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#898

By Federal Express

November 7, 2019

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

Re: GRAS Notice for the Use of Anhydrous Milk Fat (AMF) in Exempt Infant Formula

Dear Sir or Madam:

We hereby submit the enclosed GRAS notice for AMF as an ingredient in exempt infant formula. AMF is intended for use as a source of fat in exempt infant formula for term infants with calorically dense formula needs and/or requiring a fluid restriction. The maximum intended use of AMF in exempt infant formula for term infants with calorically dense formula needs or requiring a fluid restriction is 7.0% of the fat blend by weight. Hogan Lovells US LLP's conclusion of GRAS status for the intended use of AMF in exempt infant formula is based on scientific procedures in accord with 21 CFR §170.30(a) and (b).

AMF is not intended for use in any products that would require additional regulatory review by the United States Department of Agriculture. The GRAS notice does not contain any designated confidential business information. In accordance with the Agency's guidelines, we have enclosed Form 3667, one original copy of the GRAS notice, and one complete electronic copy of the GRAS notice on a compact disk (CD).

We are committed to cooperating with the Agency and believe an open dialog is one of the most effective ways to accomplish that objective. If any questions arise in the course of your review, please contact us, preferably by telephone or e-mail, so that we can provide a prompt response.

If you have any questions, please contact us.

Sincerely,

Steven B. Steinborn
steven.steinborn@hoganlovells.com
202 637 5969

Xin Tao
xin.tao@hoganlovells.com
202 637 6986

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000898	Jan 9, 2020
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

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

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd):* _____

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person Steven B. Steinborn		Position or Title Partner	
Organization *(if applicable)* Hogan Lovells US LLP			
Mailing Address *(number and street)* 555 13 ST NW			
City Washington	State or Province District of Columbia	Zip Code/Postal Code 20004	Country United States of America
Telephone Number 202 637 5969	Fax Number 202 637 5910	E-Mail Address steven.steinborn@hoganlovells.com	

Name of Contact Person		Position or Title	
Organization *(if applicable)*			
Mailing Address *(number and street)*			
City	State or Province	Zip Code/Postal Code	Country
Telephone Number	Fax Number	E-Mail Address	

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Anhydrous Milk Fat (AMF)

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

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☒ Paper

If applicable give number and type of physical media

1 CD

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

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

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

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

AMF is intended for use as a source of fat in exempt infant formula for term infants with calorically dense formula needs and/or requiring a fluid restriction. The maximum intended use of AMF in exempt infant formula for term infants with calorically dense formula needs or requiring a fluid restriction is 7.0 wt% of the fat blend

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

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

☐ Yes ☒ No

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

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Hogan Lovells US LLP

(name of notifier)

has concluded that the intended use*(s)* of Anhydrous Milk Fat (AMF)

(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Hogan Lovells US LLP agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

(name of notifier)

555 13th St, NW, Washington DC

(address of notifier or other location)

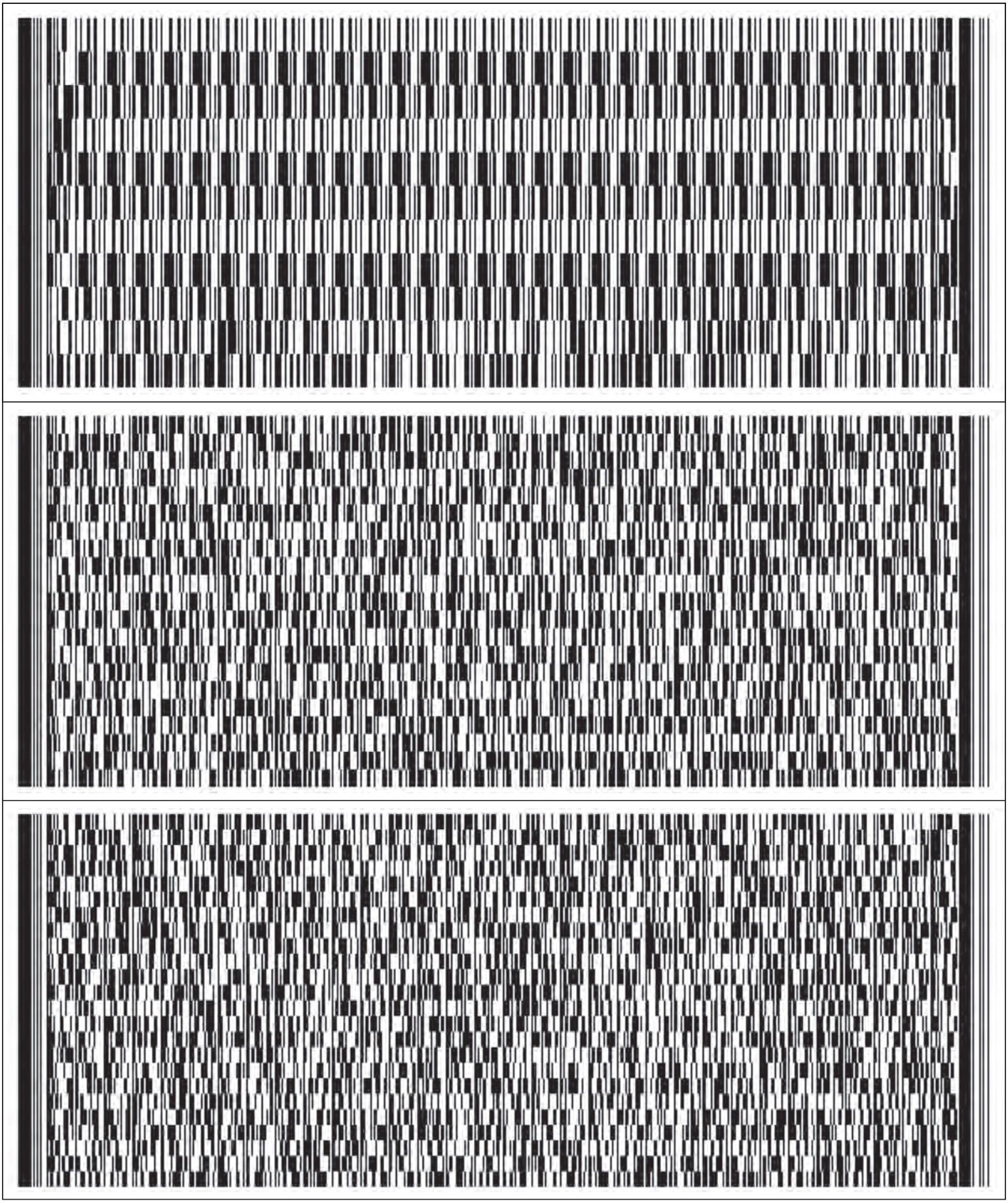
The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

