.

GRAS NOTICE FOR CETYLATED FATTY ACIDS

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

SUBMITTED BY:
Pharmanutra S.p.A.
Via Delle Lenze 216/b
56122 Pisa
Italy

DATE:
24 November 2021

GRAS Notice for Cetylated Fatty Acids

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GRAS Notice for Cetylated Fatty Acids

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Pharmanutra S.p.A (Pharmanutra) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of cetylated fatty acids, as manufactured by Pharmanutra, in various conventional food and beverage products as described in Section 1.3 below, are not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Pharmanutra's view that these notified uses of cetylated fatty acids are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Pharmanutra, the undersigned hereby certifies that all data and information presented in this notice represents a complete and balanced submission that is representative of the generally available literature. Pharmanutra considered all unfavorable as well as favorable information that is publicly available and/or known to Pharmanutra and that is pertinent to the evaluation of the safety and GRAS status of cetylated fatty acids as a food ingredient for addition to various conventional food and beverage products, as described herein.

Signed,

Germano Tarantino
Chief Scientific Officer
Pharmanutra S.p.A.
Via Delle Lenze 216/b
56122 Pisa
Italy

Date

1.1 Name and Address of Notifier

Pharmanutra S.p.A.
Via Delle Lenze 216/b
56122 Pisa
Italy

1.2 Common Name of Notified Substance

Common or Usual Name:	Cetylated fatty acids
Chemical name:	Fatty acids, C8-C18 and C18-unsatd., C12-18 alkyl esters/refined olive oil
Synonyms and abbreviations:	Refined olive oil/cetyl myristate/cetyl oleate; Cetylated fatty acid esters; Cetilmeristic oil; Paryol Cetmirol CETILAR R.M.
Trade name:	LIPOCET®

1.3 Conditions of Use

Cetylated fatty acids is intended for use as a source of dietary fatty acids by adults in the general population. The ingredient is not intended for consumption by infants or young children, nor is it intended for use in infant formula or products under the jurisdiction of the U.S. Department of Agriculture. Cetylated fatty acids is intended to be added as an ingredient to ice cream, energy or protein bars, meal replacement bars, drinkable yogurts, flavored milk, milk drinks and mixes, milk-based meal replacement, nutritional and protein beverages, and plain or flavored yogurt. A summary of the food categories and use levels in which cetylated fatty acids is intended for use is provided in Table 1.3-1 below. Food uses are organized according to 21 CFR §170.3 (U.S. FDA, 2021).

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for Cetylated Fatty Acids in the U.S.

Food Category (21 CFR 170.3) (U.S. FDA, 2021)	Food Uses	Cetylated Fatty Acids Level (g/serving)	RACC[a] (g or mL)	Cetylated Fatty Acids Use Levels (g/100 g or mL)
Frozen Dairy Desserts	Ice Cream	0.3	130 (or 2/3 cup)[b]	0.23
Grain Products and Pastas	Energy Bars or Protein Bars	1.0	40	2.50
	Meal Replacement Bars	1.0	40	2.50
Milk Products	Drinkable Yogurts	1.0	93 to 207[c]	1.08
	Flavored Milk, Milk Drinks, and Mixes	0.3	240	0.13
	Milk-Based Meal Replacement, Nutritional, and Protein Beverages	1.0	240	0.42
	Plain or Flavored Yogurt	0.3	170	0.18

CFR = *Code of Federal Regulations*; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.
[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2021). RACCs are included for reference, however the assessment was conducted with use levels expressed on a g/100 g basis.
[b] Calculated based on food item density using unit converter (https://www.aqua-calc.com/calculate/food-volume-to-weight).
[c] RACC has not been established for yogurt drinks; however, an approximate serving size was established based on products currently on the U.S. market.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2021), Pharmanutra has concluded that the intended uses of cetylated fatty acids as described herein are GRAS on the basis of scientific procedures.

This GRAS evaluation has used data pertaining to the safety of cetylated fatty acids which are generally available in the public domain. All information presented within this Notification was reviewed by a panel of experts (GRAS Panel) who are qualified in their field by scientific training and experience to evaluate the safety of cetylated fatty acids as a component of food. The GRAS Panel concurred with Pharmanutra GRAS evaluation of cetylated fatty acids and the consensus statement of the GRAS Panel is provided in Appendix A entitled "*GRAS Panel Evaluation of Cetylated Fatty Acids for Use as a Food Ingredient*".

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Pharmanutra S.p.A.
Via Delle Lenze 216/b
56122 Pisa
Italy

Should the FDA have any questions or additional information requests regarding this Notification, Pharmanutra will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

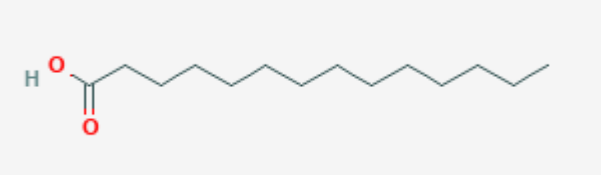
It is Pharmanutra's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

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

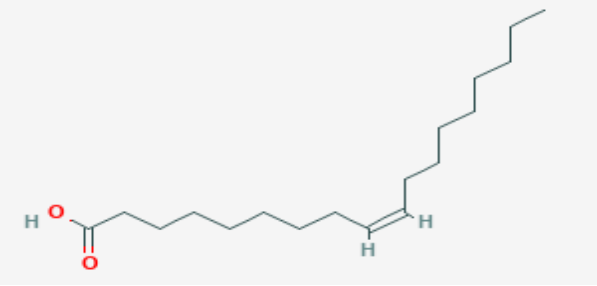
2.1 Identity

Chemical name:	Fatty acids, C8-C18 and C18-unsatd., C12-18 alkyl esters/refined olive oil
Chemical Abstracts Service (CAS) number:	Cetylated fatty acids: 90990-24-2/8001-25-0 Myristic acid: 544-63-8 Oleic acid: 112-80-1
Synonyms and abbreviations:	Refined olive oil/cetyl myristate/cetyl oleate; Cetylated fatty acid esters; Cetilmeristic oil; Paryol Cetmirol CETILAR R.M.
Trade name:	LIPOCET®
Molecular and structural formulae; stereochemistry:	Cetylated fatty acids: Not applicable (structures for the major fatty acid components provided below)

Myristic acid: $C_{14}H_{28}O_2$



Oleic acid: $C_{18}H_{34}O_2$



Molecular mass (Da):	Cetylated fatty acids: Not applicable (molecular masses of major fatty acid components provided below) Myristic acid: 228.3732 Oleic acid: 282.465
Physicochemical properties:	Pale yellow solid (25°C)
Solubility:	Insoluble in water; soluble in oils
Melting point:	47°C

2.2 Manufacturing

2.2.1 Raw Materials

The raw materials and processing aids used in the production of cetylated fatty acids are detailed in Table 2.2.1-1 along with their respective chemical formulae and CAS numbers, where applicable. Palm seed oil and vegetable-derived fatty acids are used as raw materials in the production of cetylated fatty acids (see Appendix B for declaration of origin of raw materials). All raw materials and processing aids used in the production of cetylated fatty acids are food-grade.

Table 2.2.1-1 Raw Materials and Processing Aids Used in the Production of Cetylated Fatty Acids

Material	Chemical Formula	CAS Number	Purpose
Cetyl alcohol (1-Hexadecanol)	$C_{16}H_{34}O$	36653-82-4	Cetylated fatty acid synthesis
Refined olive oil	NA	8001-25-0	
Myristic acid (99%)	$C_{14}H_{28}O_2$	544-63-8	
Vegetable oleic acid (78%)	$C_{18}H_{34}O_2$	112-80-1	
Zinc powder	Zn	7440-66-6	Catalyst
Mono t-butyl hydroquinone (MTBHQ)	$C_{10}H_{14}O_2$	1948-33-0	Antioxidant
Activated carbon	NA	NA	Decolorizer

CAS = Chemical Abstracts Service; NA = not applicable.

2.2.2 Manufacturing Process

Cetylated fatty acids is manufactured in accordance with current Good Manufacturing Practice (cGMP) in a facility that is accredited with International Organization for Standardization (ISO) 9001:2015, 22000:2005, 14001:2015, and 18001:2007. It is not anticipated that any undesirable substances will arise from the production process. The manufacturing process does not use any extraction solvents and includes heating and filtration steps to remove any potential contaminants. Furthermore, all starting materials used in the production of cetylated fatty acids are food-grade. A schematic overview of the manufacturing process is presented as Figure 2.2-1.

The manufacturing process begins with the synthesis of the cetyl ester. Cetyl alcohol and myristic acid are dissolved and mixed in a 600 kg tank at 60°C. After they are dissolved, the mixture is pumped into a reactor and placed under vacuum for approximately 3 hours. During this time, zinc powder is added as a catalyst,

and the reaction yields cetyl myristate. The quantities of cetyl alcohol and myristic acid used to produce cetyl myristate are 52.2% and 47.8%, respectively. Cetyl alcohol and oleic acid with zinc powder as a catalyst are combined in a similar manner to produce cetyl oleate. The quantities of cetyl alcohol and oleic acid used to produce cetyl oleate are 46.9% and 53.1%, respectively.

Cetyl myristate and cetyl oleate are loaded into a mixer and filled with nitrogen to bring the mixer to atmospheric pressure. A nitrogen flow of 0.5 m^3/hour is maintained to facilitate the removal of water and to create an inert environment. The mixer is activated and heated to 160°C over the course of 4 hours and is kept at this temperature for 1 hour. The temperature is then gradually increased to 220°C over the course of 3 hours. Periodically (approximately every 2 hours) the acidity is measured, and when it is between 5 and 8 mg potassium hydroxide (KOH)/g, the reaction is considered to have stopped (this takes approximately 8 hours). The flow of nitrogen is stopped and vacuum is applied (50 mbar of residual pressure), allowing the reaction to continue for approximately 2 hours until the acidity is 4 mg KOH/g. The reaction is then considered complete, the vacuum pump is switched off, and the system is filled with nitrogen to return to atmospheric pressure.

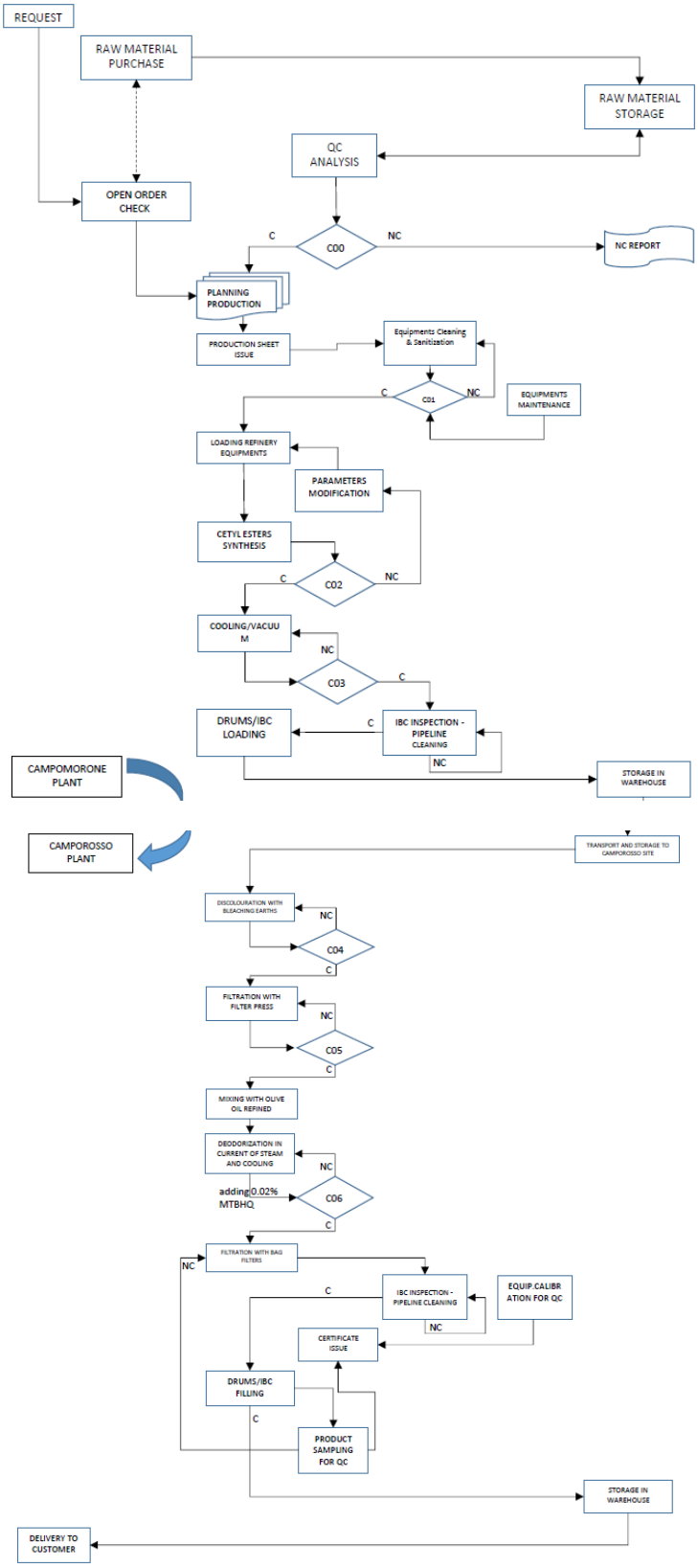
To prevent the synthesized esters from solidifying, the system is cooled with 90°C water and is eventually filtered prior to transport to the refinery.

At the refinery, the synthesized esters are transferred using vacuum to a 6,000 L decolorizer and they are heated to 90°C. A set quantity of activated carbon (typically approximately 0.5% each of Acticarbone and Filtercarb PHA, but this may be adjusted based on analyzed acidity and color) is added under vacuum while the temperature increases from 100 to 110°C; note that the activated carbon is referred to as “bleaching earth” in Figure 2.2-1. Decolorization takes approximately 30 minutes, at which point the vacuum pump stops, and the system is filled with nitrogen so that the decolorized ester is pushed into the filter press, before the cetyl esters are collected in an attached tank.

In approximate quantities of 75% refined cetyl esters and 25% olive oil, this mixture is placed under high vacuum (3 to 5 mbar), the temperature is increased to 180°C, and the product is deodorized for 2 to 3 hours in a flow of steam. After the deodorization period, the steam is turned off and the mixture is cooled with water down to 60°C. The antioxidant mono t-butyl hydroquinone (equal to 0.02% of the total quantity of the fatty acid-based product) is suctioned into the deodorizer using vacuum and mixed using nitrogen to yield the final product.

The refined ester is transferred through a sock filter into a 25 kg steel container and is stored at 10 to 15°C.

Figure 2.2-1 Schematic Overview of the Manufacturing Process for Cetylated Fatty Acids



2.3 Product Specifications and Batch Analyses

2.3.1 Specifications

The product specifications for cetylated fatty acids are presented in Table 2.3.1-1. The specification parameters are assessed using internationally recognized methods or internally developed and validated methods. Parameters for physical and compositional characteristics of the ingredient, as well as appropriate limits for microbiological parameters and heavy metals, have been established to ensure the quality of the ingredient. Aluminum was included as a specification and analyzed, as the ingredient is stored in aluminum boxes.

Table 2.3.1-1 Proposed Specifications for Cetylated Fatty Acids

Parameter	Specification	Method of Analysis
Physical status at 25°C	Solid (waxy flakes)	Visual
Color (APHA color)	≤600 (beige to light yellow)	AOCS Ea9-65
Acid value (mg KOH/g)	≤5	AOCS Cd3d-63
Iodine value (g I_2/100)	30 to 50	AOCS Cd1-25
Saponification value (mg KOH/g)	130 to 150	AOCS Cd3-25
Hydroxyl value (mg KOH/g)	≤20	AOCS Cd13-60
Ester content (%)	70 to 80	GC-FID
Cetyl oleate (%)	22 to 30	GC-FID
Cetyl myristate (%)	41 to 56	GC-FID
Aluminum (ppm)	≤2	ICP-MS
Mono t-butyl hydroquinone (%)	≤0.02	FCC
Heavy Metals		
Arsenic (ppm)	<0.1	ICP-MS
Lead (ppm)	<0.1	ICP-MS
Microbiological Criteria		
Total aerobic microbial count (CFU/g)	≤10,000	ISO 4833-1:2013
Yeasts and molds (CFU/g)	≤100	ISO 21527-1:2008

AOCS = American Oil Chemists' Society; APHA = American Public Health Association; CFU = colony-forming units; FCC = Food and Chemicals Codex; GC-FID = gas chromatography with flame ionization detection; ICP-MS = inductively-coupled plasma mass spectroscopy; ISO = International Organization for Standardization; KOH = potassium hydroxide; ppm = parts per million.

2.3.2 Batch Analysis

Batch analysis of 5 independent representative lots of cetylated fatty acids are presented below in Table 2.3.2-1. The data demonstrate that the manufacturing process results in a consistent final ingredient that meets the established specifications.