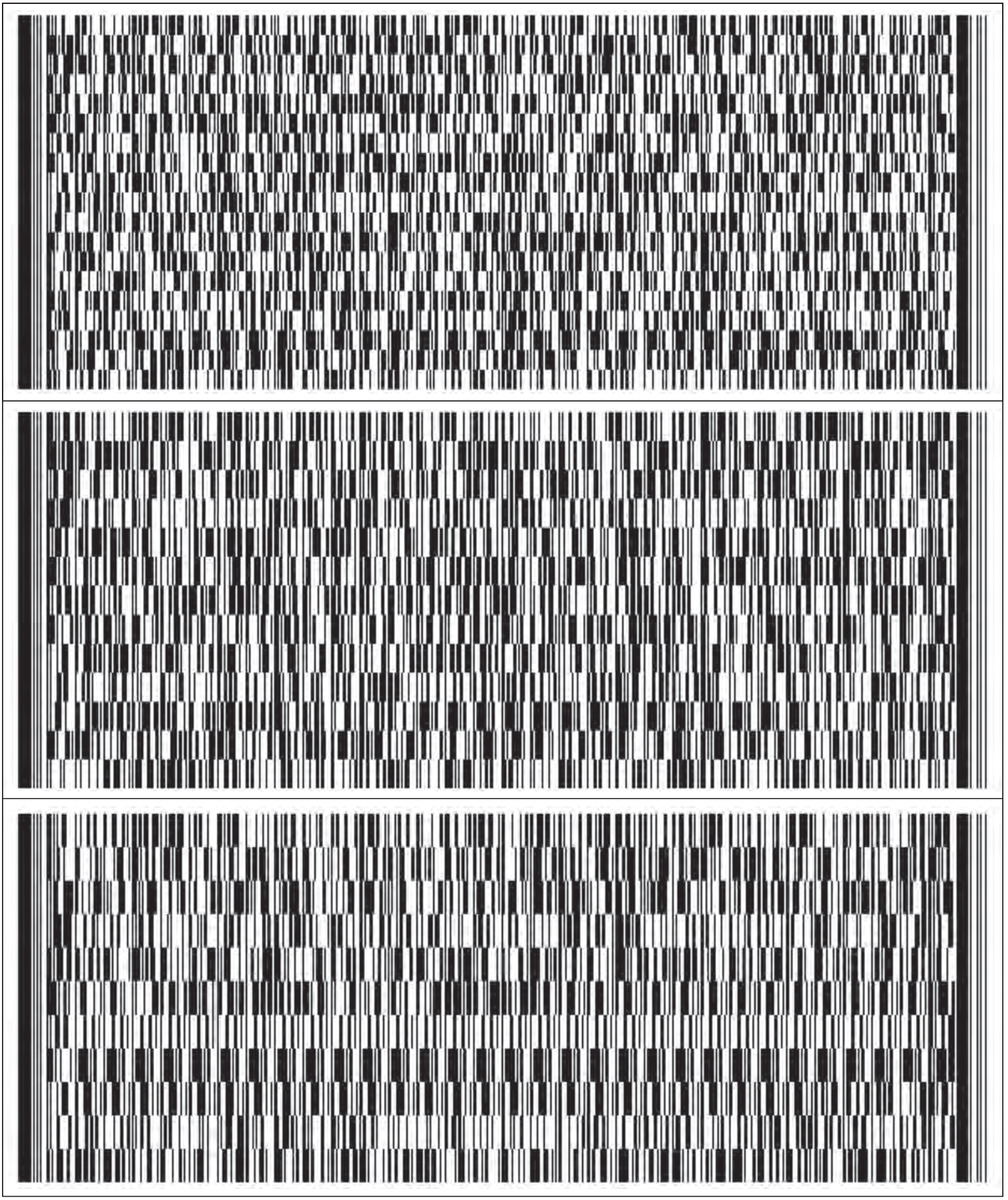
3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Steve Steinborn, Partner	11/06/2019