Table 2.3.2-1 Analytical Data for 5 Independent Representative Batches of Cetylated Fatty Acids

Parameter	Method of Analysis	Specification Limit	Batch Number				
			1090l19/01	1090l19/02	1090l19/03	1090l19/04	1090l19/05
Physical status at 25°C	Visual	Solid (waxy flakes)	Pass	Pass	Pass	Pass	Pass
Color (APHA color)	AOCS Ea9-65	≤600 (beige to light yellow)	<600 (beige to light yellow)	<600 (beige to light yellow)	<600 (beige to light yellow)	<600 (beige to light yellow)	<600 (beige to light yellow)
Acid value (mg KOH/g)	AOCS Cd3d-63	≤5	0.8	0.7	0.7	0.4	0.8
Iodine value (g I_2/100)	AOCS Cd1-25	30 to 50	30.3	30.5	32.2	30.1	30.1
Saponification value (mg KOH/g)	AOCS Cd3-25	130 to 150	134.4	138.2	138.6	141.3	136.6
Hydroxyl value (mg KOH/g)	AOCS Cd13-60	≤20	6.3	7.1	6.0	4.1	7.4
Ester content (%)	GC-FID	70 to 80	75.14	74.38	74.23	72.19	73.49
Cetyl oleate (%)	GC-FID	22 to 30	23.95	23.80	23.77	23.10	23.48
Cetyl myristate (%)	GC-FID	41 to 56	49.37	48.82	48.69	47.39	48.25
Aluminum (ppm)	ICP-MS	≤2	1.57	1.26	1.03	1.49	1.70

AOCS = American Oil Chemists' Society; APHA = American Public Health Association; GC-FID = gas chromatography with flame ionization detection; ICP-MS = inductively coupled plasma mass spectrometry; KOH = potassium hydroxide; ppm = parts per million.

2.3.3 Microbiological Analysis

Analyses of 5 independent representative lots of cetylated fatty acids are presented below in Table 2.3.3-1. The data demonstrate that microbiological contaminants are consistently low and below the proposed microbiological specifications.

Table 2.3.3-1 Microbiological Analyses of 5 Independent Representative Lots of Cetylated Fatty Acids

Parameter	Method of Analysis	Specification	Batch Number				
			1090l19/01	1090l19/02	1090l19/03	1090l19/04	1090l19/05
Total aerobic microbial count (CFU/g)	ISO 4833	<10,000	<1,000	<100	<1,000	1,000	1,000
Escherichia coli (negative/g)	ISO 16649-2	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella (negative/25 g)	ISO 21528-1/2	Negative	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus (negative/g)	ISO 6888-1	Negative	Negative	Negative	Negative	Negative	Negative
Yeasts and molds (CFU/g)	ISO 21527-1/2	<100	<100	<10	<100	<100	<100
Enterobacter (CFU/g)	ISO 6579	<100	<100	<100	<100	<100	<100

CFU = colony-forming units; ISO = International Organization for Standardization.

2.3.4 Heavy Metals Analysis

A summary of the heavy metals results for 3 independent representative lots of cetylated fatty acids is presented below in Table 2.3.4-1.

The results demonstrate that heavy metals in cetylated fatty acids are consistently well below proposed specifications and the limits established by the Codex Alimentarius (CODEX, 2019). It was therefore deemed unnecessary to include additional heavy metal specifications in the ingredient specifications.

Table 2.3.4-1 Heavy Metal Results for 3 Independent Representative Lots of Cetylated Fatty Acids

Parameter	Specification	Codex Alimentarius Limit as a Reference[a]	Batch Number			Method of Analysis
			15F16411	19M19907	2007310-001	
Arsenic (ppm)	0.1	0.1	<0.005	NT	<0.02	ICP-MS
Cadmium (ppm)	NA	0.4	<0.005	0.008	<0.02	
Mercury (ppm)	NA	0.1	<0.005	<0.005	<0.02	
Lead (ppm)	0.1	0.1	0.008	<0.005	<0.02	
Nickel (ppm)	NA	NA	0.020	NT	NT	

ICP-MS = inductively coupled plasma mass spectrometry; NA = not applicable; NT = not tested; ppm = parts per million.
[a] Maximum levels according to CODEX Stan 193-1995: for arsenic (edible fats and oils), cadmium (rice, polished), mercury (salt, food grade), lead (edible fats and oils).

2.3.5 Additional Product Analyses

2.3.5.1 Fatty Acid Analysis

The total fatty acid composition of cetylated fatty acids is presented in Table 2.3.5.1-1.

Table 2.3.5.1-1 Cetylated Fatty Acids Total Fatty Acid Composition

Fatty Acid	Results
Oleic acid (%)	45.98
Myristic acid (%)	40.96
Linoleic acid (%)	7.97
Palmitic acid (%)	3.24
Stearic acid (%)	0.80
Palmitoleic acid (%)	0.44
Eicosenoic acid (%)	0.30
Lauric acid (%)	0.21
Eicosanoic acid (%)	0.11

In addition, triglycerides from the same 5 independent representative batches analyzed in Section 2.3.2 were determined using gas chromatography with flame ionization detection (GC-FID). Analytical data expressed as a relative percentage have been summarized in Table 2.3.5.1-2. The data demonstrate that the manufacturing process employed by Pharmanutra reliably produces cetylated fatty acids that has a consistent triglyceride composition.

Table 2.3.5.1-2 Triglyceride Analysis for 5 Independent Representative Batches of Cetylated Fatty Acids

Parameter	Batch Number				
	1090l19/01	1090l19/02	1090l19/03	1090l19/04	1090l19/05
Total triglycerides (%)	22.83	23.81	24.82	24.85	22.84

2.3.5.2 Other Potential Contaminants of Fats and Oils

Analysis of [3-monochloropropanediol (3-MCPD)] and glycidyl fatty acid esters in 5 independent representative batches of cetylated fatty acids, and of dioxins and polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and erucic acid, including erucic acid bound in fat in 1 representative batch of cetylated fatty acids, has been conducted. The results and European Union (EU) maximum levels (in absence of specific limits in the U.S.) for these contaminants in final food products, taken from the closest relevant food categories detailed in Commission Regulation (EC) No 1881/2006, as amended (EC, 2006), are presented in Tables 2.3.5.2-1 and 2.3.5.2-2.

The results show that amounts of all of these potential contaminants in cetylated fatty acids are far below the EU limits, which is justification for why they are not required in the specifications for cetylated fatty acids.

Table 2.3.5.2-1 Results for 3-MCPD and Glycidyl Fatty Acid Esters in 5 Independent Representative Batches of Cetylated Fatty Acids

Parameter	EU Limit as a Reference[a]	Batch Number				
		20E03517	1090l19/02	1090l19/03	1090l19/04	1090l19/05
3-MCPD and glycidyl fatty acid esters						
3-MCPD (µg/kg)	20	<10	-	-	-	-
Sum of 3-monochloropropanediol (3-MCPD) and 3-MCPD fatty acid esters, expressed as 3-MCPD (µg/kg)	1,250	-	210	230	260	240
Glycidyl fatty acid esters expressed as glycidol (µg/kg)	1,000	340	300	400	320	290

3-MCPD = 3-monochloropropanediol; EU = European Union.

[a] Maximum levels according to Commission Regulation (EC) No. 1881/2006 for 3-MCPD (hydrolysed vegetable protein); sum of 3-monochloropropanediol (3-MCPD) and 3-MCPD fatty acid esters, expressed as 3-MCPD (vegetable oils and fats, fish oils and oils from other marine organisms placed on the market for the final consumer or for use as an ingredient in food); and glycidyl fatty acid esters expressed as glycidol (vegetable oils and fats placed on the market for the final consumer or for use as an ingredient in food, with the exception of vegetable oils and fats destined for the production of baby food and processed cereal-based food for infants and young children) (EC, 2006).

Table 2.3.5.2-2 Results for Other Contaminants in an Independent Representative Batch of Cetylated Fatty Acids

Parameter	EU Limit as a Reference[a]	Batch 20E03517
Dioxins and PCBs		
Sum of dioxins (WHO-PCDD/F-TEQ) (pg/g fat)	0.75	0.098
Sum of dioxins and dioxin-like PCBS (WHO-PCDD/F-PCB-TEQ) (pg/g fat)	1.25	0.125
Sum of PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180 (ng/g fat)	40	0.061

Table 2.3.5.2-2 Results for Other Contaminants in an Independent Representative Batch of Cetylated Fatty Acids

Parameter	EU Limit as a Reference[a]	Batch 20E03517
Polycyclic aromatic hydrocarbons		
Benzo(a)pyrene (µg/kg)	2	<0.5
Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene (µg/kg)	10	1.3
Erucic acid, including erucic acid bound in fat		
Erucic acid (g/kg)	20	<0.5

EU = European Union; PCB = polychlorinated biphenyl; WHO-PCDD/F-TEQ = the sum of the toxic equivalencies of the 17 most toxicologically significant dioxins and furans; WHO-PCDD/F-PCB-TEQ = total dioxin equivalency.
[a] Maximum levels according to Commission Regulation (EC) No. 1881/2006 for dioxins and PCBs (vegetable oils and fats), polycyclic aromatic hydrocarbons (oils and fats, excluding cocoa butter and coconut oil); and erucic acid, including erucic acid bound in fat (vegetable oils and fats placed on the market for the final consumer or for use as an ingredient in food, with the exception of camelina oil, mustard oil, and borage oil) (EC, 2006).

2.4 Stability

The stability of cetylated fatty acids was assessed when stored under real-time (25±2°C) conditions for 18 months and under accelerated (40±2°C) conditions for 9 months. The results for 5 independent representative batches are provided in Tables 2.4.1.1-1 to 2.4.3.2-1.

Results for all parameters remained within specifications under all environmental conditions and at all time-points, with no significant changes observed. This demonstrates that cetylated fatty acids is stable for at least 18 months when stored under real-time conditions and for at least 9 months under accelerated conditions.

2.4.1 Physicochemical Stability

2.4.1.1 Real-Time Stability

Table 2.4.1.1-1 Real-time Physicochemical Stability Results for Cetylated Fatty Acids During Storage at 25±2°C for 18 Months

Parameter	Specification	Batch Number	Time (Months)						
			0	1	3	6	9	12	18
Physical status at 25°C	Solid	1090I19/01	Solid	Solid	Solid	Solid	Solid	Solid	Solid
		1090I19/02	Solid	Solid	Solid	Solid	Solid	Solid	Solid
		1090I19/03	Solid	Solid	Solid	Solid	Solid	Solid	Solid
		1090I19/04	Solid	Solid	Solid	Solid	Solid	Solid	Solid
		1090I19/05	Solid	Solid	Solid	Solid	Solid	Solid	Solid
Color (APHA)	≤600	1090I19/01	<600	<600	<600	<600	<600	<600	<600
		1090I19/02	<600	<600	<600	<600	<600	<600	<600
		1090I19/03	<600	<600	<600	<600	<600	<600	<600
		1090I19/04	<600	<600	<600	<600	<600	<600	<600
		1090I19/05	<600	<600	<600	<600	<600	<600	<600

Table 2.4.1.1-1 Real-time Physicochemical Stability Results for Cetylated Fatty Acids During Storage at 25±2°C for 18 Months

Parameter	Specification	Batch Number	Time (Months)						
			0	1	3	6	9	12	18
Color	Beige to light yellow	1090I19/01	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/02	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/03	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/04	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/05	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow

APHA = American Public Health Association.

2.4.1.2 Accelerated Stability

Table 2.4.1.2-1 Accelerated Physicochemical Stability Results for Cetylated Fatty Acids During Storage at 40±2°C for 9 Months

Parameter	Specification	Batch Number	Time (Months)				
			0	1	3	6	9
Physical status at 25°C	Solid	1090I19/01	Solid	Solid	Solid	Solid	Solid
		1090I19/02	Solid	Solid	Solid	Solid	Solid
		1090I19/03	Solid	Solid	Solid	Solid	Solid
		1090I19/04	Solid	Solid	Solid	Solid	Solid
		1090I19/05	Solid	Solid	Solid	Solid	Solid
Color (APHA)	≤600	1090I19/01	<600	<600	<600	<600	<600
		1090I19/02	<600	<600	<600	<600	<600
		1090I19/03	<600	<600	<600	<600	<600
		1090I19/04	<600	<600	<600	<600	<600
		1090I19/05	<600	<600	<600	<600	<600
Color	Beige to light yellow	1090I19/01	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/02	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/03	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/04	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow
		1090I19/05	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow	Beige to light yellow

APHA = American Public Health Association.

2.4.2 Biochemical Stability

2.4.2.1 Real-Time Stability

Table 2.4.2.1-1 Real-time Biochemical Stability Results for Cetylated Fatty Acids During Storage at 25±2°C for 18 Months

Parameter	Specification	Batch Number	Time (Months)						
			0	1	3	6	9	12	18
Acid value (mg KOH/g)	≤5	1090I19/01	0.8	1.1	0.9	0.8	1.6	0.9	1.0
		1090I19/02	0.7	0.9	1.2	1.2	0.5	1.0	1.1
		1090I19/03	0.7	0.9	1.0	0.8	1.2	1.0	1.2
		1090I19/04	0.4	0.9	0.9	1.3	1.1	1.0	1.0
		1090I19/05	0.8	0.9	0.8	0.8	0.5	1.0	1.1
Iodine value (g I_2/100)	30 to 50	1090I19/01	30.3	32.3	31.8	33.5	38.0	33.4	37.9
		1090I19/02	30.5	33.2	33.4	33.7	37.0	34.2	39.0
		1090I19/03	32.2	33.8	32.9	33.3	34.2	34.6	38.7
		1090I19/04	30.1	32.0	33.7	33.9	33.9	34.4	38.3
		1090I19/05	30.1	32.3	32.8	33.3	32.4	33.7	38.9
Saponification value (mg KOH/g)	130 to 150	1090I19/01	134.4	134.4	133.0	132.1	133.3	134.0	139.6
		1090I19/02	138.2	137.5	137.3	141.7	138.0	137.1	141.3
		1090I19/03	138.6	140.6	132.3	144.5	135.2	138.9	138.0
		1090I19/04	141.3	140.7	135.5	145.9	140.0	140.2	140.7
		1090I19/05	136.6	137.4	135.0	148.1	137.6	137.7	139.3
Hydroxyl value (mg KOH/g)	≤20	1090I19/01	6.3	6.8	11.3	5.6	8.3	10.2	17.7
		1090I19/02	7.1	7.1	7.0	8.8	3.0	8.3	15.8
		1090I19/03	6.0	5.7	5.6	7.7	6.3	10.1	9.8
		1090I19/04	4.1	7.7	9.6	6.9	5.7	10.9	16.1
		1090I19/05	7.4	10.6	5.0	7.1	3.3	10.2	16.9
Ester content (%)	70 to 80	1090I19/01	75.14	75.69	76.49	75.73	73.67	72.85	73.95
		1090I19/02	74.38	73.16	74.37	74.44	73.18	71.52	74.37
		1090I19/03	74.23	71.25	74.04	73.30	73.08	72.47	72.20
		1090I19/04	72.19	71.76	72.60	73.06	72.35	71.30	73.13
		1090I19/05	73.49	75.26	75.39	73.91	72.95	73.60	74.52
Palmitoyl oleate (%)	20 to 30	1090I19/01	23.95	24.10	24.20	24.18	23.45	22.95	23.63
		1090I19/02	23.80	23.41	24.98	23.66	23.39	22.64	23.87
		1090I19/03	23.77	22.77	23.53	23.46	23.39	22.85	23.03
		1090I19/04	23.10	22.98	23.09	23.32	23.13	22.60	23.36
		1090I19/05	23.48	24.05	23.87	23.61	23.30	23.40	23.68
Palmitoyl myristate (%)	≥15	1090I19/01	49.37	49.61	50.39	49.65	48.38	48.08	48.48
		1090I19/02	48.82	47.93	48.94	48.91	47.96	47.09	48.64
		1090I19/03	48.69	46.71	48.67	48.02	47.87	47.80	47.36
		1090I19/04	47.39	47.01	47.68	47.92	47.42	46.90	47.93
		1090I19/05	48.25	49.33	49.64	48.45	47.83	48.35	48.96

Table 2.4.2.1-1 Real-time Biochemical Stability Results for Cetylated Fatty Acids During Storage at 25±2°C for 18 Months

Parameter	Specification	Batch Number	Time (Months)						
			0	1	3	6	9	12	18
Aluminum (mg/kg)	≤2	1090I19/01	1.57	0.468	0.268	1.00	0.411	0.413	0.381
		1090I19/02	1.26	0.258	0.490	1.07	1.25	0.352	0.34
		1090I19/03	1.03	0.573	0.461	0.188	1.41	0.608	0.455
		1090I19/04	1.49	0.549	0.269	0.300	0.695	0.824	0.454
		1090I19/05	1.70	0.931	0.440	0.258	0.455	0.771	0.317

KOH = potassium hydroxide.