SECTION G – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Appendix A. Analytical Data on AMF	Submission
	Appendix B. Statement of Quality Assurance	Submission
	Appendix C. Certificates of Analysis on 5 Lots of AMF	Submission
	Appendix D. Monitoring for Potential Contaminants	Submission
	Appendix E. PubMed Literature Searches	Submission
	Appendix F. Supportive Data on Concentrations of Components in Human Milk	Submission
	Appendix G. Animal Studies of the Effect of Milk Fat in Infant Formula	Submission





GRAS Conclusion for the Use of Anhydrous Milk Fat (AMF) in Exempt Infant Formula

SUBMITTED BY:

Hogan Lovells US LLP.
555 13th St NW
Washington, DC 20004

SUBMITTED TO:

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
5001 Campus Drive
College Park, MD 20740

CONTACT FOR TECHNICAL OR OTHER INFORMATION:

Hogan Lovells US LLP.
555 13th St NW
Washington, DC 20004

November 7, 2019

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List of Acronyms

°C	degrees Celsius
µg	microgram
µm	micrometer
%	percent
ADI	acceptable daily intake
ALA	a-linoleic acid
AMF	anhydrous milk fat
ARA	arachidonic acid
BCFA	branched-chain fatty acid
bw	body weight
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CFU	colony forming unit
cGMP	current Good Manufacturing Practice
CLA	conjugated linoleic acid
d	day
DHA	docosahexaenoic acid
DL	dairy lipids
EC	European Commission
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
F	female
FA	fatty acid
FDA	U.S. Food and Drug Administration
FOIA	Freedom of Information Act
g	gram
GI	gastrointestinal
GRAS	Generally Recognized As Safe
GRN	GRAS Notice
h	hour
kcal	kilocalorie
kg	kilogram
LA	linoleic acid
LCPUFA	long chain polyunsaturated fatty acid
LOQ	limit of quantification
LSRO	Life Sciences Research Office
M	male

MFGM	milkfat globule membrane
mg	milligram
mL	milliliter
n	number
NA	not applicable
NLT	not less than
NMT	not more than
NOAEL	no observed adverse effect level
NOEL	no observed effect level
PAH	polyaromatic hydrocarbon
PICU	pediatric intensive care unit
PCB	Polychlorinated biphenyl
ppm	parts per million
RBC	red blood cell
RCT	randomized controlled trial
SCFA	short-chain fatty acid
SD	Standard deviation
SRC	Sulphite Reducing Clostridia
TFA	trans fatty acid
U.S.	United States
USDA	U.S. Department of Agriculture
VL	vegetable lipids
VO	vegetable oil
wk	Week
y	year

Part 1: Signed Statements and Certification

Hogan Lovells US LLP submits to the U.S. Food and Drug Administration (FDA) this generally recognized as safe (GRAS) notice in accordance with 21 CFR part 170, subpart E.

Name and Address of Notifier

Hogan Lovells US LLP
555 13th St NW
Washington, DC 20004

Name of GRAS Substance

The substance that is the subject of this GRAS notice is anhydrous milk fat (AMF).

Intended Use and Consumer Exposure

AMF is intended for use as a source of fat in exempt infant formula for term infants with calorically dense formula needs and/or requiring a fluid restriction. The maximum intended use of AMF in exempt infant formula for term infants with calorically dense formula needs or requiring a fluid restriction is 7.0% of the fat blend by weight.

Basis for Conclusion of GRAS Status

Hogan Lovells US LLP's conclusion of GRAS status for the intended use of AMF in exempt infant formula is based on scientific procedures in accord with 21 CFR §170.30(a) and (b).

Pre-Market Approval Exclusion Claim

The intended use of AMF in exempt infant formula is not subject to the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act because Hogan Lovells US LLP has concluded that such use is generally recognized as safe (GRAS) through scientific procedures.

Availability of Information

The data and information that serve as the basis for this GRAS conclusion, as well as the information that has become available since the GRAS conclusion, will be sent to the FDA upon request, or are available for the FDA's review and copying during customary business hours at the office of Hogan Lovells US LLP located at:

555 13th St NW

Washington, DC 20004

Exemptions from Disclosure

It is our view that none of the data and information in Parts 2 through 7 of the GRAS notice are exempt from disclosure under the Freedom of Information Act (FOIA).

Certification Statement

On behalf of Hogan Lovells US LLP, I hereby certify that, to the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

11/07/2019

Name: Steven B. Steinborn

Date

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

Identity

The substance of this dossier is anhydrous milk fat (AMF).