2.4.2.2 Accelerated Stability

Table 2.4.2.2-1 Accelerated Biochemical Stability Results for Cetylated Fatty Acids During Storage at 40±2°C for 9 Months

Parameter	Specification	Batch Number	Time (Months)				
			0	1	3	6	9
Acid value (mg KOH/g)	≤5	1090I19/01	0.8	1.1	0.9	0.9	1.0
		1090I19/02	0.7	1.0	0.7	0.7	1.1
		1090I19/03	0.7	0.9	0.9	1.0	1.1
		1090I19/04	0.4	1.5	0.9	1.1	1.1
		1090I19/05	0.8	1.0	0.9	1.3	0.5
Iodine value (g I_2/100)	30 to 50	1090I19/01	30.3	33.0	31.9	32.4	39.1
		1090I19/02	30.5	32.7	33.4	33.3	32.0
		1090I19/03	32.2	30.8	33.4	33.9	37.5
		1090I19/04	30.1	31.7	34.0	33.9	34.9
		1090I19/05	30.1	31.8	31.9	33.0	35.9
Saponification value (mg KOH/g)	130 to 150	1090I19/01	134.4	132.0	137.6	146.1	140.4
		1090I19/02	138.2	134.7	141.6	142.1	139.5
		1090I19/03	138.6	138.3	139.5	144.7	141.2
		1090I19/04	141.3	137.9	139.7	147.8	140.3
		1090I19/05	136.6	131.3	137.7	146	137.7
Hydroxyl value (mg KOH/g)	≤20	1090I19/01	6.3	8.2	7.7	12.8	5.4
		1090I19/02	7.1	9.9	4.0	3.8	6.0
		1090I19/03	6.0	7.0	8.6	12.7	5.7
		1090I19/04	4.1	11.1	6.5	10.1	5.6
		1090I19/05	7.4	12.0	7.5	12.1	3.0
Ester content (%)	70 to 80	1090I19/01	75.14	73.97	75.74	75.19	74.48
		1090I19/02	74.38	72.14	73.72	73.68	73.56
		1090I19/03	74.23	72.61	73.50	73.26	73.65
		1090I19/04	72.19	76.69	72.94	72.97	73.33
		1090I19/05	73.49	73.26	75.03	73.97	73.70

Table 2.4.2.2-1 Accelerated Biochemical Stability Results for Cetylated Fatty Acids During Storage at 40±2°C for 9 Months

Parameter	Specification	Batch Number	Time (Months)				
			0	1	3	6	9
Palmitoyl oleate (%)	20 to 30	1090I19/01	23.95	23.47	23.73	23.87	23.65
		1090I19/02	23.80	22.92	23.08	23.35	23.16
		1090I19/03	23.77	23.06	23.14	23.20	23.36
		1090I19/04	23.10	23.00	22.98	23.20	23.24
		1090I19/05	23.48	23.08	23.60	23.50	23.27
Palmitoyl myristate (%)	≥15	1090I19/01	49.37	48.65	50.12	49.44	48.96
		1090I19/02	48.82	47.42	48.81	48.49	48.55
		1090I19/03	48.69	47.74	48.53	48.23	48.43
		1090I19/04	47.39	47.88	48.15	47.94	48.23
		1090I19/05	48.25	48.35	49.56	48.61	48.57
Aluminum (mg/kg)	≤2	1090I19/01	1.57	0.504	0.325	0.433	0.378
		1090I19/02	1.26	0.516	0.476	0.686	0.541
		1090I19/03	1.03	0.573	0.360	0.175	0.977
		1090I19/04	1.49	0.397	0.271	0.292	1.29
		1090I19/05	1.70	0.397	0.466	0.104	0.668

KOH = potassium hydroxide.

2.4.3 Microbiological Stability

2.4.3.1 Real-Time Stability

Table 2.4.3.1-1 Real-time Microbiological Stability Results for Cetylated Fatty Acids During Storage at 25±2°C for 18 Months

Parameter	Specification	Batch Number	Time (Months)						
			0	1	3	6	9	12	18
Total aerobic microbial count (CFU/g)	<10,000	1090I19/01	<1,000	<100	<100	<1,000	<1,000	<100	<1,000
		1090I19/02	<100	<100	<100	<1,000	<1,000	<100	<1,000
		1090I19/03	<1,000	<100	<100	<1,000	<1,000	<100	<1,000
		1090I19/04	1,000	<1,000	<100	<1,000	<1,000	<100	<1,000
		1090I19/05	1,000	<100	<100	<1,000	1,000	<100	<1,000
Escherichia coli (negative/g)	Negative	1090I19/01	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/02	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/03	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/04	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/05	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella (negative/25 g)	Negative	1090I19/01	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/02	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/03	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/04	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/05	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 2.4.3.1-1 Real-time Microbiological Stability Results for Cetylated Fatty Acids During Storage at 25±2°C for 18 Months

Parameter	Specification	Batch Number	Time (Months)						
			0	1	3	6	9	12	18
Staphylococcus aureus (negative/g)	Negative	1090I19/01	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/02	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/03	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/04	Negative	Negative	Negative	Negative	Negative	Negative	Negative
		1090I19/05	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Yeasts and molds (CFU/g)	<100	1090I19/01	<100	<100	<100	<100	<100	<100	<100
		1090I19/02	<10	<100	<100	<100	<100	<100	<100
		1090I19/03	<100	<100	<100	<100	<100	<100	<100
		1090I19/04	<100	<100	<100	<100	<100	<100	<100
		1090I19/05	<100	<100	<100	<100	<100	<100	<100
Enterobacter (CFU/g)	<100	1090I19/01	<100	<100	<10	<10	<100	<100	<100
		1090I19/02	<100	<100	<100	<10	<100	<100	<100
		1090I19/03	<100	<100	<100	<10	<100	<100	<100
		1090I19/04	<100	<100	<100	<10	<100	<100	<100
		1090I19/05	<100	<100	<100	<10	<100	<100	<100

CFU = colony-forming units.

2.4.3.2 Accelerated Stability

Table 2.4.3.2-1 Accelerated Microbiological Stability Results for Cetylated Fatty Acids During Storage at 40±2°C for 9 Months

Parameter	Specification	Batch Number	Time (Months)				
			0	1	3	6	9
Total aerobic microbial count (CFU/g)	<10,000	1090I19/01	<1,000	<100	<100	<1,000	<1,000
		1090I19/02	<100	<100	<1,000	<1,000	<1,000
		1090I19/03	<1,000	<100	<100	<1,000	<1,000
		1090I19/04	1,000	<1,000	<1,000	<1,000	<1,000
		1090I19/05	1,000	<100	<100	<1,000	<10,000
Escherichia coli (negative/g)	Negative	1090I19/01	Negative	Negative	Negative	Negative	Negative
		1090I19/02	Negative	Negative	Negative	Negative	Negative
		1090I19/03	Negative	Negative	Negative	Negative	Negative
		1090I19/04	Negative	Negative	Negative	Negative	Negative
		1090I19/05	Negative	Negative	Negative	Negative	Negative
Salmonella (negative/25 g)	Negative	1090I19/01	Negative	Negative	Negative	Negative	Negative
		1090I19/02	Negative	Negative	Negative	Negative	Negative
		1090I19/03	Negative	Negative	Negative	Negative	Negative
		1090I19/04	Negative	Negative	Negative	Negative	Negative
		1090I19/05	Negative	Negative	Negative	Negative	Negative

Table 2.4.3.2-1 Accelerated Microbiological Stability Results for Cetylated Fatty Acids During Storage at 40±2°C for 9 Months

Parameter	Specification	Batch Number	Time (Months)				
			0	1	3	6	9
Staphylococcus aureus (negative/g)	Negative	1090I19/01	Negative	Negative	Negative	Negative	Negative
		1090I19/02	Negative	Negative	Negative	Negative	Negative
		1090I19/03	Negative	Negative	Negative	Negative	Negative
		1090I19/04	Negative	Negative	Negative	Negative	Negative
		1090I19/05	Negative	Negative	Negative	Negative	Negative
Yeasts and molds (CFU/g)	<100	1090I19/01	<100	<100	<100	<100	<100
		1090I19/02	<10	<100	<100	<100	<100
		1090I19/03	<100	<100	<100	<100	<100
		1090I19/04	<100	<100	<100	<100	<100
		1090I19/05	<100	<100	<100	<100	<100
Enterobacter (CFU/g)	<100	1090I19/01	<100	<100	<100	<10	<100
		1090I19/02	<100	<100	<100	<10	<100
		1090I19/03	<100	<100	<100	<10	<100
		1090I19/04	<100	<100	<100	<10	<100
		1090I19/05	<100	<100	<100	<10	<100

CFU = colony-forming units.

Part 3. §170.235 Dietary Exposure

3.1 Estimated Intake of Cetylated Fatty Acids from Proposed Food Uses

The ingredient cetylated fatty acids is intended for use as a source of dietary fatty acids by adults in the general population. The ingredient is not intended for consumption by infants or young children, nor is it intended for use in infant formula or products under the jurisdiction of the U.S. Department of Agriculture. Dietary exposure to cetylated fatty acids was estimated under the proposed food uses and use levels. Exposure to the primary saturated fatty acids in cetylated fatty acids (myristic acid and palmitic acid) from the consumption of cetylated fatty acids under the intended conditions of use was also estimated.

3.1.1 Methods

An assessment of the anticipated intake of cetylated fatty acids as an ingredient under the intended conditions of use (see Table 1.3-1) was conducted using data available in the 2015-2016 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (USDA, 2019; CDC, 2020a,b).

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2015-2016. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (USDA, 2019; CDC, 2020a,b). The NHANES data were employed to assess the mean and 90th percentile intake of cetylated fatty acids for each of the following population groups:

- Infants and young children, up to and including 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (ages 2 years and older, and both gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of cetylated fatty acids by the U.S. population[1]. Estimates for the daily intake of cetylated fatty acids represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of cetylated fatty acids averaged over all individuals surveyed, regardless of whether they consumed food products in which cetylated fatty acids is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing cetylated fatty acids during the 2 survey days). "Consumer-only" intake

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives, and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

refers to the estimated intake of cetylated fatty acids by those individuals who reported consuming food products in which the use of cetylated fatty acids is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which cetylated fatty acids is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of cetylated fatty acids were generated using the maximum use level indicated for each intended food use, as presented in Table 1.3-1, together with food consumption data available from the 2015-2016 NHANES datasets. The results for these assessments are presented in Section 3.1.2. In addition, a summary of the estimated daily intakes of myristic acid and palmitic acid (the major saturated fatty acids of cetylated fatty acids) was calculated based on the intake estimates of cetylated fatty acids from proposed food uses, and is provided in Section 3.1.3.

3.1.2 Intake Estimates for Cetylated Fatty Acids

A summary of the estimated daily intake of cetylated fatty acids from proposed food uses is provided in Table 3.1.2-1 on an absolute basis (mg/person/day), and in Table 3.1.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers ranged from 36.3 to 64.7% among all age groups evaluated in the current intake assessment (*i.e.*, greater than 36.3% of the population groups consisted of consumers of food products in which cetylated fatty acids is currently proposed for use) (Table 3.1.2-1). The consumer-only estimates are more relevant to risk assessments, as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (2 years and older), the mean and 90th percentile consumer-only intakes of cetylated fatty acids were determined to be 423 and 959 mg/person/day, respectively. Of the individual population groups, male adults had the highest mean and 90th percentile consumer-only intakes of cetylated fatty acids on an absolute basis, at 547 and 1,294 mg/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 211 and 557 mg/person/day, respectively (Table 3.1.2-1).

Table 3.1.2-1 Summary of the Estimated Daily Intake of Cetylated Fatty Acids from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	77	246	36.3	208	211	557
Children	3 to 11	180	467	64.7	751	279	598
Female Teenagers	12 to 19	113	334	42.7	205	264	531
Male Teenagers	12 to 19	165	502	42.9	208	385	764
Female Adults	20 and older	194	576	46.9	924	414	1,060
Male Adults	20 and older	223	576	40.7	716	547	1,294
Total Population	2 and older	196	552	46.3	2,898	423	959

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (2 years and older) mean and 90th percentile consumer-only intakes of cetylated fatty acids were 7 and 16 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 17 and 46 mg/kg body weight/day, respectively. Female teenagers had the lowest mean and 90th percentile consumer-only intakes of 5 and 10 mg/kg body weight/day, respectively (Table 3.1.2-2).

Table 3.1.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Cetylated Fatty Acids from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	6	19	36.4	206	17	46
Children	3 to 11	7	19	64.8	749	11	24
Female Teenagers	12 to 19	2	6	42.6	202	5	10
Male Teenagers	12 to 19	3	8	43.0	208	6	12
Female Adults	20 and older	3	8	47.0	920	6	14
Male Adults	20 and older	3	7	40.2	701	6	16
Total Population	2 and older	3	9	46.3	2,873	7	16

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.1.3 Intake Estimates of Saturated Fatty Acids (Myristic Acid and Palmitic Acid) from Cetylated Fatty Acids

As described in Section 2.3.5, cetylated fatty acids consist of multiple fatty acids, primarily oleic acid (45.98%) and myristic acid (40.96%), with lesser amounts of linoleic acid (7.97%), palmitic acid (3.24%), and other fatty acids (<2%). The U.S. Food and Drug Administration (FDA) has established a daily value of 78 g/day for total fat and 20 g/day for saturated fat (U.S. FDA, 2016). Myristic acid and palmitic acid are the primary saturated fatty acids in cetylated fatty acids; therefore, a summary of the estimated daily intakes of myristic acid and palmitic acid was calculated based on the intake estimates of cetylated fatty acids from proposed food uses, and is provided in Table 3.1.3-1 on an absolute basis (mg/person/day), and in Table 3.1.3-2 on a body weight basis (mg/kg body weight/day).

Table 3.1.3-1 Summary of the Estimated Daily Intake of Myristic Acid and Palmitic Acid from Proposed Food Uses of Cetylated Fatty Acids in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	%	n	Consumer-Only Intake (mg/day)			
				Myristic Acid		Palmitic Acid	
				Mean	90th Percentile	Mean	90th Percentile
Infants and Young Children	0 to 2	36.3	208	86	228	7	18
Children	3 to 11	64.7	751	114	245	9	19
Female Teenagers	12 to 19	42.7	205	108	217	9	17
Male Teenagers	12 to 19	42.9	208	158	313	12	25
Female Adults	20 and older	46.9	924	170	434	13	34
Male Adults	20 and older	40.7	716	224	530	18	42

Table 3.1.3-1 Summary of the Estimated Daily Intake of Myristic Acid and Palmitic Acid from Proposed Food Uses of Cetylated Fatty Acids in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	%	n	Consumer-Only Intake (mg/day)			
				Myristic Acid		Palmitic Acid	
				Mean	90th Percentile	Mean	90th Percentile
Total Population	2 and older	46.3	2,898	173	393	14	31

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Among the total population (2 years and older), the mean and 90th percentile consumer-only intakes of myristic acid were 173 and 393 mg/person/day, respectively, and of palmitic acid were 14 and 31 mg/person/day, respectively. Of the individual population groups, male adults had the highest mean consumer-only intakes of myristic acid and palmitic acid on an absolute basis, at 224 and 18 mg/person/day, respectively. Similarly, male adults were also observed to have the highest 90th percentile consumer-only intakes of myristic acid and palmitic acid at 530 and 42 mg/person/day, respectively.

On a body weight basis, the total population (2 years and older) mean and 90th percentile consumer-only intakes of myristic acid were 2.8 and 6.7 mg/kg body weight/day, respectively, and of palmitic acid were 0.2 and 0.5 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group of myristic acid (7.1 and 18.7 mg/kg body weight/day, respectively) and palmitic acid (0.6 and 1.5 mg/kg body weight/day, respectively).

Table 3.1.3-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Myristic Acid and Palmitic Acid from Proposed Food Uses of Cetylated Fatty Acids in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	%	n	Consumer-Only Intake (mg/kg body weight/day)			
				Myristic Acid		Palmitic Acid	
				Mean	90th Percentile	Mean	90th Percentile
Infants and Young Children	0 to 2	36.4	206	7.1	18.7	0.6	1.5
Children	3 to 11	64.8	749	4.5	9.8	0.4	0.8
Female Teenagers	12 to 19	42.6	202	1.9	3.9	0.2	0.3
Male Teenagers	12 to 19	43.0	208	2.5	5.0	0.2	0.4
Female Adults	20 and older	47.0	920	2.4	5.5	0.2	0.5
Male Adults	20 and older	40.2	701	2.5	6.5	0.2	0.5
Total Population	2 and older	46.3	2,873	2.8	6.7	0.2	0.5

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.1.4 Summary and Conclusions

Consumption data and information pertaining to the intended food uses of cetylated fatty acids were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain cetylated fatty acids at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the

food product and it is unlikely that cetylated fatty acids will have 100% market penetration in all identified food categories.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of cetylated fatty acids by the total U.S. population from proposed food uses in the U.S. were estimated to be 423 mg/person/day (7 mg/kg body weight/day) and 959 mg/person/day (16 mg/kg body weight/day), respectively. Among the individual population groups, male adults had the highest mean and 90th percentile consumer-only intakes of 547 mg/person/day (6 mg/kg body weight/day) and 1,294 mg/person/day (16 mg/kg body weight/day), respectively. While infants and young children had the lowest mean and 90th percentile consumer-only intakes of 211 and 557 mg/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest daily mean and 90th percentile intakes of 17 and 46 mg/kg body weight/day.

In terms of the intakes of the primary saturated fatty acids in cetylated fatty acids (myristic acid and palmitic acid), the mean and 90th percentile consumer-only intakes by the total U.S. population were estimated to be 173 and 393 mg/person/day, respectively, for myristic acid, and 14 and 31 mg/person/day, respectively, for palmitic acid. Of the individual population groups, male adults had the highest mean consumer-only intakes of myristic acid and palmitic acid on an absolute basis, at 224 and 18 mg/person/day, respectively. Similarly, male adults were also observed to have the highest 90th percentile consumer-only intakes of myristic acid and palmitic acid at 530 and 42 mg/person/day, respectively. Therefore, even the highest estimated 90th percentile intakes of saturated fatty acids from consumption of cetylated fatty acids are well below the daily value of 20 g/day for saturated fatty acids established by the U.S. FDA.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with cetylated fatty acids.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

The pivotal data to support the safety of cetylated fatty acids under their intended conditions of use are derived from product-specific toxicological studies commissioned by Pharmanutra. These include a bacterial reverse mutation test, an *in vitro* mammalian cell micronucleus test, and a repeated dose 90-day oral (gavage) toxicity study in rats, which have all been published as Brilli and Tarantino (2021). These studies were all conducted in compliance with Good Laboratory Practice (GLP) following the relevant Organisation for Economic Co-operation and Development (OECD) Test Guidelines (TG).

To obtain other publications relevant to the safety of cetylated fatty acids, comprehensive and detailed searches of the published scientific literature were conducted by Intertek Health Sciences Inc. through February 2020. Databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and ToxFile® served as the primary sources of published literature pertinent to the safety of cetylated fatty acids.

6.2 Absorption, Distribution, Metabolism and Excretion

The absorption, distribution, metabolism, and elimination (ADME) of a different ingredient that contains cetylated fatty acids (Cetyl Myristoleate Complex) was briefly discussed by the European Food Safety Authority (EFSA) in their Scientific Opinion on its use as a food ingredient in 2013, where EFSA concluded that cetylated fatty acids either remain intact in the gastrointestinal tract or are partially or completely hydrolyzed into the cetyl alcohol and fatty acid moieties (EFSA, 2013). The ADME of fatty acids is well-understood; briefly, they are absorbed from the gastrointestinal tract before being catabolized (either immediately or after incorporation into chylomicrons) *via* the ß-oxidation pathway and the tricarboxylic acid cycle, yielding carbon dioxide, which is excreted in expired air (EFSA, 2017; Gyamfi *et al*., 2019).

Cetyl alcohol follows a similar metabolic pathway as fatty acids after absorption, firstly being oxidized to hexadecanal, which is rapidly oxidized to palmitic acid and in turn is metabolized *via* the fatty acid and tricarboxylic acid pathways (Williams, 1959; JECFA, 1999a). Studies in rats have demonstrated that cetyl alcohol is primarily distributed to the thoracic duct lymph, predominantly after being oxidized to palmitic acid, although a small amount (~15%) remains unchanged (Blomstrand and Rumpf, 1954; Baxter *et al*., 1967). Unchanged cetyl alcohol is predominantly eliminated in feces, although a minor excretion route is believed to be *via* urine following conjugation with glucuronic acid (McIsaac and Williams, 1958).

6.3 Toxicological Studies

6.3.1 Short-Term Study

A 14-day dose range finding study was conducted to evaluate the potential short-term toxicity of cetylated fatty acids and to select dose levels for the subsequent 90-day study.

Methods

Sprague-Dawley rats (5/sex/group) were administered cetylated fatty acids by oral gavage once daily at doses of 0 (vehicle – corn oil), 1,600, 1,900, 2,200, 2,600, or 4,500 mg/kg body weight/day. All formulations were heated to 51°C before dosing, due to nature of the test article and reference control, which both solidified at lower temperatures.

All animals were observed once daily for changes in clinical condition and behavior. Body weight and food consumption were recorded before the first dose and twice weekly thereafter. Blood samples were collected at necropsy for analysis of hematology (red and white blood cell count, differential leukocyte count, hematocrit, platelet count, hemoglobin concentration, mean corpuscular hemoglobin concentration, related corpuscular volumes, and prothrombin time) and blood chemistry (sodium, potassium, glucose, total cholesterol, triglycerides, urea, creatinine, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, *gamma* glutamyl transferase, and high, low, and very low density lipoproteins) parameters. At the end of the dosing period, all animals were subjected to a gross macroscopic necropsy.

Results

There were no deaths, no test item-related clinical signs, and no differences in body weight or food consumption between controls and test item groups. The only noteworthy clinical pathology result was a reduction in glucose at 4,500 mg/kg body weight/day, which was considered to be biologically irrelevant due to a lack of a dose-response. No macroscopic findings related to the test item were observed at necropsy.

Conclusion

Administration of cetylated fatty acids once daily for 14 days was well tolerated in rats at doses up to 4,500 mg/kg body weight/day. Therefore, 4,500 mg/kg body weight/day was established as the no-observed-adverse-effect-level (NOAEL) and was considered to be a suitable high-dose for the subsequent 90-day study.

6.3.2 Subchronic Study

A 90-day repeat dose toxicity study was conducted to evaluate the potential subchronic toxicity of cetylated fatty acids after oral gavage administration to Crl:WI(Han) rats once daily for at least 90 days (Brilli and Tarantino, 2021). The study was conducted in compliance with the OECD principles of GLP (OECD, 1998) and according to the most recently revised OECD TG 408 (*Repeated dose 90-day oral toxicity study in rodents*) (OECD, 2018).

Methods

Groups of 10 male and 10 female Sprague-Dawley rats received 0 (vehicle – corn oil), 1,500, 3,000, or 4,500 mg/kg body weight/day cetylated fatty acids, by oral gavage at a dose volume of 10 mL/kg body weight, each as 2 separate daily doses (60 to 150 minutes apart) for at least 90 days, until the day before necropsy. An additional reference control group (comprising the same number of animals) received a combination of commonly consumed starting materials used to produce cetylated fatty acids (*i.e.*, myristic acid, oleic acid, olive oil, and cetyl alcohol) at 4,500 mg/kg body weight/day under the same conditions, to allow for direct comparison against the high-dose cetylated fatty acids group and identify any effects related to general consumption of high doses of fats. All formulations were heated to 51°C before

dosing (dosing temperature of approximately 47°C), due to nature of the test article and reference control, which both solidified at lower temperatures. A further 5 males and 5 females in each of the vehicle control, high-dose, and reference control groups were also dosed once daily for at least 90 days and then kept undosed for 4 weeks, to assess the reversibility of any observed effects after 90 days.

All animals were observed daily for changes in clinical condition, with detailed standard arena observations conducted once weekly and a functional observation battery (including assessment of sensory reactivity to different stimuli, grip strength and motor activity) performed in Week 11. Body weights and food consumption were recorded once weekly throughout the dosing period. Ophthalmoscopic examinations were performed before the start of dosing (all animals) and again in Week 13.

Blood samples were collected for hematology (red and white blood cell counts, differential leukocyte count, hematocrit, platelet counts, hemoglobin, mean corpuscular hemoglobin concentration, and related corpuscular volumes), blood chemistry (thyroid hormones, sodium, potassium, glucose, total cholesterol, triglycerides, urea, creatine, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, *gamma* glutamyl transferase, and high, low, and very-low lipoproteins) in Week 13. Urine samples were collected for urinalysis (density, sediment, pH, leukocytes, nitrites, proteins, glucose, ketones, urobilinogen, bilirubin, erythrocytes) in Week 13.

At the end of the dosing period, a gross macroscopic necropsy was conducted, specified organs were weighed for animals in all groups and vaginal smears were collected for all females. A full list of tissues was examined microscopically for animals in the control and high-dose groups. Additionally, the stomach, small and large intestine, lungs, salivary glands, and aorta from animals in the low- and mid-dose groups, were also microscopically examined.

Results

There were no deaths, no test item-related clinical signs, and no ocular changes. Functional observation battery assessments were unaffected by cetylated fatty acids. There were no differences in body weight or food consumption between test item groups and controls.

At the end of the dosing period, the only statistically significant differences in clinical pathology parameters between controls and test item groups—reduced mean corpuscular hemoglobin concentration for low-dose males, reference control males, and for males and females given 4,500 mg/kg body weight/day; lengthened prothrombin time for low-dose males and females, mid-dose males, and reference control males and females; increased platelets for mid-dose females; increased glucose for low-dose females; reduced total protein, albumin, and urea for low- and mid-dose males; and reduced urea and increased sodium and potassium for females given 4,500 mg/kg body weight/day—were not associated with a dose response and were therefore considered to be unrelated to administration of cetylated fatty acids. There were no statistically significant differences in urinalysis parameters or thyroid hormones between controls and test item groups.

There were no test item-related differences in organ weights. Statistically significant differences reported—increased absolute and body weight-relative pancreas weights for females in all dose groups, and reduced urinary bladder weight for low-dose females—were not associated with a dose response. No test item-related macroscopic findings were observed.

Noteworthy histopathological findings are summarized in Table 6.3.2-1. The histopathology findings in the glandular stomach, intestine, lungs, and salivary glands were seen across all groups including the vehicle and

reference controls, with a possible increased incidence for superficial disruption of the gastric mucosa and emphysematous pictures in cetylated fatty acid groups and of acinar cell degeneration in females. It should be noted that although the dosing temperature was kept the same across all groups, the viscosity of vehicle control formulations (containing only olive oil) would have been less than that of the test item and reference control groups (containing olive oil and the additional fatty acids), thus reducing the time of contact of vehicle control formulations with the gastric and gut mucosae of vehicle controls.

The range of histopathology findings reported in the glandular stomach could be related to a potential local irritant effect of the formulations administered at 47°C. In any case, given the poor dose-response—4, 10, 6, 9, and 10 in males of the control, low-, mid-, high-dose, and reference control groups, respectively, with respective incidences of 7, 5, 7, 10, and 9 in females—and the finding of 5/5 in recovery group vehicle controls and 5/5 males and 2/5 females in the high-dose recovery group, the lesions are more likely of spontaneous origin, with a variable incidence that is not related to the test article.

Emphysematous pictures were more frequent in the cetylated fatty acid groups, but there was no clear dose-response and the incidences varied widely. With respect to the acinar cell atrophy of salivary glands, it is possible that such a finding could be due to a physiological response to the gavage administration of the high viscosity test material; however, the poor-dose response, the lack of any noticeable effect in males, the findings in the vehicle controls (3/10 in both males and females *versus* 4/10 and 6/10 in high-dose males and females, respectively), and the finding of a similar or higher incidence of acinar cell degeneration in vehicle controls (3/5 and 1/5 in males and females, respectively) relative to the high-dose group (0/5 in both sexes) at recovery, collectively indicates that the acinar cell findings, as with the other histopathological findings, are background variations unrelated to dosing.

Conclusion

Cetylated fatty acids were generally well tolerated at doses up to 4,500 mg/kg body weight/day (the highest dose tested), and was just as well tolerated as the reference control material, which comprised a simple mixture of fatty acids that have a long history of consumption in the regular diet. While histopathological findings in the gastrointestinal tract and lungs were noted, they generally did not correlate with increasing dose and were likely incidental. The NOAEL was therefore established as 4,500 mg/kg body weight/day, the highest dose tested.

Table 6.3.2-1 Histopathological Findings at End of Dosing Period and End of Recovery Period in 90-Day Study with Cetylated Fatty Acids

Tissue and Finding	End of Dosing Period (No. of Animals Affected/No. of Animals Examined)										End of Recovery Period (No. of Animals Affected/No. of Animals Examined)					
	Dose (mg/kg bw/day)										Dose (mg/kg bw/day)					
	0 (Control)		1,500		3,000		4,500		Reference Control		0 (Control)		4,500		Reference Control	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Stomach																
Superficial disruption of gastric mucosa	4/10	7/10	10/10	5/10	6/10	7/10	9/10	10/10	10/10	9/10	5/5	4/5	5/5	2/5	3/5	4/5
Small intestine																
Activated GALT																
Light activation	3/10	4/10	1/10	1/10	1/10	2/10	2/10	2/10	4/10	3/10	1/5	2/5	1/5	0/5	2/5	0/5
Mild activation	1/10	1/10	1/10	2/10	1/10	3/10	3/10	2/10	3/10	2/10	0/5	0/5	0/5	1/5	1/5	3/5
Moderate activation	1/10	0/10	5/10	5/10	3/10	3/10	2/10	0/10	1/10	0/10	2/5	2/5	0/5	2/5	0/5	1/5
Large intestine																
Activated GALT																
Light activation	2/10	1/10	0/10	1/10	0/10	0/10	0/10	1/10	4/10	1/10	1/5	2/5	1/5	0/5	2/5	0/5
Mild activation	1/10	1/10	1/10	2/10	1/10	1/10	2/10	1/10	0/10	0/10	0/5	0/5	0/5	2/5	1/5	3/5
Moderate activation	1/10	0/10	4/10	5/10	0/10	2/10	0/10	0/10	0/10	0/10	2/5	2/5	0/5	2/5	0/5	1/5
Severe activation	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/5	0/5	0/5	0/5	0/5	0/5
Lungs																
Emphysematous pictures	0/10	1/10	5/10	5/10	4/10	6/10	4/10	5/10	4/10	7/10	3/5	4/5	4/5	3/5	4/5	4/5
Pulmonary emphysema	0/10	0/10	1/10	1/10	0/10	1/10	1/10	1/10	0/10	1/10	0/5	0/5	0/5	0/5	0/5	0/5
Salivary glands																
Acinar cell degeneration and/or atrophy	3/10	3/10	0/10	1/10	1/10	0/10	4/10	6/10	4/10	2/10	3/5	1/5	0/5	0/5	1/5	1/5
Inflammatory infiltrates	0/10	2/10	0/10	1/10	0/10	0/10	3/10	0/10	2/10	3/10	0/5	0/5	0/5	0/5	0/5	1/5
Aorta																
Area of degeneration	0/10	0/10	1/10	1/10	1/10	0/10	2/10	2/10	3/10	0/10	1/5	0/5	0/5	0/5	1/5	1/5

bw = body weight; F = females; GALT = gut-associated lymphoid tissue; M = males.

6.3.3 Genotoxicity

6.3.3.1 Bacterial Reverse Mutation Test

The potential mutagenicity of cetylated fatty acids was evaluated in a bacterial reverse mutation test (Ames test), which was performed in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD TG 471 (*Bacterial reverse mutation test. In: OECD Guidelines for the Testing of Chemicals*) (OECD, 1997), Commission Regulation (EC) No 440/2008 (EC, 2008a) B13/14 and United States Environmental Protection Agency Health Effects Test Guidelines OPPTS 870.5100 (U.S. EPA, 1998; Brilli and Tarantino, 2021).

Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2*uvrA* were exposed to cetylated fatty acids using the plate incorporation and pre-incubation methods at up to 8 dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolizing system (S9 mix).

The experimental design is summarized in Table 6.3.3.1-1 below. The initial plate incorporation and pre-incubation experiments were followed by 3 confirmatory tests, 2 using the pre-incubation method, in triplicate, in order to assess the reproducibility of non-dose related increases in WP2*uvrA* revertant colony frequency, seen in the absence and presence of S9, in the first pre-incubation test (Experiment 2), and the other using the plate incorporation method, in triplicate, to assess the reproducibility of small, non-dose related increases in TA98 revertant colony frequency (in the absence of S9), observed in the first plate incorporation test (Experiment 1).

In all tests, concentrations up to the maximum recommended concentration in OECD TG 471 (*In Vitro mammalian cell micronucleus test*) (5,000 µg/plate) were used (OECD, 1997).

Table 6.3.3.1-1 Experimental Design for Bacterial Reverse Mutation Test with Cetylated Fatty Acids

Experiment	Bacterial Strains	Dose Concentrations (µg/plate)	With/Without S9
Experiment 1 – Plate incorporation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, *Escherichia coli* WP2*uvrA*	1.5, 5, 15, 50, 150, 500, 1,500, 5,000	With and without S9
Experiment 2 – Pre-incubation	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2*uvrA*	15, 50, 150, 500, 1,500, 5,000	With and without S9
Experiment 3 – Pre-incubation (Confirmatory Test 1)	*E. coli* WP2*uvrA*	15, 50, 150, 500, 1,500, 5,000	With and without S9
Experiment 4 – Pre-incubation (Confirmatory Test 2)	*E. coli* WP2*uvrA*	15, 50, 150, 500, 1,500, 5,000	With and without S9
Experiment 5 – Plate incorporation (Confirmatory Test 3)	*S. typhimurium* TA98	1.5, 5, 15, 50, 150, 500, 1,500, 5,000	Without S9

S9 = metabolic activation.

Results for vehicle (tetrahydrofuran) controls were within historical control ranges for all experiments and all positive controls induced marked increases in the frequency of revertant colonies, with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9 mix were validated. A test item precipitate (greasy in appearance) was noted at ≥500 µg/plate, but this observation did not prevent the scoring of revertant colonies.

No biologically relevant, reproducible, or dose-related increases in revertant colony numbers over control counts were observed with any of the tester strains (in any of mutation or confirmation tests) following exposure to cetylated fatty acids at any concentration, in either the presence or absence of metabolic activation.

It was therefore concluded that cetylated fatty acids is non-mutagenic at concentrations up to 5,000 µg/plate (the maximum recommended concentration in OECD TG 471), in the absence or presence of metabolic activation (OECD, 1997).

6.3.3.2 In Vitro Mammalian Cell Micronucleus Test

The potential ability of cetylated fatty acids to induce micronuclei was evaluated in an *in vitro* micronucleus test conducted in accordance with the OECD principles of GLP (OECD, 1998) and using the test methods described in OECD TG 487 (OECD, 2016; Brilli and Tarantino, 2021).

Human lymphocytes in whole blood culture, stimulated to divide by addition of phytohemagglutinin 48 hours prior to treatment, were exposed to cetylated fatty acids for 4 hours in both the absence and presence of S9 mix, and for 20 hours in the absence of S9 mix only. Vehicle (tetrahydrofuran) and positive control (mitomycin C, demecolcine, and cyclophosphamide) cultures were included in all appropriate test conditions.

Due to formulation difficulties, the maximum achievable concentration was 625 µg/mL and the dose range for the preliminary toxicity test was set at 2.44 to 625 µg/mL. Concentration selection thereafter was dictated by precipitation (in accordance with OECD TG 487) (OECD, 2016). Precipitation observed at the higher concentrations in the preliminary test restricted the high concentration in the main experiment to 160 µg/mL. Precipitation observed at and above 40 µg/mL in the main experiment limited the upper concentration selected for scoring; therefore, final concentrations scored for micronucleus analysis were 10, 20, 40, and 80 µg/mL.

The mean numbers of binucleate cells containing micronuclei in the vehicle control groups were within historical negative control ranges. Positive control compounds induced biologically relevant and statistically significant increases in the number of binucleate cells containing micronuclei under appropriate conditions (with values within historical positive control ranges), which demonstrated the sensitivity of the assay and metabolic activity of the S9 preparations.

Cetylated fatty acids did not induce any marked cytotoxicity and did not induce any biologically relevant or statistically significant increases in the frequency of binucleate cells containing micronuclei, compared with vehicle controls, with values falling within the vehicle historical control range.

It was therefore concluded that cetylated fatty acids is non-clastogenic and non-aneugenic, in the absence and presence of metabolic activation, using a dose range which included the lowest precipitating dose level (in accordance with OECD TG 487).

6.4 Authoritative Reviews

In July 2021, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) published a Scientific Opinion on the use of cetylated fatty acids as a novel food, concluding that it is safe under the intended conditions of use (EFSA, 2021).