Composition of Typical AMF

AMF is derived from cow's milk and consists nearly entirely of milk fat through processes that result in almost complete removal of water and nonfat solids. As defined by the Codex Alimentarius Commission's standard CODEX STAN 280-1973, AMF contains at least 99.8% milk fat and a maximum of 0.1% water. The Code of Federal Regulations (7 CFR §58.347) for AMF, which defines specifications for finished products to bear USDA Official Identification, also specifies limits of at least 99.8% milk fat and a maximum of 0.1% water.

The lipids in cow's milk are present predominantly in the form of globules approximately 0.1 to 15 μm in diameter (Huerou-Luron et al., 2018; Illingworth et al., 2009). These fat globules are surrounded by the milk fat globule membrane (MFGM), which consists of phospholipids including glycerolphospholipids and sphingolipids, enzymes, proteins, glycolipids, total and partial glycerides, free fatty acids and cholesterol (Avalli and Contarini, 2005; Cavaletto et al., 2008; Contarini and Milena Povolo, 2013). In the production of AMF, the MFGM is disrupted and lipid in the form of triglycerides is separated from the MFGM and its constituents as well as other components in milk, resulting in the AMF product that typically contains a minimum of 99.8% total solids in the form of triglycerides or little or no components from the MFGM (Huppertz and Kelly, 2006). Analysis of AMF shows that the AMF fraction of milk contains less than 0.01% phospholipids and less than 0.1% each of phospholipid and sphingolipid present in raw milk from which AMF is derived (Rombaut et al., 2006).

Representative levels of key fatty acids in AMF are shown in Table 1 below. Concentrations of fat-soluble vitamins are also summarized in Table 1.

Table 1. Typical Composition of Anhydrous Milk Fat (AMF)

Nutrient	Typical Concentration based on Analytical Data (mean, range) [a]
Moisture, g/100 g	0.07 (0.06 - 0.07)
Fat, g/100 g	100
C4:0, Butyric	2.46 (2.43 - 2.50)

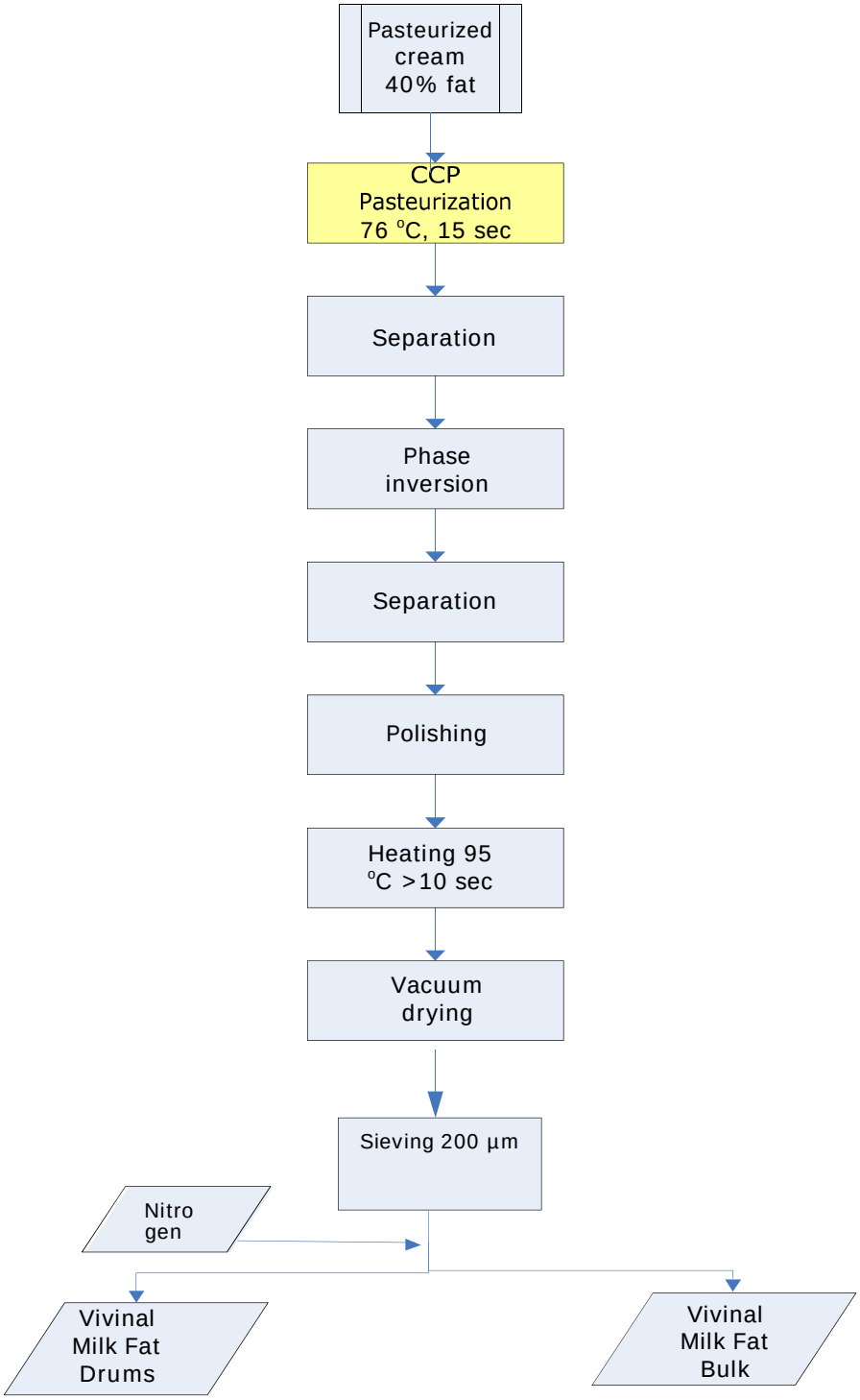
Nutrient	Typical Concentration based on Analytical Data (mean, range) [a]
C6:0, Caproic	2.11 (2.09 - 2.12)
C8:0, Caprylic	1.35 (1.34 - 1.35)
C10:0, Capric	3.28 (3.27 - 3.28)
C12:0, Lauric	4.26 (4.23 - 4.31)
C14:0, Myristic	12.23 (12.16 - 12.29)
C16:0. Palmitic	33.85 (33.73 - 33.98)
C18:0, Stearic	10.14 (10.10 - 10.18)
C18:1, Oleic	19.05 (18.96 - 19.19)
C18:2, Linoleic	1.45 (1.44 - 1.46)
C18:3, α-Linolenic	0.57 (0.56 - 0.58)
trans fatty acids	3.07 (2.98 - 3.12)
Cholesterol, mg/100 g	236 (232 - 241)
Vitamin A, mcg/100 g	879 (834 - 909)
Vitamin E, mg/100 g	1.97 (1.95 – 2.00)
Vitamin K1, mcg/100 g	9.0
[a]Analytical data; see Appendix A.	