6.5 Safety of the Constituents

Several fatty acids and their salts, as well as cetyl alcohol, are authorized as food additives in numerous jurisdictions worldwide, and thus their use as food additives has been subject to safety evaluations from the appropriate Scientific Committees. These evaluations have been summarized below and demonstrate that there is no safety concern regarding the use of the individual constituents of cetylated fatty acids in foods.

6.5.1 Regulatory Status and Safety Evaluations from Scientific Committees

6.5.1.1 Fatty Acids

U.S. FDA

Pursuant with 21 CFR §172.860 (U.S. FDA, 2021), the food additive fatty acids may be used in foods and in the manufacture of food components, provided that the following conditions are met:

- It must consist of 1 or any mixture of the following straight-chain monobasic carboxylic acids and their associated fatty acids manufactured from fats and oils derived from edible sources: myristic acid, oleic acid, palmitic acid, stearic acid, capric acid, caprylic acid and lauric acid;
- The unsaponifiable matter must not exceed 2%; and
- It must be free of chick-edema factor.

Synthetic fatty alcohols may be safely used in food and in the synthesis of food components in accordance with the following prescribed conditions described in 21 CFR §172.864 (U.S. FDA, 2021):

- The food additive consists of any 1 of the following fatty alcohols:
    - Hexyl, octyl, decyl, lauryl, myristyl, cetyl, and stearyl; manufactured by fractional distillation of alcohols obtained by a sequence of oxidation and hydrolysis of organo-aluminums generated by the controlled reaction of low molecular weight trialkylaluminum with purified ethylene (minimum 99% by volume), and utilizing the hydrocarbon solvent, such that:
        - Hexyl, octyl, decyl, lauryl, and myristyl alcohols contain at least 99% of total alcohols and at least 96% of straight chain alcohols.
        - Cetyl and stearyl alcohol s contain at least 98% of total alcohols and at least 94% of straight chain alcohols.
        - The synthetic fatty alcohols contain no more than 0.1% w/w of total diols.

JECFA

In 1974, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) of "not limited" for myristic, palmitic, and stearic acid and their salts, concluding that they are normal products of fat metabolism and their metabolic fate is well established, and that there is no need to consider the use of these compounds in any different manner to that of dietary fatty acids, provided that (in the case of their salts) the cations do not add excessively to the normal body load (JECFA, 1974). Regarding the use of oleic acid as flavoring agent, in the 51st meeting of JECFA it was concluded that there is "*no safety concern at current levels of intake when used as a flavoring agent*", and that the 1988 group ADI of "not specified" for the salts of oleic acid was to be maintained (JECFA, 1999b).

Scientific Committee on Food (SCF)

As part of the evaluation of food additives of various technological functions in 1991, the Scientific Committee on Food (SCF) established a group ADI of "not specified" for myristic, oleic, stearic, and palmitic acid, based on them all being constituents of biological fat and therefore present in food generally, as well as being produced in the metabolism of fats (SCF, 1991).

EFSA

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) published a scientific opinion on the re-evaluation of fatty acids (E 570) as a food additive in 2017 (EFSA, 2017). Fatty acids (E 570) is a group of substances comprising oleic, myristic, palmitic, stearic, caprylic, capric, and lauric acid and is authorized as a food additive in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives (EC, 2008b). In the re-evaluation, EFSA concluded that fatty acids used as a food additive (E 570) are of "*no safety concern at the reported uses and use levels*", as they are absorbed and metabolized in the same way as fatty acids derived from lipids present in the regular diet, there was no genotoxicity concern, and no test article-related adverse effects were observed in the available subchronic toxicity studies (EFSA, 2017).

6.5.1.2 Cetyl Alcohol

JECFA

JECFA concluded that cetyl alcohol is of "*no safety concern at current levels of intake when used as a flavouring agent*" at the 49th meeting in 1997 (JECFA, 1999a).

EFSA

In the EFSA Scientific Opinion on the evaluation of the substances currently on the list in the annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils – part II of III, EFSA concluded that cetyl alcohol is of no toxicological concern when used as a previous cargo, nor is there any concern regarding its possible allergenicity, and that no reaction products of toxicological concern are known or anticipated (EFSA, 2012).

Cosmetic Ingredient Review (CIR) Expert Panel

The Cosmetic Ingredient Review (CIR) Expert Panel assessed the safety of cetyl alcohol and other long-chain aliphatic alcohols (cetearyl alcohol, isostearyl alcohol, myristyl alcohol, and behenyl alcohol) in 1988 (CIR, 1988). The CIR assessment included thorough review of numerous published toxicity data on

cetyl alcohol, and concluded that "*cetearyl, cetyl, isostearyl, myristyl and behenyl alcohols are long-chain aliphatic alcohols that are, at most, only slightly toxic when administered orally at doses of 5 g/kg and greater*" (CIR, 1988).

6.6 GRAS Panel Evaluation

Pharmanutra has concluded that cetylated fatty acids is GRAS for use in various conventional food and beverage products, as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of cetylated fatty acids, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Tom Brenna, Ph.D. (Cornell University), and Gary M. Williams, MD, (New York Medical College).

The GRAS Panel, convened by Pharmanutra, independently and critically evaluated all data and information presented herein, and also concluded that cetylated fatty acids is GRAS for use in conventional food and beverage products as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses of cetylated fatty acids, is presented in Appendix A.

6.7 Conclusion

Based on the above data and information presented herein, Pharmanutra has concluded that cetylated fatty acids is GRAS, on the basis of scientific procedures, for use in food and beverage products as described in Section 1. General recognition of Pharmanutra's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of cetylated fatty acids in food and beverage products, who similarly concluded that the proposed uses of cetylated fatty acids as described herein are GRAS on the basis of scientific procedures.

Cetylated fatty acids therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the *Code of Federal Regulations*.

Part 7. §170.255 List of Supporting Data and Information

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Part	Section §	Section Title
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.860	Fatty acids
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RESPONSES TO FDA QUESTIONS REGARDING GRN 001043

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Responses to FDA Questions Regarding GRN 001043

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RESPONSES TO FDA QUESTIONS REGARDING GRN 001043

QUESTION 1

Question from FDA:

"*Cetylated fatty acids (CFA) is stated to be a mixture consisting primarily of myristic acid and oleic acid esterified with cetyl alcohol (p. 8). Other cetylated fatty acids may also be present. Please clarify the ratio of cetyl myristate/cetyl oleate/other minor cetylated fatty acids as a percent of the total cetylated fatty acid ester composition.*"

Response to Question 1:

The total fatty acid composition of cetylated fatty acid esters is presented in Table 1 below.

Table 1 Cetylated Fatty Acid Esters Total Fatty Acid Composition

Fatty Acid	Results
Oleic acid (%)	29.04
Myristic acid (%)	64
Linoleic acid (%)	4.29
Palmitic acid (%)	2.01
Stearic acid (%)	0.49
Palmitoleic acid (%)	0.39
Lauric acid (%)	0.28

QUESTION 2

Question from FDA:

"*A specification for the antioxidant mono-t-butylhydroquinone (TBHQ) was proposed in Table 2.3.1-1 (p. 12). Please provide data from the analyses of 5 nonconsecutive batches to demonstrate that CFA can be manufactured to conform with the proposed specification for TBHQ.*"

Response to Question 2:

Additional analytical data will be provided at the earliest possible time, pending generation of new analytical results.

QUESTION 3

Question from FDA:

"*You provided the results from the analyses of 5 representative batches of CFA (p. 13). Please clarify if the data are derived from consecutive or nonconsecutive batches. If the analyses are from consecutive batches, please provide the analyses of 5 nonconsecutive batches.*"

Response to Question 3:

Additional analytical data will be provided at the earliest possible time, pending generation of new analytical results.

QUESTION 4

Question from FDA:

"*In the Conditions of Use (p. 6), you state that CFA is intended for use as a source of dietary fatty acids by adults in the general population and is not intended for consumption by infants and young children. However, the foods in which you intend to use CFA can be consumed by the general population, including infants and young children. In addition, you provided estimates of dietary exposure to multiple subpopulations, including infants and young children, in Part 3. Please clarify the intended uses of your ingredient, including the populations that would be expected to consume the ingredient.*"

Response to Question 4:

The target population for Pharmanutra's CFA is adults in the general population. This target population was selected purely on the basis of the intended marketing of the ingredient (in products which are not typically consumed by infants or young children, other than ice cream) rather than potential safety concerns. CFA is safe for consumption by the general population, hence all population groups were included in the exposure assessment. The estimated high-level exposure in young children (0 to 2 and 3 to 11 years of age), coupled with the existing clinical and pre-clinical data pertaining to the metabolic fate and safety of CFA, supports that there is no indication that CFA would be nutritionally disadvantageous in young children, nor is there any biologically plausible mechanism by which the consumption of CFA would be expected to result in adverse effects in young children (further details provided in GRN 1043). Furthermore, it must be noted that the exposure estimate is extremely conservative, as it assumes all foods which are consumed contain the maximum proposed level of CFA; in reality, the levels added to specific foods will vary depending on the nature of the food product, and it is unlikely that CFA will have 100% market penetration in all identified food categories.

QUESTION 5

Question from FDA:

"*In the batch analyses (Table 2.3.4-1, p. 14), data were only provided from the analyses of two batches for arsenic. Please provide the analyses of at least one additional batch for arsenic*."

Response to Question 5:

Additional analytical data will be provided at the earliest possible time, pending generation of new analytical results.

QUESTION 6

Question from FDA:

"*Please provide the limit of detection (LOD) for the method(s) used for the analyses of heavy metals by inductively coupled plasma mass spectrometry (ICP-MS) (p. 14). In addition, the results of the batch analyses for certain heavy metals were listed as both <0.005 mg/kg and <0.02 mg/kg, which seems to imply that two different methods with different LODs were used. Please clarify this inconsistency in the results for the heavy metal analyses.*"

Response to Question 6:

A certificate of validation (for limit of quantification) from the laboratory responsible for analysis of batches 15F16411 and 19M19907 included in GRN 001043 is provided in Attachment 1. As shown in this certificate, the limit of quantification for heavy metals analysis was determined to be 0.005 mg/kg. Batch 2007310-001 was analyzed at a different testing laboratory; at this laboratory, the ICP-MS equipment has a limit of detection of 0.02 mg/kg. Pharmanutra provides assurance that they are currently using the laboratory with the limit of quantification of 0.005 mg/kg for heavy metal testing, and will continue to do so in the future.

QUESTION 7

Question from FDA:

"*On pg. 30, the notifier states that literature searches were conducted through February 2020. Since the cut-off date is more than one year since your submission (November 2021), please confirm that no other publicly available information relevant to your GRAS conclusion has been found since the literature search was conducted. If additional information was found, please provide a brief narrative as to how these newer publications do or do not impact your GRAS conclusion.*"

Response to Question 7:

The literature search was updated using the same databases as those searched previously (*i.e.,* Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and ToxFile®). Search terms relevant to cetyl alcohol, myristic acid, and oleic acid were used with terms pertaining to oral exposure and pre-clinical and clinical safety endpoints. No publications relevant to the safety of CFA (or cetyl alcohol, myristic acid, and oleic acid

individually) were identified; thus, no publications are available that would affect the GRAS conclusion for Pharmanutra's CFA ingredient.

QUESTION 8

Question from FDA:

"*The notifier states on pg. 37: 'In July, 2021, the EFSA Panel on Nutrition Novel Foods and Food Allergens (NDA) published a Scientific Opinion on the use of cetylated fatty acids as a novel food, concluding that it is safe under the intended conditions of use (EFSA, 2021).'*

We note:

- *It appears that this EFSA panel evaluated the use of cetylated fatty acids as a dietary supplement, not necessarily as a direct food ingredient.*

- *EFSA concluded: 'By applying the default uncertainty factor of 200 as suggested by the EFSA Scientific Committee (2012), and considering a default body weight of 70 kg for the adult target population, this would result in an intake of 1.6 g per day, which is lower than the maximum intake proposed by the application (i.e., 2.1 g per day) … The Panel concludes that the NF, cetylated fatty acids, is safe at an intake of 1.6 g per day for the intended target population, i.e., adults.' Thus, it appears that EFSA's conclusion was that there was no data to support a use level greater than 1.6 g/day for food supplements.*

- *While a GRAS Panel evaluation is not necessary and/or sufficient to reach a GRAS conclusion (see FDA's draft guidance on convening GRAS panels), it appears that the EFSA evaluation occurred after the GRAS Panel was convened (December 2020).*

Please provide a narrative clearly describing how EFSA's most recent evaluation supports your GRAS conclusion."

Response to FDA Question 8:

In July 2021, EFSA published a scientific opinion on the safety of Pharmanutra's CFA as a novel food (*i.e.,* as an ingredient in food supplements) (EFSA, 2021[1]). EFSA considered the 90-day study of CFA in rats (Brilli and Tarantino, 2021[2]) in the calculation of their safe intake of CFA. In their novel food application dossier, Pharmanutra noted that CFA was well-tolerated in this study at doses up to 4,500 mg/kg body weight/day (the highest dose tested), but cited a conservative no-observed-adverse-effect level (NOAEL) of 3,000 mg CFA/kg body weight/day based on histopathological findings in the gastrointestinal tract and lungs, noting that these findings generally did not correlate with increasing dose and were likely incidental. However, in EFSA's evaluation of the 90-day study, the NDA Panel considered the highest dose tested, 4,500 mg CFA/kg body weight/day, as the NOAEL for this study (EFSA, 2021).

[1] EFSA (2021). Scientific Opinion on the safety of Cetylated Fatty Acids as a Novel Food pursuant to Regulation (EU) 2015/2283 (EFSA Panel on Nutrition, Novel Foods and Food Allergens/NDA) (Question no EFSA-Q-2020-00422, adopted: 26 May 2021 by European Food Safety Authority). EFSA J 6670(19):6670 [14pp]. DOI:10.2903/j.efsa.2021.6670. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/6670.

[2] Brilli E, Tarantino G (2021). Genotoxicity and subchronic toxicity studies of Lipocet®, a novel mixture of cetylated fatty acids. J Appl Toxicol 41(7):1148-1162. DOI:10.1002/jat.4101.

Pharmanutra used the conservative NOAEL of 3,000 mg/kg body weight/day with a standard uncertainty factor (UF) of 100 (*i.e.,* 10 for interspecies variation and 10 for inter-human variation) to calculate their intended human consumption of 30 mg/kg body weight/day, or 2.1 g/day for a 70-kg individual. However, EFSA used a higher NOAEL (4,500 mg/kg body weight/day) from the 90-day study of CFA but a higher uncertainty factor of 200, which was chosen based on EFSA's Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels, and Units in the absence of actual measured data (EFSA, 2012). In this guidance, it is stated that when using a NOAEL from an animal study to calculate an acceptable intake level for humans, an UF of 2 (in addition to the standard UF of 100) is recommended to be used when the NOAEL is based on a 90-day study rather than a chronic toxicity study (*i.e.,* generally considered to be ≥1 year in duration). The basis for the use of this additional UF is stated as "*considering the extent of investigations usually performed in 90-day studies*." Thus, the justification for the use of the UF of 200 appears to be based on the notion that the parameters evaluated in most 90-day studies are not as extensive as those evaluated in studies ≥1 year in duration. However, the 90-day study of CFA was conducted in accordance with OECD Test Guideline 408 (OECD, 2018[3]), and included a comprehensive safety assessment as described in GRN 1043. In addition, according to EFSA's tiered approach[4], further toxicity testing is not required to support the safety of CFA based on the existing safety data on the ingredient (*i.e.,* the lack of safety concerns regarding metabolism, genotoxicity, and subchronic toxicity).

Using the NOAEL of 4,500 mg/kg body weight/day and an uncertainty factor of 200, EFSA calculated a safe intake level of 1.6 g CFA/day, or 22.8 mg/kg body weight/day for a 70-kg individual. However, given that 4,500 mg/kg body weight/day was the highest dose tested, the true NOAEL, and therefore the safe level of consumption for humans, may be higher than the calculated values. In addition, JECFA stated in their review of myristic, palmitic, and stearic acids and their salts that "*they are normal products of fat metabolism and their metabolic fate is well established, and that there is no need to consider the use of these compounds in any different manner to that of dietary fatty acids*" (JECFA, 1974[5]). Therefore, given the well-documented metabolic fate of the constituents of CFA, their natural presence in the human diet, and that the NOAEL in the 90-day study of CFA is the highest dose tested, the use of an additional UF of 2 by EFSA, and subsequent calculation of a lower safe daily intake than that stated in GRN 1043, is considered to be excessively conservative. Consumption of CFA at the level indicated in GRN 1043 (2.1 g/day) is therefore not expected to be associated with safety concerns.

[3] OECD (2018). Repeated dose 90-day oral toxicity study in rodents. In: OECD Guidelines for the Testing of Chemicals. (OECD Guideline no 408) [Updated & Adopted: 27 June 2018]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en.

[4] As stated in Section 2.10.1 of the *Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283*, the tiered approach to the safety assessment of food additives [described in the EFSA *Guidance for submission for food additive evaluations* (EFSA ANS Panel, 2012)] is the also the default approach for safety assessment of novel foods.

[5] JECFA (1974). Salts of myristic, palmitic, and stearic acids. In: Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents. Seventeenth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switz. (WHO Food Additives Series No. 5; WHO Technical Report Series, No. 539; Nutrition Meetings Report Series, No. 53). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je03.htm.

QUESTION 9

Question from FDA:

"*On pg. 15, relating to 3-monochloro-2-pro-panediol (3-MCPD) and glycidyl fatty acid esters (GEs) levels, you state: "The results show that amounts of all of these potential contaminants in cetylated fatty acids are far below the EU limits, which is justification for why they are not required in the specifications for cetylated fatty acids.*

We note that your food categories (i.e., ice cream, milk products) contain products expected to be consumed by infants and children as indicated in your dietary exposure assessment. Given that we do not have established limits in the U.S. at this time for GEs and 3-MCPD, please provide a brief narrative on how you are taking steps to monitor and minimize formation of these process contaminants and that potential dietary exposure to these contaminants from your intended use is safe, especially for young children and infants."

Response to Question 9:

Fatty acid esters of glycidol and 3-MCPD are products of heat-processing that have been identified in many types of oil-based foods and food ingredients (MacMahon *et al.*, 2013[6]). Based on a pre-clinical no-observed-effect level of 1.1 mg 3-MCPD/kg body weight/day, and a lack of *in vivo* genotoxicity potential, the Scientific Committee on Food derived a tolerable daily intake (TDI) of 2 µg 3-MCPD/kg body weight/day (EC, 2004[7]). Limits for specific foods were set only for hydrolyzed vegetable protein and soy sauce (20 µg/kg each) based on the contribution of these foods to overall consumption of 3-MCPD (EC, 2006[8]). As shown in GRN 1043, the concentration of 3-MCPD in a representative batch of CFA was <10 µg/kg. Using the calculated 90th percentile consumer-only intake of CFA in infants and young children (46 mg/kg body weight/day) and a concentration of <10 µg 3-MCPD/kg in CFA, a potential maximum intake of <0.00046 µg 3-MCPD/kg body weight/day was calculated. Based on the TDI of 2 µg/kg body weight/day, a margin of safety of >4,348 was calculated, supporting the safety of CFA for consumption by infants and children with respect to potential 3-MCPD content.

With respect to estimated consumption by infants and young children, there were a number of assumptions included in the intakes assessment for CFA which render estimates of exposure to 3-MCPD suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain CFA at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that CFA will have 100% market penetration in all identified food categories. Therefore, the estimation of potential consumption of foods containing CFA by infants and children, while remaining within levels considered to be safe, are conservative and likely to be higher than actual intake levels.

[6] MacMahon S, Begley TH, Diachenko GW (2013). Occurrence of 3-MCPD and glycidyl esters in edible oils in the United States. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 30(12):2081-2092. DOI:10.1080/19440049.2013.840805.
[7] EC (2004). *Collection and Collation of Data on Levels of 3-Monochloropropanediol (3-MCPD) and Related Substances in Food*. (Reports on Tasks for Scientific Cooperation – Report of Experts Participating in Task 3.2.9). European Commission (EC), Directorate-General Health and Consumer Protection. Available at: https://ec.europa.eu/food/system/files/2016-10/cs_contaminants_catalogue_mcpd_scoop_3-2-9_final_report_chloropropanols_en.pdf [June 2004].
[8] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20140701&from=EN.

The European Commission (EC) also has set limits for glycidol in foods; as glycidyl fatty acid esters are hydrolyzed in the gastrointestinal tract to glycidol and the respective fatty acids, these limits are expressed as glycidol. A limit 1,000 µg glycidol/kg has been established for vegetable oils and fats used as ingredients in food products, while a limit of 500 µg/kg has been established for vegetable oils and fats used in baby food and processed cereal-based foods for infants and young children (EU, 2018[9]). No TDI on a body weight basis was identified. The content of glycidyl fatty acid esters (as glycidol) in a representative batch of CFA is within the EC limits for vegetable oils and fats (*i.e.,* 340 µg/kg), and therefore is not considered to pose safety concerns.

As further support for the safety of CFA with respect to 3-MCPD and glycidyl ester content, MacMahon *et al.* (2013) compared the 3-MCPD and glycidyl ester contents of 94 processed oils commercially available in the U.S. Using a liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analytical method, concentrations of 3-MCPD were reported to range from 0.005 to 7.2 mg/kg in 94 refined oils. Concentrations of glycidyl esters were reported to range from below the limit of quantification to 10.5 mg/kg. These levels of 3-MCPD and glycidyl esters identified in commercially available processed oil products are up to 720 and 30.9 times higher than the levels measured in Pharmanutra's CFA ingredient, respectively, indicating that the 3-MCPD and GE levels in CFA are well within the range of processed oil products currently on the market in the U.S. and are not expected to pose safety concerns.

Pharmanutra has provided a declaration from the manufacturer of CFA stating that the ingredient is monitored for the presence of glycidol esters (expressed as glycidol) and 3-MCPD by analysis of the ingredient and review of certificates of analysis for the olive oil raw material. This declaration is provided in Attachment 2.

[9] EU (2018). Commission Regulation (EU) 2018/290 of 26 February 2018 amending Regulation (EC) No 1881/2006 as regards maximum levels of glycidyl fatty acid esters in vegetable oils and fats, infant formula, follow-on formula and foods for special medical purposes intended for infants and young children. Off J Eur Union 61(L55):27-29. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32018R0290&qid=1654024753926.

ATTACHMENT 1
Certificate of Validation

Validation parameters:

Selectivity and Specificity: Parameter not applicable. The ICP-MS equipment has a resolution of 1/250 UMA, so the overlap of a signal adjacent to the analyte is impossible. Furthermore, the system is equipped with a double system of interferences abatement: helium reaction cell and energy discriminator. These devices make the existence of molecular compounds in the detector impossible.

Detection Limit: L.D. = (Standard. Deviation. Reagent blank *3) *See Table 1*

Quantification Limit: L.Q. = (Standard. Deviation. reagent blank *6) *See Table 1*

Table 1 (L.D. & L.Q.) **Scope of application**: **Validated matrices as reported in the Test method M-05/ICP-MS**

Element	Li	Be	B	Al	Sc	Ti	Cr	V	Co	Ni	Cu	Zn	As
L.D. µg/L	0,009	0,004	0,018	0,017	0,022	0,031	0,009	0,008	0,005	0,021	0,035	0,028	0,032
L.Q. µg/L	0,018	0,007	0,036	0,033	0,045	0,062	0,017	0,017	0,011	0,043	0,071	0,055	0,064

Element	As in	As or	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn
L.D. µg/L	0,022	0,050	0,021	0,014	0,011	0,033	0,009	0,009	0,014	0,018	0,016	0,010	0,010
L.Q. µg/L	0,045	0,100	0,042	0,028	0,022	0,066	0,018	0,019	0,028	0,035	0,031	0,020	0,020

Element	Sn or	Sb	Te	Ba	Hg	MHg	Pb	Tl	Bi	U			
L.D. µg/L	0,011	0,007	0,018	0,017	0,030	0,032	0,007	0,018	0,005	0,054			
L.Q. µg/L	0,021	0,014	0,037	0,033	0,060	0,065	0,014	0,036	0,011	0,109			

The limits of quantification reported in the Test Reports are referred to food matrix and they are rounded up to 0,005 mg/kg.

Table 1b (L.D. & L.Q.) **Scope of application**: **Waters**

Element	Li	Be	B	Al	Sc	Ti	Cr	V	Co	Ni	Cu	Zn	As
L.Quant µg/L	1,0	1,0	1,0	5,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Element	As in	As or	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn
L.Quant µg/L	1,0	1,0	1,0	1,0	1,0	5,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Element	Sb	Te	Ba	Hg	Pb	Tl	Bi	U					
L.Quant µg/L	1,0	1,0	1,0	0,2	1,0	1,0	1,0	1,0					

Range: 0,005 mg/kg <= Range <= 1000 mg/kg, for all the elements, the limit of 1000 mg/kg is determined by the opportunity to make dilutions that do not compromise the precision of the result.

Linearity: All the elements have a linearity reported as correlation coefficient $R^2 > 0,999$

The minimum condition of $R^2 > 0,995$ is more than satisfactory.

Precision: Limits of repeatability summarized in *Table 2*.

Table 2: Relative limits of repeatability

Element	Li	Be	B	Al	Sc	Ti	Cr	V	Co	Ni	Cu	Zn	As
Rel. limit % Repeatability high levels	8,9	12,3	12,6	8,3	10,0	11,0	8,7	5,8	10,9	3,6	10,5	9,4	11,3
Rel. limit % Repeatability medium levels	9,6	12,7	12,7	12,2	12,2	14,0	12,3	14,3	14,8	12,9	9,2	10,5	10,0
Rel. limit % Repeatability low levels	13,3	16,3	7,2	14,2	15,9	12,8	12,7	15,3	12,9	10,3	14,5	12,3	12,8

Element	As in	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn	Sn or
Rel. limit % Repeatability high levels	7,5	6,8	3,8	12,6	12,5	11,0	11,8	11,1	5,7	4,0	9,0	10,2	7,7
Rel. limit % Repeatability medium levels	8,0	8,3	11,3	11,4	11,8	10,8	12,7	11,9	14,5	12,8	12,3	15,2	11,6
Rel. limit % Repeatability low levels	12,0	7,4	11,3	13,6	11,9	13,2	12,7	11,6	14,0	12,7	11,5	18,2	17,9

Element	Sb	Te	Ba	Hg	MHg	Pb	Tl	Bi	U				
Rel. limit % Repeatability high levels	8,0	12,5	6,2	7,4	10,3	4,4	5,5	6,6	7,5				
Rel. limit % Repeatability medium levels	16,2	13,9	18,2	8,3	8,8	12,5	12,6	5,0	5,4				
Rel. limit % Repeatability low levels	16,4	13,6	18,6	8,2	14,6	10,2	12,9	16,0	12,2				

All the limits of repeatability are rounded up to 20%.

Accuracy: Mean Recovery and T test summarized in *Table 3*.

Table 3: Mean Rec. % & T-test (T limit = 2,26)

Element	**Li**	**Be**	**B**	**Al**	**Sc**	**Ti**	**Cr**	**V**	**Co**	**Ni**	**Cu**	**Zn**	**As**
Mean Rec %	100,8	99,5	98,7	98,0	102,6	100,4	98,7	99,2	101,0	101,6	99,1	99,0	102,5
T-test < 2,26	0,71	0,57	1,49	1,44	1,66	0,35	1,54	1,20	1,31	1,63	1,44	1,08	1,38

Element	**As in**	**Se**	**Mo**	**Mn**	**Fe**	**Sr**	**Zr**	**Rb**	**Ag**	**Pd**	**Cd**	**Sn**	**Sn or**
Mean Rec %	101,0	100,5	101,3	99,2	101,0	98,8	99,7	101,6	98,2	99,0	99,0	100,5	102,0
T-test < 2,26	0,60	0,46	1,24	1,06	0,88	0,99	0,27	1,57	1,14	0,53	0,83	0,30	1,14

Element	**Sb**	**Te**	**Ba**	**Hg**	**MHg**	**Pb**	**Tl**	**Bi**	**U**				
Mean Rec %	100,2	100,8	96,3	99,0	99,0	98,7	100,4	100,4	100,1				
T-test < 2,26	0,15	0,61	1,61	0,72	1,14	1,11	0,33	0,39	0,10				

Sensitivity: The slope of the calibration curve for each element is summarized in *Table 4*.

Table 4: Sensitivity (Sens) cps/µg/l

Element	**Li**	**Be**	**B**	**Al**	**Sc**	**Ti**	**Cr**	**V**	**Co**	**Ni**	**Cu**	**Zn**	**As**
Sen. Cps/µg/l	9920	12347	3529	4290	3575	3501	10467	8880	15599	4226	2522	2358	2491

Element	As in	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn	Sn or
Sen. Cps/µg/l	2491	1248	4473	5239	2618	10371	10653	7717	4879	4907	5446	8745	8745

Element	Sb	Te	Ba	Hg	MHg	Pb	Tl	Bi	U				
Sen. Cps/µg/l	9581	3254	4097	556	556	8852	6247	15838	15694				

Traceability: In this procedure the traceability is attributable to the certificate of guarantee and expiration of the reference material where the concentration of the analyte has a tolerance of 0,2%.

Robustness: Parameter not applicable. During the mineralisation phase, the maximum temperature is the boiling temperature of HNO3 (or NH4OH for I and Br), temperature lower at the evaporation temperature of the minerals contained in the matrix. In case of too low temperature of the mineraliser, the redox reaction with the matrix would not be triggered and the matrix would remain visually unchanged.
The ICP-MS system has a sensitivity higher than what is necessary to satisfy the quantification limits that are all rounded up. The procedure foresees minimum usage conditions, that, if satisfied, enable the correct execution of the analyses. This is all demonstrated in the verification of the instrumental performances in the relevant control chart.

Expanded Uncertainty:

The expanded uncertainty applied for each parameter is that calculated with the Horwitz / Thompson model, because it is considered the most correct estimated uncertainty for a multiresidual determination at different concentrations, unless otherwise indicated by the customer or provided for by legislative / mandatory aspects

Nature of the modifications:

- *Page 1: introduced Table 1b with the specific limits of quantification for the water matrix*
- *Iodine and Bromine parameters removed from all tables.*

Approval:

Considering the specifications defined during the planning phase and the experimental results obtained applying the test method M-05/ICP-MS "Determination of metals in ICP-MS",

The test method is considered validated and applicable by the Laboratory.
Thus, the method is approved by the Management.

Laboratory Director:

Alberto Gatti Date: 14/05/2021

ATTACHMENT 2
Manufacturer Declaration

A.& A. Fratelli Parodi s.p.a.
www.fratelliparodi.it

R.E.A. MI 2098402
R.E.A. GE 125281
R.E.A. IM 138058
Cod. Fisc. e Part. IVA 00248230104
Cap. Soc. € 1.500.000,00 I.V.

DECLARATION

To Whom It Concerns

A&A Fratelli Parodi S.p.A. herewith declares that for the product LIPOCET, quality controls on olive oil raw material are carried out as follows:

-analysis of glycidol esters expressed as glycidol
-analysis of 3-MCPD

This control is performed both by A&A Fratelli Parodi QC laboratory and by the documentary review of the certificates of analysis of the raw material of our supplier.

Campomorone – Genova – Italia 27/05/2022
Dr. F. Scalzullo – Quality Office
A&A Fratelli Parodi Spa

RESPONSES TO FDA QUESTIONS REGARDING GRN 001043

PREPARED FOR:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
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DATE:
30 June 2022

Responses to FDA Questions Regarding GRN 001043

TABLE OF CONTENTS

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RESPONSES TO FDA QUESTIONS REGARDING GRN 001043

QUESTION 1

Question from FDA:

"*Cetylated fatty acids (CFA) is stated to be a mixture consisting primarily of myristic acid and oleic acid esterified with cetyl alcohol (p. 8). Other cetylated fatty acids may also be present. Please clarify the ratio of cetyl myristate/cetyl oleate/other minor cetylated fatty acids as a percent of the total cetylated fatty acid ester composition.*"

Response to Question 1:

The total fatty acid composition of cetylated fatty acid esters is presented in Table 1 below.

Table 1 Cetylated Fatty Acid Esters Total Fatty Acid Composition

Fatty Acid	Results
Oleic acid (%)	29.04
Myristic acid (%)	64
Linoleic acid (%)	4.29
Palmitic acid (%)	2.01
Stearic acid (%)	0.49
Palmitoleic acid (%)	0.39
Lauric acid (%)	0.28

QUESTION 2

Question from FDA:

"*A specification for the antioxidant mono-t-butylhydroquinone (TBHQ) was proposed in Table 2.3.1-1 (p. 12). Please provide data from the analyses of 5 nonconsecutive batches to demonstrate that CFA can be manufactured to conform with the proposed specification for TBHQ.*"

Response to Question 2:

The notifier has provided the results of analysis for TBHQ in 5 non-consecutive batches of cetylated fatty acid esters. These results demonstrate that TBHQ levels are consistently well below the specification value of <0.02%. Please refer to Attachment 1 for these results.

QUESTION 3

Question from FDA:

"*You provided the results from the analyses of 5 representative batches of CFA (p. 13). Please clarify if the data are derived from consecutive or nonconsecutive batches. If the analyses are from consecutive batches, please provide the analyses of 5 nonconsecutive batches.*"

Response to Question 3:

The notifier has provided the results of analysis of 5 non-consecutive and representative batches of cetylated fatty acid esters. These results demonstrate that the manufacturing process results in a final product that consistently meets ingredient specifications established by the notifier. Please refer to Attachment 2 for certificates of analysis.

QUESTION 4

Question from FDA:

"*In the Conditions of Use (p. 6), you state that CFA is intended for use as a source of dietary fatty acids by adults in the general population and is not intended for consumption by infants and young children. However, the foods in which you intend to use CFA can be consumed by the general population, including infants and young children. In addition, you provided estimates of dietary exposure to multiple subpopulations, including infants and young children, in Part 3. Please clarify the intended uses of your ingredient, including the populations that would be expected to consume the ingredient.*"

Response to Question 4:

The target population for Pharmanutra's CFA is adults in the general population. This target population was selected purely on the basis of the intended marketing of the ingredient (in products which are not typically consumed by infants or young children, other than ice cream) rather than potential safety concerns. CFA is safe for consumption by the general population, hence all population groups were included in the exposure assessment. The estimated high-level exposure in young children (0 to 2 and 3 to 11 years of age), coupled with the existing clinical and pre-clinical data pertaining to the metabolic fate and safety of CFA, supports that there is no indication that CFA would be nutritionally disadvantageous in young children, nor is there any biologically plausible mechanism by which the consumption of CFA would be expected to result in adverse effects in young children (further details provided in GRN 1043). Furthermore, it must be noted that the exposure estimate is extremely conservative, as it assumes all foods which are consumed contain the maximum proposed level of CFA; in reality, the levels added to specific foods will vary depending on the nature of the food product, and it is unlikely that CFA will have 100% market penetration in all identified food categories.

QUESTION 5

Question from FDA:

"*In the batch analyses (Table 2.3.4-1, p. 14), data were only provided from the analyses of two batches for arsenic. Please provide the analyses of at least one additional batch for arsenic*."

Response to Question 5:

The notifier has provided the results of analysis for heavy metals, including arsenic, for 2 non-consecutive and representative batches of cetylated fatty acid esters. These results demonstrate that the ingredient consistently meets heavy metal specifications established by the notifier. Please refer to Attachment 3 for certificates of analysis.