The majority of fatty acids in AMF are saturated fatty acids, with palmitic, myristic, and stearic acids accounting for the largest proportions of saturated fatty acids. Milk fats such as AMF are further characterized by a relatively high proportion of short-chain and medium-chain saturated fatty acids (C4:0 - C12:0). Unsaturated fatty acids, primarily in the form of oleic acid, largely account for the balance. AMF is also a source of cholesterol and fat-soluble vitamins including vitamin A, vitamin E, and vitamin K.

Method of Manufacture

AMF is made from dairy cream or butter using physical processes to extract water and non-fat dry matter (Tamime 2009). The typical process beginning with cream is presented in Figure 1. In this method of manufacture, AMF is produced from pasteurized cream (40% fat) that is heated and concentrated to high-fat cream by separation of water. This step is followed by phase inversion of the oil-in-water emulsion to a water-in-oil emulsion, further concentration, vacuum drying to remove residual moisture, and sieving. The final product is blanketed with nitrogen. No additives or preservatives are use in the production of AMF and it is manufactured in compliance with quality and food safety management processes consistent with current Good Manufacturing Practices (cGMP) as noted in Appendix B.

Figure 1. Flow Diagram of the Production of Anhydrous Milk Fat



Product Specifications

The specifications for AMP intended for use in infant formula are listed in Table 2. The specifications include limits on fat, moisture, and microbiological parameters. Codex specifications for AMF include limits on fat (minimum of 99.8%) and moisture (maximum of 0.1%), which are identical to the specifications of AMF intended for use in infant formula as shown in Table 2. The Codex specifications also include recommended quality factors for free fatty acids (maximum of 0.3%), and peroxide value (maximum of 0.3 Meq O2/kg) which are consistent with quality factor specifications for the ingredient.

Certificates of analysis from non-consecutive batches of AMF (Table 3; Appendix C) demonstrate that the AMF meets food-grade specifications for the physio-chemical parameters of fat, moisture, free fatty acids, and peroxide value, and microbiological parameters established for the ingredient including anaerobic plate counts, Enterobacteriaceae, Sulphite Reducing Clostridia, mold, yeast, thermophilic thermoresistant sporeformers (aerobic and anaerobes), Bacillus cereus, and Salmonella. Analytical data on non-consecutive batches of AMF provide additional evidence that the product meets the specified limits (Appendix A). The manufacturer routinely monitors AMF and reports that samples from 2019 show non-detectable levels of *E. coli* (<10, below detection limit) and *Listeria monocytogenes* (negative) (Appendix D). The AMF is monitored periodically to ensure to ensure fatty acid concentrations are within specific limits; these ranges and representative data are shown in Table 4.

Table 2. Specifications for Anhydrous Milk Fat (AMF)

Parameter	**Unit**	**Limit**	**Test Method**
Physico-Chemical Parameters			
Fat	% (as solids)	Min 99.8	Calculate by difference
Moisture	%	Max 0.1	Karl Fischer-STD 023-2002 ISO
Free fatty acids (as oleic acid)	%	Max 0.3	IDF 6B 1989
Peroxide Value	Meq O_2/kg fat	Max 0.3	IDF 74A 1991
Microbiological Parameters			
Anaerobic plate count 30°C	/g	< 500	ISO 4833-1
Anaerobic plate count 55°C	/g	< 2500	ISO 4833
Enterobacteriaceae	/g	non detectable	DIN ISO 21528-1
Mold	/g	Max 10	ISO 6611
Yeast	/g	Max 10	ISO 6611
Staphylococci Aureus	/g	non detectable	ISO 6888-1 AMD 1
Salmonella	/25 g	non detectable	EN ISO 6579+A1
Thermophilic Thermoresistant Sporeformers, Aerobic	/g	Max 100	NEN 6809 –(100°C-30min)
Thermophilic Thermoresistant	/g	Max 100	NEN 6809 –(100°C-30min)

Parameter	Unit	Limit	Test Method
Sporeformers, Anaerobe			
Sulphite Reducing Clostridia	/g	Max 5	ISO 15213
Bacillus cereus	/g	Max 50	ISO 7932

Table 3. Analytical Results of Physico-Chemical and Microbiological Parameters in Non-Consecutive Batches of Anhydrous Milk Fat (AMF)

Parameter	**Unit**	**Limit**	**BB0XW1H**	**BB0Z9ZN**	**BB0XJBS**	**BB0Z7VD**	**BB0ZLR2**
Physico-Chemical Parameters							
Fat	% (as solids)	Min 99.8	99.9	99.9	99.9	99.9	99.9
Moisture	%	Max 0.1	0.1	0.1	0.1	0.1	0.1
FFA (as oleic acid)	%	Max 0.3	0.2	0.2	0.2	0.2	0.2
Peroxide value	Meq O_2/kg	Max 0.3	0.2	0.2	0.2	0.2	0.2
Microbiological Parameters							
Anaerobic plate count 30°C	CFU/g	<500	<500	<500	<500	<500	<500
Anaerobic plate count 55°C	CFU/g	<2500	<2500	<2500	<2500	<2500	<2500
Enterobacteriaceae	/g	non detectable	Absent	Absent	Absent	Absent	Absent
Yeast & Mold	CFU/g	Max 10	<10	<10	<10	<10	<10
Staphylococci Aureus	/g	non detectable	Absent	Absent	Absent	Absent	Absent
Salmonella	/25 g	non detectable	Absent	Absent	Absent	Absent	Absent
Thermophilic Aerobic and Anaerobic Spores	CFU/g	Max 100	<100	<100	<100	<100	<100
Sulphite Reducing Clostridia	CFU/g	Max 5	<1	<1	<1	<1	<1
Bacillus cereus	CFU/g	Max 50	<10	<10	<10	<10	<10
CFU – colony forming unit, LOQ – limit of quantification. See Appendix C.							

Table 4. Analytical Results of Fatty Acids in Samples of Anhydrous Milk Fat (AMF)

Parameter	Unit	Limit	Batch BBoWTLS	Batch BBoWT5I	Batch BBoWBZ7
C4:0, Butyric acid	% of fatty acids	2.40 to 4.50	2.43	2.50	2.44
C6:0, Caproic acid	% of fatty acids	1.60 to 3.00	2.11	2.09	2.12
C8:0, Caprylic acid	% of fatty acids	1.1 to 2.10	1.35	1.34	1.35
C10:0, Capric acid	% of fatty acids	2.40 to 4.50	3.28	3.28	3.27
C12:0, Lauric acid	% of fatty acids	2.6 to 6.75	4.31	4.24	4.23
C14:0, Myristic acid	% of fatty acids	9.2 to 18.00	12.29	12.25	12.16
C16:0, Palmitic acid	% of fatty acids	23 to 46.50	33.98	33.84	33.73
C18:0, Stearic acid	% of fatty acids	9.7 to 12.0	10.10	10.18	10.15
C18:1w9, Oleic acid	% of fatty acids	18 to 33.75	19.01	18.96	19.19
C18:2w6, Linoleic acid (LA)	% of fatty acids	1.4 to 2.0	1.44	1.46	1.45
C18:3w3, a-Linolenic acid (ALA)	% of fatty acids	0.48 to 0.90	0.56	0.58	0.57
Trans fatty acids	g/100 g fatty acids	Max 4.5	2.98	3.10	3.12
See Appendix A.					

Table 5. Analytical Results of Potential Contaminants in Samples of Anhydrous Milk Fat (AMF)

Parameter	Unit	Limit	Batch BBoWTLS	Batch BBoWT5I	Batch BBoWBZ7
Aflatoxin M1	ppb	Max 0.1	<0.005^	<0.005^	<0.005^
Mercury	ppb	20	<1	<1	<1
Lead	ppb	50	7	6	6
Cadmium	ppb	10	<1	<1	<1
Total arsenic	ppb	100	<15	<15	<15
Inorganic arsenic	ppb	75	<30	<30	<30
Non dioxin like PCBs (ndl PCBs), sum of	Ndl/g fat	Max 20	1.880	1.990	2.020
Dioxins and Furans WHO (2005)-PCDD/F TEQ (upper bound)	pg TEQ/g fat	Max 2.5	0.298	0.325	0.327
Sum WHO(2005)-PCDD/F + dl-PCBs TEQ (upper bound)	pg TEQ/g fat	Max 5.5	0.539	0.573	0.569
Activity Cs 134	Bq/kg	Max 370	<10^	<10^	<10^
Activity Cs 137	Bq/kg	Max 370	<10^	<10^	<10^

See Appendix A.
^ - limit of detection.