QUESTION 6

Question from FDA:

"*Please provide the limit of detection (LOD) for the method(s) used for the analyses of heavy metals by inductively coupled plasma mass spectrometry (ICP-MS) (p. 14). In addition, the results of the batch analyses for certain heavy metals were listed as both <0.005 mg/kg and <0.02 mg/kg, which seems to imply that two different methods with different LODs were used. Please clarify this inconsistency in the results for the heavy metal analyses.*"

Response to Question 6:

A certificate of validation (for limit of quantification) from the laboratory responsible for analysis of batches 15F16411 and 19M19907 included in GRN 001043 is provided in Attachment 4. As shown in this certificate, the limit of quantification for heavy metals analysis was determined to be 0.005 mg/kg. Batch 2007310-001 was analyzed at a different testing laboratory; at this laboratory, the ICP-MS equipment has a limit of detection of 0.02 mg/kg. Pharmanutra provides assurance that they are currently using the laboratory with the limit of quantification of 0.005 mg/kg for heavy metal testing, and will continue to do so in the future.

QUESTION 7

Question from FDA:

"*On pg. 30, the notifier states that literature searches were conducted through February 2020. Since the cut-off date is more than one year since your submission (November 2021), please confirm that no other publicly available information relevant to your GRAS conclusion has been found since the literature search was conducted. If additional information was found, please provide a brief narrative as to how these newer publications do or do not impact your GRAS conclusion.*"

Response to Question 7:

The literature search was updated using the same databases as those searched previously (*i.e.,* Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and ToxFile®). Search terms relevant to cetyl alcohol, myristic acid, and oleic acid were used with terms pertaining to oral exposure and pre-clinical and clinical

safety endpoints. No publications relevant to the safety of CFA (or cetyl alcohol, myristic acid, and oleic acid individually) were identified; thus, no publications are available that would affect the GRAS conclusion for Pharmanutra's CFA ingredient.

QUESTION 8

Question from FDA:

"*The notifier states on pg. 37: 'In July, 2021, the EFSA Panel on Nutrition Novel Foods and Food Allergens (NDA) published a Scientific Opinion on the use of cetylated fatty acids as a novel food, concluding that it is safe under the intended conditions of use (EFSA, 2021).'*

We note:

- *It appears that this EFSA panel evaluated the use of cetylated fatty acids as a dietary supplement, not necessarily as a direct food ingredient.*

- *EFSA concluded: 'By applying the default uncertainty factor of 200 as suggested by the EFSA Scientific Committee (2012), and considering a default body weight of 70 kg for the adult target population, this would result in an intake of 1.6 g per day, which is lower than the maximum intake proposed by the application (i.e., 2.1 g per day) … The Panel concludes that the NF, cetylated fatty acids, is safe at an intake of 1.6 g per day for the intended target population, i.e., adults.' Thus, it appears that EFSA's conclusion was that there was no data to support a use level greater than 1.6 g/day for food supplements.*

- *While a GRAS Panel evaluation is not necessary and/or sufficient to reach a GRAS conclusion (see FDA's draft guidance on convening GRAS panels), it appears that the EFSA evaluation occurred after the GRAS Panel was convened (December 2020).*

Please provide a narrative clearly describing how EFSA's most recent evaluation supports your GRAS conclusion."

Response to FDA Question 8:

In July 2021, EFSA published a scientific opinion on the safety of Pharmanutra's CFA as a novel food (*i.e.,* as an ingredient in food supplements) (EFSA, 2021[1]). EFSA considered the 90-day study of CFA in rats (Brilli and Tarantino, 2021[2]) in the calculation of their safe intake of CFA. In their novel food application dossier, Pharmanutra noted that CFA was well-tolerated in this study at doses up to 4,500 mg/kg body weight/day (the highest dose tested), but cited a conservative no-observed-adverse-effect level (NOAEL) of 3,000 mg CFA/kg body weight/day based on histopathological findings in the gastrointestinal tract and lungs, noting that these findings generally did not correlate with increasing dose and were likely incidental. However, in EFSA's evaluation of the 90-day study, the NDA Panel considered the highest dose tested, 4,500 mg CFA/kg body weight/day, as the NOAEL for this study (EFSA, 2021).

[1] EFSA (2021). Scientific Opinion on the safety of Cetylated Fatty Acids as a Novel Food pursuant to Regulation (EU) 2015/2283 (EFSA Panel on Nutrition, Novel Foods and Food Allergens/NDA) (Question no EFSA-Q-2020-00422, adopted: 26 May 2021 by European Food Safety Authority). EFSA J 6670(19):6670 [14pp]. DOI:10.2903/j.efsa.2021.6670. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/6670.

[2] Brilli E, Tarantino G (2021). Genotoxicity and subchronic toxicity studies of Lipocet®, a novel mixture of cetylated fatty acids. J Appl Toxicol 41(7):1148-1162. DOI:10.1002/jat.4101.

Pharmanutra used the conservative NOAEL of 3,000 mg/kg body weight/day with a standard uncertainty factor (UF) of 100 (*i.e.,* 10 for interspecies variation and 10 for inter-human variation) to calculate their intended human consumption of 30 mg/kg body weight/day, or 2.1 g/day for a 70-kg individual. However, EFSA used a higher NOAEL (4,500 mg/kg body weight/day) from the 90-day study of CFA but a higher uncertainty factor of 200, which was chosen based on EFSA's Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels, and Units in the absence of actual measured data (EFSA, 2012). In this guidance, it is stated that when using a NOAEL from an animal study to calculate an acceptable intake level for humans, an UF of 2 (in addition to the standard UF of 100) is recommended to be used when the NOAEL is based on a 90-day study rather than a chronic toxicity study (*i.e.,* generally considered to be ≥1 year in duration). The basis for the use of this additional UF is stated as "*considering the extent of investigations usually performed in 90-day studies*." Thus, the justification for the use of the UF of 200 appears to be based on the notion that the parameters evaluated in most 90-day studies are not as extensive as those evaluated in studies ≥1 year in duration. However, the 90-day study of CFA was conducted in accordance with OECD Test Guideline 408 (OECD, 2018[3]), and included a comprehensive safety assessment as described in GRN 1043. In addition, according to EFSA's tiered approach[4], further toxicity testing is not required to support the safety of CFA based on the existing safety data on the ingredient (*i.e.,* the lack of safety concerns regarding metabolism, genotoxicity, and subchronic toxicity).

Using the NOAEL of 4,500 mg/kg body weight/day and an uncertainty factor of 200, EFSA calculated a safe intake level of 1.6 g CFA/day, or 22.8 mg/kg body weight/day for a 70-kg individual. However, given that 4,500 mg/kg body weight/day was the highest dose tested, the true NOAEL, and therefore the safe level of consumption for humans, may be higher than the calculated values. In addition, JECFA stated in their review of myristic, palmitic, and stearic acids and their salts that "*they are normal products of fat metabolism and their metabolic fate is well established, and that there is no need to consider the use of these compounds in any different manner to that of dietary fatty acids*" (JECFA, 1974[5]). Therefore, given the well-documented metabolic fate of the constituents of CFA, their natural presence in the human diet, and that the NOAEL in the 90-day study of CFA is the highest dose tested, the use of an additional UF of 2 by EFSA, and subsequent calculation of a lower safe daily intake than that stated in GRN 1043, is considered to be excessively conservative. Consumption of CFA at the level indicated in GRN 1043 (2.1 g/day) is therefore not expected to be associated with safety concerns.

[3] OECD (2018). Repeated dose 90-day oral toxicity study in rodents. In: OECD Guidelines for the Testing of Chemicals. (OECD Guideline no 408) [Updated & Adopted: 27 June 2018]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en.

[4] As stated in Section 2.10.1 of the *Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283*, the tiered approach to the safety assessment of food additives [described in the EFSA *Guidance for submission for food additive evaluations* (EFSA ANS Panel, 2012)] is the also the default approach for safety assessment of novel foods.

[5] JECFA (1974). Salts of myristic, palmitic, and stearic acids. In: Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents. Seventeenth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switz. (WHO Food Additives Series No. 5; WHO Technical Report Series, No. 539; Nutrition Meetings Report Series, No. 53). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je03.htm.

QUESTION 9

Question from FDA:

"*On pg. 15, relating to 3-monochloro-2-pro-panediol (3-MCPD) and glycidyl fatty acid esters (GEs) levels, you state: "The results show that amounts of all of these potential contaminants in cetylated fatty acids are far below the EU limits, which is justification for why they are not required in the specifications for cetylated fatty acids.*

We note that your food categories (i.e., ice cream, milk products) contain products expected to be consumed by infants and children as indicated in your dietary exposure assessment. Given that we do not have established limits in the U.S. at this time for GEs and 3-MCPD, please provide a brief narrative on how you are taking steps to monitor and minimize formation of these process contaminants and that potential dietary exposure to these contaminants from your intended use is safe, especially for young children and infants."

Response to Question 9:

Fatty acid esters of glycidol and 3-MCPD are products of heat-processing that have been identified in many types of oil-based foods and food ingredients (MacMahon *et al.*, 2013[6]). Based on a pre-clinical no-observed-effect level of 1.1 mg 3-MCPD/kg body weight/day, and a lack of *in vivo* genotoxicity potential, the Scientific Committee on Food derived a tolerable daily intake (TDI) of 2 µg 3-MCPD/kg body weight/day (EC, 2004[7]). Limits for specific foods were set only for hydrolyzed vegetable protein and soy sauce (20 µg/kg each) based on the contribution of these foods to overall consumption of 3-MCPD (EC, 2006[8]). As shown in GRN 1043, the concentration of 3-MCPD in a representative batch of CFA was <10 µg/kg. Using the calculated 90th percentile consumer-only intake of CFA in infants and young children (46 mg/kg body weight/day) and a concentration of <10 µg 3-MCPD/kg in CFA, a potential maximum intake of <0.00046 µg 3-MCPD/kg body weight/day was calculated. Based on the TDI of 2 µg/kg body weight/day, a margin of safety of >4,348 was calculated, supporting the safety of CFA for consumption by infants and children with respect to potential 3-MCPD content.

With respect to estimated consumption by infants and young children, there were a number of assumptions included in the intakes assessment for CFA which render estimates of exposure to 3-MCPD suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain CFA at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that CFA will have 100% market penetration in all identified food categories. Therefore, the estimation of potential consumption of foods containing CFA by infants and children, while remaining within levels considered to be safe, are conservative and likely to be higher than actual intake levels.

[6] MacMahon S, Begley TH, Diachenko GW (2013). Occurrence of 3-MCPD and glycidyl esters in edible oils in the United States. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 30(12):2081-2092. DOI:10.1080/19440049.2013.840805.
[7] EC (2004). *Collection and Collation of Data on Levels of 3-Monochloropropanediol (3-MCPD) and Related Substances in Food*. (Reports on Tasks for Scientific Cooperation – Report of Experts Participating in Task 3.2.9). European Commission (EC), Directorate-General Health and Consumer Protection. Available at: https://ec.europa.eu/food/system/files/2016-10/cs_contaminants_catalogue_mcpd_scoop_3-2-9_final_report_chloropropanols_en.pdf [June 2004].
[8] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20140701&from=EN.

The European Commission (EC) also has set limits for glycidol in foods; as glycidyl fatty acid esters are hydrolyzed in the gastrointestinal tract to glycidol and the respective fatty acids, these limits are expressed as glycidol. A limit 1,000 µg glycidol/kg has been established for vegetable oils and fats used as ingredients in food products, while a limit of 500 µg/kg has been established for vegetable oils and fats used in baby food and processed cereal-based foods for infants and young children (EU, 2018[9]). No TDI on a body weight basis was identified. The content of glycidyl fatty acid esters (as glycidol) in a representative batch of CFA is within the EC limits for vegetable oils and fats (*i.e.,* 340 µg/kg), and therefore is not considered to pose safety concerns.

As further support for the safety of CFA with respect to 3-MCPD and glycidyl ester content, MacMahon *et al.* (2013) compared the 3-MCPD and glycidyl ester contents of 94 processed oils commercially available in the U.S. Using a liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analytical method, concentrations of 3-MCPD were reported to range from 0.005 to 7.2 mg/kg in 94 refined oils. Concentrations of glycidyl esters were reported to range from below the limit of quantification to 10.5 mg/kg. These levels of 3-MCPD and glycidyl esters identified in commercially available processed oil products are up to 720 and 30.9 times higher than the levels measured in Pharmanutra's CFA ingredient, respectively, indicating that the 3-MCPD and GE levels in CFA are well within the range of processed oil products currently on the market in the U.S. and are not expected to pose safety concerns.

Pharmanutra has provided a declaration from the manufacturer of CFA stating that the ingredient is monitored for the presence of glycidol esters (expressed as glycidol) and 3-MCPD by analysis of the ingredient and review of certificates of analysis for the olive oil raw material. This declaration is provided in Attachment 5.

[9] EU (2018). Commission Regulation (EU) 2018/290 of 26 February 2018 amending Regulation (EC) No 1881/2006 as regards maximum levels of glycidyl fatty acid esters in vegetable oils and fats, infant formula, follow-on formula and foods for special medical purposes intended for infants and young children. Off J Eur Union 61(L55):27-29. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32018R0290&qid=1654024753926.

ATTACHMENT 1
TBHQ Analysis

	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
ALESCO	Date	Revision N°	Issued by	
	30/06/2022	01	QAQC	

Cetilar r.m.	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090C20/01
	BEST BEFORE:	03/2022
	MANUFACTURING DATE:	03/2020

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
TBHQ	<0,0006	%	<0,02%	HPLC-PDA	✓	

Analytical final result: batch n. 1090C20/01 complies with product specification.

Simona Stagi
Quality Assurance & Quality Control

ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	30/06/2022	01	QAQC	

Cetilar r.m.		
	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090I19/01
	BEST BEFORE:	09/2021
	MANUFACTURING DATE:	09/2019

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
TBHQ	<0,0006	%	<0,02%	HPLC-PDA	✓	

Analytical final result: batch n. 1090I19/01 complies with product specification.

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	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	30/06/2022	01	QAQC	

	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090L18/01
	BEST BEFORE:	10/2020
	MANUFACTURING DATE:	10/2018

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
TBHQ	0,00998	%	<0,02%	HPLC-PDA	✓	

Analytical final result: batch n. 1090L18/01 complies with product specification.

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ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	30/06/2022	01	QAQC	

Cetilar r.m.	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090N20/01
	BEST BEFORE:	12/2022
	MANUFACTURING DATE:	12/2020

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
TBHQ	0,0199	%	<0,02%	HPLC-PDA	✓	

Analytical final result: batch n. 1090N20/01 complies with product specification.

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	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	30/06/2022	01	QAQC	

	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	10033E22/01
	BEST BEFORE:	05/2024
	MANUFACTURING DATE:	05/2022

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
TBHQ	<0,00019	%	<0,02%	HPLC-PDA	✓	

Analytical final result: batch n. 10033E22/01 complies with product specification.

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ATTACHMENT 2
Batch Analysis

ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	29/05/2020	5	QAQC	

Cetilar r.m.		
	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090C20/01
	BEST BEFORE:	03/2022
	MANUFACTURING DATE:	03/2020

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
Physical status at 25° C	Solid	--	Solid	Visual	✓	
Acid value	1,1	mgKOH/g	≤ 5,0	AOCS Cd3d-63	✓	
Iodine value	36,3	gI_2/100	30,0 – 50,0	AOCS Cd1-25	✓	
Saponification value	132,8	mgKOH/g	130,0 – 150,0	AOCS Cd3-25	✓	
Colour	<600	APHA	≤ 600	AOCS Ea9-65	✓	
Hydroxyl value	3,5	mgKOH/g	≤ 20,0	AOCS Cd13-60	✓	
Ester content	73,12	% w/w	70 – 80	GC-FID	✓	
Olive oil	25,91	% w/w	20 – 30	GC-FID	✓	
Cetyl Myristate	48,14	% w/w	≥ 15,0	GC-FID	✓	
Alluminium	0,167	ppm	< 2,0	ICP-MS	✓	
Cadmium	0,011	ppm	<1	ICP-Mass	✓	
Lead	<0,005	ppm	<3	ICP-Mass	✓	
Mercury	<0,005	ppm	<0,1	ICP-Mass	✓	
Arsenic	<0,005	ppm	<1	ICP-Mass	✓	
Total Aerobic Microbial Count	<10	CFU/g	$<10^4$	ISO 4833	✓	
Molds and Yeasts	<10	CFU/g	$<10^2$	ISO 21527-1/2	✓	
Enterobacteria	<10	CFU/g	$<= 10^2$	ISO 21528-1/2	✓	
Escherichia coli	Absent	in 1 g	Absent	ISO 16649-3:2015	✓	
Salmonella	Absent	in 25 g	Absent	ISO 6579	✓	
Staphylococcus aureus	Absent	in 25 g	Absent	ISO 6579	✓	

Analytical final result: batch n. 1090C20/01 complies with product specification.