Analytical data from three non-consecutive batches of AMF also demonstrate that the product meets appropriate limits for environmental contaminants including aflatoxin M1, heavy metals, polychlorinated biphenyl (PCB) compounds, dioxins, radioactive substances, and polyaromatic hydrocarbons (PAHs) to ensure a safe food ingredient (Table 5 and Appendix A). Additional analytical data show that "other pesticide residues" are present at a level below the limit of detection.

Milk and dairy products produced by the supplier providing the AMF are routinely monitored to ensure that potential contaminants of concern, including metals (e.g., lead, arsenic, cadmium, mercury), aflatoxins, radioactive substances, PCB compounds, dioxins, PAHs, and veterinary drugs meet appropriate specifications to ensure that all milk and milk-derived products are food-grade. The specifications established for the monitoring program established by the supplier and documentation demonstrating that sample products meet the specifications are provided in Appendix D.

Technical Effect

Lipids are the predominant source of energy for infants, accounting for approximately 45-55% of total energy intake in human milk and formula (Koletzko et al. 2005). Infant formulas are produced using a combination of fat sources, predominantly vegetable oils, designed to mimic the fatty acid pattern and absorption of human milk (Heird 2007). The intended technical effect of AMF in calorically-dense, ready-to-feed exempt infant formula is to contribute fatty acids primarily in the form of palmitic acid to the fat blend.

Part 3. Dietary Exposure

Proposed Use and Level

The intended use of AMF in infant formula is to provide a source of fat. The proposed maximum use of AMF in exempt infant formula for term infants with calorically dense formula needs and/or requiring a fluid restriction is 7.0% of the fat blend. Calorically dense infant formula provides 100 kcal 100 mL while standard infant formulas and human milk typically provide 67 kcal per 100 mL and 65 kcal per 100 mL, respectively (Green Corkins and Shurley, 2016; IOM, 2005).

Estimated Daily Intakes

Formula Intake

The contribution of fat to total energy intake of human milk or formula is approximately 48 to 50% (IOM, 2005; Martin et al., 2016). The daily intake of AMF from the proposed use in calorically dense formula was estimated assuming (1) the fat blend accounts for 50% of total energy in the formula, which provides a conservatively high estimate of energy as fat, (2) a maximum concentration of 7.0% AMF by weight in the fat blend, and (3) formula intake representative of intakes among the population of term infants requiring a calorically dense infant formula or with fluid restrictions.

Formula intake among populations of term infants administered calorically dense infant formula has been examined in clinical trials and in a retrospective study of infants in the pediatric intensive care unit (PICU). These data can be used to estimate intake of AMF from the proposed use in calorically dense infant formula.

As summarized in Table 6, the target intake of calorically dense formula, as documented in the identified published literature, ranges from 130 kcal per kilogram bodyweight per day (kcal/kg bw/day) while in the intensive care unit to 200 kcal/kg bw/day over longer periods of intake (i.e., 3-6 weeks). Target daily formula intakes in interventions spanning multiple weeks were based on estimated energy needs on a per kg bw basis with stress factors to support catch-up growth, such as the factors of 1.5 to 2.0 times basal metabolic needs as recommended in the Schofield equations (e.g., Clarke et al., 2007; Eveleens et al., 2018).

Reported intake of formula by infants in the identified clinical studies was consistently lower than the targeted intake. Among the two 5-day interventions, mean formula intake was 119 kcal/kg bw/day in one study and between 55 to 120 kcal/kg bw/day in the second (Cui et al., 2017; de Betue et al., 2011). In the retrospective study, mean formula intake was reported at 105 kcal/kg bw/day (Eveleens et al., 2018), which is consistent with daily formula intake at baseline in an unpublished study (INGROTO, 2012). Based on these four studies, intake of formula at a level of 120 kcal/kg bw/day provides a conservative estimate of typical intake. This estimate of intake is consistent with reference energy needs of 113 to 123 kcal/kg bw/day for catch-up growth in children assuming a rate of gain of 10 g/kg bw/day (IOM, 2005; Table 5-32). Calorically dense term infant formulas provide 100 kcal per 100 mL; therefore, 120 kcal/kg bw/day is equivalent to 120 mL/kg bw/day of formula.

The 6-week intervention reported higher intakes, with a median formula intake of 140 kcal/kg bw/day and intakes ranging from 103 to 175 kcal/kg bw/day (Clarke et al., 2007). The highest achieved formula intake of 175 kcal/kg bw per 24 h in the 6-week intervention provides a conservative estimate for evaluating high infant formula intake and in turn, constituents in the formula. With a caloric density of 100 kcal per 100 mL, intake of 175 kcal/kg bw/day is equivalent to 175 mL/kg bw/day of formula.