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ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	27/04/2022	5	QC	

Cetilar r.m.	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090C22/01
	BEST BEFORE:	03/2024
	MANUFACTURING DATE:	03/2022

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
Physical status at 25° C	Conform	--	Solid	Visual	✓	
Acid value	0,2	mgKOH/g	≤ 5,0	AOCS Cd3d-63	✓	
Iodine value	34,1	gI_2/100	30,0 – 50,0	AOCS Cd1-25	✓	
Saponification value	136,4	mgKOH/g	130,0 – 150,0	AOCS Cd3-25	✓	
Colour	<500	APHA	≤ 600	AOCS Ea9-65	✓	
Hydroxyl value	7,1	mgKOH/g	≤ 20,0	AOCS Cd13-60	✓	
Ester content	71,17	% w/w	70 – 80	GC-FID	✓	
Olive oil	26,92	% w/w	20 – 30	GC-FID	✓	
Cetyl Myristate	47,44	% w/w	≥ 15,0	GC-FID	✓	
Alluminium	1,28	ppm	< 2,0	ICP-MS	✓	
Cadmium	0,005	ppm	<1	ICP-Mass	✓	
Lead	<0,005	ppm	<3	ICP-Mass	✓	
Mercury	<0,005	ppm	<0,1	ICP-Mass	✓	
Arsenic	<0,005	ppm	<1	ICP-Mass	✓	
Total Aerobic Microbial Count	<10	CFU/g	$<10^4$	ISO 4833	✓	
Molds and Yeasts	<10	CFU/g	$<10^2$	ISO 21527-1/2	✓	
Enterobacteria	<10	CFU/g	$<= 10^2$	ISO 21528-1/2	✓	
Escherichia coli	Absent	in 1 g	Absent	ISO 16649-3:2015	✓	
Salmonella	Absent	in 25 g	Absent	ISO 6579	✓	
Staphylococcus aureus	Absent	in 25 g	Absent	ISO 6579	✓	

Analytical final result: batch n. 1090C22/01 complies with product specification.

Federico Ricci
Quality Control

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ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	22/06/2022	1	QAQC	

Cetilar r.m.		
	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090I19/01
	BEST BEFORE:	09/2021
	MANUFACTURING DATE:	09/2019

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
Physical status at 25° C	Solid	--	Solid	Visual	✓	
Acid value	0,9	mgKOH/g	≤ 5,0	AOCS Cd3d-63	✓	
Iodine value	32,9	gI_2/100	30,0 – 50,0	AOCS Cd1-25	✓	
Saponification value	142,2	mgKOH/g	130,0 – 150,0	AOCS Cd3-25	✓	
Colour	<600	APHA	≤ 600	AOCS Ea9-65	✓	
Hydroxyl value	19,7	mgKOH/g	≤ 20,0	AOCS Cd13-60	✓	
Ester content	71,74	% w/w	70 – 80	GC-FID	✓	
Olive oil	22,89	% w/w	20 – 30	GC-FID	✓	
Cetyl Myristate	47,06	% w/w	≥ 15,0	GC-FID	✓	
Alluminium	0,627	ppm	< 2,0	ICP-MS	✓	
Total Aerobic Microbial Count	<10	CFU/g	$<10^4$	ISO 4833	✓	
Molds and Yeasts	<10	CFU/g	$<10^2$	ISO 21527-1/2	✓	
Enterobacteria	$<10^2$	CFU/g	$<= 10^2$	ISO 21528-1/2	✓	
Escherichia coli	Absent	in 1 g	Absent	ISO 16649-3:2015	✓	
Salmonella	Absent	in 25 g	Absent	ISO 6579	✓	
Staphylococcus aureus	Absent	in 25 g	Absent	ISO 6579	✓	

Analytical final result: batch n. 1090I19/01 complies with product specification.

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Quality Assurance & Quality Control

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ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	25/11/2021	01	QAQC	

Cetilar r.m.	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090M21/01
	BEST BEFORE:	11/2023
	MANUFACTURING DATE:	11/2021

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
Physical status at 25° C	Solid	--	Solid	Visual	✓	
Acid value	1,0	mgKOH/g	≤ 5,0	AOCS Cd3d-63	✓	
Iodine value	33,8	gI_2/100	30,0 – 50,0	AOCS Cd1-25	✓	
Saponification value	133,8	mgKOH/g	130,0 – 150,0	AOCS Cd3-25	✓	
Colour	285	APHA	≤ 600	AOCS Ea9-65	✓	
Hydroxyl value	14,0	mgKOH/g	≤ 20,0	AOCS Cd13-60	✓	
Ester content	75,0	%	70 – 80	GC-FID	✓	
Olive oil	25,0	%	20 – 30	GC-FID	✓	
Cetyl Myristate	46,2	%	≥ 15,0	GC-FID	✓	
Alluminium	1,16	ppm	< 2,0	ICP-MS	✓	
Cadmium	<0,005	ppm	<1	ICP-Mass	✓	
Lead	<0,005	ppm	<3	ICP-Mass	✓	
Mercury	<0,005	ppm	<0,1	ICP-Mass	✓	
Arsenic	<0,005	ppm	<1	ICP-Mass	✓	
Total Aerobic Microbial Count	<10	CFU/g	$<10^4$	ISO 4833	✓	
Molds and Yeasts	<10	CFU/g	$<10^2$	ISO 21527-1/2	✓	
Enterobacteria	<10	CFU/g	$<= 10^2$	ISO 21528-1/2	✓	
Escherichia coli	Absent	in 1 g	Absent	ISO 16649-3:2015	✓	
Salmonella	Absent	in 25 g	Absent	ISO 6579	✓	
Staphylococcus aureus	Absent	in 25 g	Absent	ISO 6579	✓	

Analytical final result: batch n. 1090M21/01 complies with product specification.

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ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	15/02/2021	5	QAQC	

Cetilar r.m.		
	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090N20/01
	BEST BEFORE:	12/2022
	MANUFACTURING DATE:	12/2020

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
Physical status at 25° C		--	Solid	Visual	✓	
Acid value	1	mgKOH/g	≤ 5,0	AOCS Cd3d-63	✓	
Iodine value	40	gI_2/100	30,0 – 50,0	AOCS Cd1-25	✓	
Saponification value	137,2	mgKOH/g	130,0 – 150,0	AOCS Cd3-25	✓	
Colour	<500	APHA	≤ 600	AOCS Ea9-65	✓	
Hydroxyl value	6,6	mgKOH/g	≤ 20,0	AOCS Cd13-60	✓	
Ester content	71,43	%	70 – 80	GC-FID	✓	
Olive oil	27,63	%	20 – 30	GC-FID	✓	
Cetyl Myristate	46,35	%	≥ 15,0	GC-FID	✓	
Alluminium	0,96	ppm	< 2,0	ICP-MS	✓	
Cadmium	<0,020	ppm	<1	ICP-Mass	✓	
Lead	<0,020	ppm	<3	ICP-Mass	✓	
Mercury	<0,020	ppm	<0,1	ICP-Mass	✓	
Arsenic	<0,020	ppm	<1	ICP-Mass	✓	
Total Aerobic Microbial Count	<10	CFU/g	$<10^4$	ISO 4833	✓	
Molds and Yeasts	<10	CFU/g	$<10^2$	ISO 21527-1/2	✓	
Enterobacteria	<10	CFU/g	$<= 10^2$	ISO 21528-1/2	✓	
Escherichia coli	Absent	in 1 g	Absent	ISO 16649-3:2015	✓	
Salmonella	Absent	in 25 g	Absent	ISO 6579	✓	
Staphylococcus aureus	Absent	in 25 g	Absent	ISO 6579	✓	

Analytical final result: batch n. 1090N20/01 complies with product specification.

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Quality Assurance and Quality Control

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ATTACHMENT 3
Heavy Metals Analysis

ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	30/06/2022	01	QAQC	

Cetilar r.m.		
	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090C20/01
	BEST BEFORE:	03/2022
	MANUFACTURING DATE:	03/2020

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
Alluminium	0,167	ppm	< 2,0	ICP-Mass	✓	
Cadmium	0,011	ppm	<1	ICP-Mass	✓	
Lead	<0,005	ppm	<3	ICP-Mass	✓	
Mercury	<0,005	ppm	<0,1	ICP-Mass	✓	
Arsenic	<0,005	ppm	<1	ICP-Mass	✓	

Analytical final result: batch n. 1090C20/01 complies with product specification.

Simona Stagi
Quality Assurance & Quality Control

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ALESCO	CERTIFICATE OF ANALYSIS			Pag. 1 di 1
	Date	Revision N°	Issued by	
	30/06/2022	01	QAQC	

Cetilar r.m.		
	PRODUCT NAME:	CETILAR® R.M
	PRODUCT CODE:	S01301R
	BATCH:	1090M21/01
	BEST BEFORE:	11/2023
	MANUFACTURING DATE:	11/2021

ANALYTICAL RESULTS

TEST DESCRIPTION	RESULT	UNIT	SPECIFICATION	METHOD	COMPLINAT	NOT COMPLINAT
Alluminium	1,16	ppm	< 2,0	ICP-Mass	✓	
Cadmium	<0,005	ppm	<1	ICP-Mass	✓	
Lead	<0,005	ppm	<3	ICP-Mass	✓	
Mercury	<0,005	ppm	<0,1	ICP-Mass	✓	
Arsenic	<0,005	ppm	<1	ICP-Mass	✓	

Analytical final result: batch n. 1090M21/01 complies with product specification.

Simona Stagi
Quality Assurance & Quality Control

This Certificate of Analysis has been produced electronically and is valid without signature

ATTACHMENT 4
Certificate of Validation

Validation parameters:

Selectivity and Specificity: Parameter not applicable. The ICP-MS equipment has a resolution of 1/250 UMA, so the overlap of a signal adjacent to the analyte is impossible. Furthermore, the system is equipped with a double system of interferences abatement: helium reaction cell and energy discriminator. These devices make the existence of molecular compounds in the detector impossible.

Detection Limit: L.D. = (Standard. Deviation. Reagent blank *3) *See Table 1*

Quantification Limit: L.Q. = (Standard. Deviation. reagent blank *6) *See Table 1*

Table 1 (L.D. & L.Q.) **Scope of application**: **Validated matrices as reported in the Test method M-05/ICP-MS**

Element	Li	Be	B	Al	Sc	Ti	Cr	V	Co	Ni	Cu	Zn	As
L.D. µg/L	0,009	0,004	0,018	0,017	0,022	0,031	0,009	0,008	0,005	0,021	0,035	0,028	0,032
L.Q. µg/L	0,018	0,007	0,036	0,033	0,045	0,062	0,017	0,017	0,011	0,043	0,071	0,055	0,064

Element	As in	As or	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn
L.D. µg/L	0,022	0,050	0,021	0,014	0,011	0,033	0,009	0,009	0,014	0,018	0,016	0,010	0,010
L.Q. µg/L	0,045	0,100	0,042	0,028	0,022	0,066	0,018	0,019	0,028	0,035	0,031	0,020	0,020

Element	Sn or	Sb	Te	Ba	Hg	MHg	Pb	Tl	Bi	U			
L.D. µg/L	0,011	0,007	0,018	0,017	0,030	0,032	0,007	0,018	0,005	0,054			
L.Q. µg/L	0,021	0,014	0,037	0,033	0,060	0,065	0,014	0,036	0,011	0,109			

The limits of quantification reported in the Test Reports are referred to food matrix and they are rounded up to 0,005 mg/kg.

Table 1b (L.D. & L.Q.) **Scope of application**: **Waters**

Element	Li	Be	B	Al	Sc	Ti	Cr	V	Co	Ni	Cu	Zn	As
L.Quant µg/L	1,0	1,0	1,0	5,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Element	As in	As or	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn
L.Quant µg/L	1,0	1,0	1,0	1,0	1,0	5,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Element	Sb	Te	Ba	Hg	Pb	Tl	Bi	U					
L.Quant µg/L	1,0	1,0	1,0	0,2	1,0	1,0	1,0	1,0					

Range: 0,005 mg/kg <= Range <= 1000 mg/kg, for all the elements, the limit of 1000 mg/kg is determined by the opportunity to make dilutions that do not compromise the precision of the result.

Linearity: All the elements have a linearity reported as correlation coefficient $R^2 > 0,999$

The minimum condition of $R^2 > 0,995$ is more than satisfactory.

Precision: Limits of repeatability summarized in *Table 2*.

Table 2: Relative limits of repeatability

Element	Li	Be	B	Al	Sc	Ti	Cr	V	Co	Ni	Cu	Zn	As
Rel. limit % Repeatability high levels	8,9	12,3	12,6	8,3	10,0	11,0	8,7	5,8	10,9	3,6	10,5	9,4	11,3
Rel. limit % Repeatability medium levels	9,6	12,7	12,7	12,2	12,2	14,0	12,3	14,3	14,8	12,9	9,2	10,5	10,0
Rel. limit % Repeatability low levels	13,3	16,3	7,2	14,2	15,9	12,8	12,7	15,3	12,9	10,3	14,5	12,3	12,8

Element	As in	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn	Sn or
Rel. limit % Repeatability high levels	7,5	6,8	3,8	12,6	12,5	11,0	11,8	11,1	5,7	4,0	9,0	10,2	7,7
Rel. limit % Repeatability medium levels	8,0	8,3	11,3	11,4	11,8	10,8	12,7	11,9	14,5	12,8	12,3	15,2	11,6
Rel. limit % Repeatability low levels	12,0	7,4	11,3	13,6	11,9	13,2	12,7	11,6	14,0	12,7	11,5	18,2	17,9

Element	Sb	Te	Ba	Hg	MHg	Pb	Tl	Bi	U				
Rel. limit % Repeatability high levels	8,0	12,5	6,2	7,4	10,3	4,4	5,5	6,6	7,5				
Rel. limit % Repeatability medium levels	16,2	13,9	18,2	8,3	8,8	12,5	12,6	5,0	5,4				
Rel. limit % Repeatability low levels	16,4	13,6	18,6	8,2	14,6	10,2	12,9	16,0	12,2				

All the limits of repeatability are rounded up to 20%.

Accuracy: Mean Recovery and T test summarized in *Table 3*.

Table 3: Mean Rec. % & T-test (T limit = 2,26)

Element	**Li**	**Be**	**B**	**Al**	**Sc**	**Ti**	**Cr**	**V**	**Co**	**Ni**	**Cu**	**Zn**	**As**
Mean Rec %	100,8	99,5	98,7	98,0	102,6	100,4	98,7	99,2	101,0	101,6	99,1	99,0	102,5
T-test < 2,26	0,71	0,57	1,49	1,44	1,66	0,35	1,54	1,20	1,31	1,63	1,44	1,08	1,38

Element	**As in**	**Se**	**Mo**	**Mn**	**Fe**	**Sr**	**Zr**	**Rb**	**Ag**	**Pd**	**Cd**	**Sn**	**Sn or**
Mean Rec %	101,0	100,5	101,3	99,2	101,0	98,8	99,7	101,6	98,2	99,0	99,0	100,5	102,0
T-test < 2,26	0,60	0,46	1,24	1,06	0,88	0,99	0,27	1,57	1,14	0,53	0,83	0,30	1,14

Element	**Sb**	**Te**	**Ba**	**Hg**	**MHg**	**Pb**	**Tl**	**Bi**	**U**				
Mean Rec %	100,2	100,8	96,3	99,0	99,0	98,7	100,4	100,4	100,1				
T-test < 2,26	0,15	0,61	1,61	0,72	1,14	1,11	0,33	0,39	0,10				

Sensitivity: The slope of the calibration curve for each element is summarized in *Table 4*.

Table 4: Sensitivity (Sens) cps/µg/l

Element	**Li**	**Be**	**B**	**Al**	**Sc**	**Ti**	**Cr**	**V**	**Co**	**Ni**	**Cu**	**Zn**	**As**
Sen. Cps/µg/l	9920	12347	3529	4290	3575	3501	10467	8880	15599	4226	2522	2358	2491

Element	As in	Se	Mo	Mn	Fe	Sr	Zr	Rb	Ag	Pd	Cd	Sn	Sn or
Sen. Cps/µg/l	2491	1248	4473	5239	2618	10371	10653	7717	4879	4907	5446	8745	8745

Element	Sb	Te	Ba	Hg	MHg	Pb	Tl	Bi	U				
Sen. Cps/µg/l	9581	3254	4097	556	556	8852	6247	15838	15694				

Traceability: In this procedure the traceability is attributable to the certificate of guarantee and expiration of the reference material where the concentration of the analyte has a tolerance of 0,2%.

Robustness: Parameter not applicable. During the mineralisation phase, the maximum temperature is the boiling temperature of HNO3 (or NH4OH for I and Br), temperature lower at the evaporation temperature of the minerals contained in the matrix. In case of too low temperature of the mineraliser, the redox reaction with the matrix would not be triggered and the matrix would remain visually unchanged.
The ICP-MS system has a sensitivity higher than what is necessary to satisfy the quantification limits that are all rounded up. The procedure foresees minimum usage conditions, that, if satisfied, enable the correct execution of the analyses. This is all demonstrated in the verification of the instrumental performances in the relevant control chart.

Expanded Uncertainty:

The expanded uncertainty applied for each parameter is that calculated with the Horwitz / Thompson model, because it is considered the most correct estimated uncertainty for a multiresidual determination at different concentrations, unless otherwise indicated by the customer or provided for by legislative / mandatory aspects

Nature of the modifications:

- *Page 1: introduced Table 1b with the specific limits of quantification for the water matrix*
- *Iodine and Bromine parameters removed from all tables.*

Approval:

Considering the specifications defined during the planning phase and the experimental results obtained applying the test method M-05/ICP-MS "Determination of metals in ICP-MS",

The test method is considered validated and applicable by the Laboratory.
Thus, the method is approved by the Management.

Laboratory Director:

Alberto Gatti Date: 14/05/2021

ATTACHMENT 5
Manufacturer Declaration

A.& A. Fratelli Parodi s.p.a.
www.fratelliparodi.it

R.E.A. MI 2098402
R.E.A. GE 125281
R.E.A. IM 138058
Cod. Fisc. e Part. IVA 00248230104
Cap. Soc. € 1.500.000,00 I.V.

DECLARATION

To Whom It Concerns

A&A Fratelli Parodi S.p.A. herewith declares that for the product LIPOCET, quality controls on olive oil raw material are carried out as follows:

-analysis of glycidol esters expressed as glycidol
-analysis of 3-MCPD

This control is performed both by A&A Fratelli Parodi QC laboratory and by the documentary review of the certificates of analysis of the raw material of our supplier.

Campomorone – Genova – Italia 27/05/2022
Dr. F. Scalzullo – Quality Office
A&A Fratelli Parodi Spa