Table 6. Formula Intake in Studies of Term Infants Consuming a Calorically Dense Infant Formula

Study	Study Population; Number of infants on test formula; Duration of Intervention	Age (mean ± SD) / Bodyweight (bw) at Baseline	Target Daily Formula Intake	Reported Daily Formula Intake
de Betue et al., 2011 (also van Waardenburg et al., 2009)	Infants admitted to the pediatric intensive care unit with respiratory failure due to viral bronchiolitis n = 8; 5 days	age: 2.7 ± 1.4 months bw: 3.97 ± 0.94 kg	130 kcal/kg bw/day	Mean reported intake (day 5): 119±25 kcal/kg bw/day Range of intake: 105-147% of recommended intake for energy (as cited by Butte 2005)
Clarke et al., 2007	Infants with faltering growth due to cardiac lesions, cystic fibrosis, or other causes n = 26; 6 weeks	age: 5.6 (2.4 - 31.0) months (median, range) bw: Not reported	150-200 kcal/kg bw/day (based on Schofield equation with factors for catch up growth)	Median: 140 kcal/kg bw/day Range of intake: 103-175 kcal/kg bw/day
Cui et al.,	Infants admitted to	age: 4.69 ± 3.54	130 kcal/kg	Range of intake: 55-

Study	Study Population; Number of infants on test formula; Duration of Intervention	Age (mean ± SD) / Bodyweight (bw) at Baseline	Target Daily Formula Intake	Reported Daily Formula Intake
2017	cardiac intensive care unit after congenital heart surgery n = 26; 5 days	months bw: 5.24 ± 1.66 kg	bw/day	120 kcal/kg bw/day
Eveleens et al., 2018	Retrospective study of infants admitted to a pediatric intensive care unit n = 76; 30 (21-54) days on formula (median, interquartile range)	age: 76 (30-182) days bw: 3.94 (3.29-5.80) kg (median, interquartile range)	2 x calculated resting energy requirement (based on Schofield equation for weight)	Mean reported intake: 104.6 ± 19.4 kcal/kg bw/day
INGROTO, 2012 (un-published)	Infants requiring calorically dense formula, including: congenital heart disease, chronic lung disease, non-organic failure to thrive, or other conditions n = 14; 12 weeks	age: 19.7 ± 8.2 weeks at screening bw: 4.29 ± 1.04 kg at baseline	No target intake recommendation; intake was based on clinical practice.	105 kcal/kg bw/day at baseline

Based on data in these clinical studies, the estimated daily intake of calorically dense infant formula for the average or typical infant therefore is assumed to be 120 kcal/kg bw/day, while the estimated daily intake of calorically dense infant formula for an infant representative of a high consumer is assumed to be 175 kcal/kg bw/day. The estimate of typical intake of the calorically dense formula (120 kcal/kg bw/day) in this assessment is consistent with mean formula intake for formula-fed infants with the highest intake per kg bw as reported by Fomon (1993), namely 121.1 kcal/kg bw/day for boys age 14-27 days. Fomon reported a 90th percentile formula intake by this male population of 141.3 kcal/kg bw/day. The estimate of high intake of the calorically dense formula of 175 kcal/kg bw/day exceeds a conservatively high average intake among healthy infants by a factor of up to 1.5 (175 kcal/kg bw/day vs 120 - 140 kcal/kg bw/day), which is a reflection of the higher energy needs of the target population.

Anhydrous Milk Fat (AMF) Intake

Assuming the fat blend accounts for 50% of total energy in the formula and AMF is a maximum of 7.0% in the fat blend, AMF provides 0.39 g per 100 kcal formula. This estimate is calculated

assuming that for each 100 kcal formula, 50% of energy (i.e., 50 kcal) is provided by fat. At 9 kcal/g, each 100 kcal of formula contains approximately 5.56 g of fat; 7% of the fat is therefore equivalent to 0.39 g. The estimated intake of AMF is 0.47 g/kg bw/day for an infant consuming formula at a typical rate of 120 mL/kg bw/day, and 0.68 g/kg bw/day for an infant consuming formula at a high rate of 175 mL/kg bw/day (Table 7).

The calorically dense infant formula is intended for infants weighing up to 9 kg. Assuming this maximum body weight, consumption of formula at the typical rate of 120 mL/kg bw/day will deliver 4.2 g AMF and consumption of formula at the high rate of 175 mL/kg bw/day will deliver 6.1 g AMF. For infants weighing in the range of 4-5 kg (e.g., 4.5 kg), intake of AMF is estimated at 2.1 or 3.1 g for a typical or high consumer of the infant formula, respectively.

Table 7. Estimated Daily Intake of Anhydrous Milk Fat from the Maximum Proposed Use

Calorically Dense Formula Intake			**AMF Intake** [c]
Level of intake	**kcal/kg bw/day**	**Total fat (g/kg bw/day)** [b]	**g/kg bw/day**
Typical [a]	120	6.7	0.47
High	175	9.7	0.68

[a] 100 kcal per 100 mL
[b] Assume fat accounts for 50% of kcal, and 9 kcal per gram of fat
[c] Assume maximum use of 7.0% AMF in fat blend

Part 4. Self-Limiting Levels of Use

AMF is intended for use as a component of the fat blend in exempt infant formula for term infants requiring a calorically dense formula and/or fluid restriction at a concentration not to exceed 7.0% of the fat blend by weight. We are not aware of technological or palatability issues associated with the proposed use levels. Self-limiting levels of use are not applicable to this notice.

Part 5. Experience Based on Common Use in Food before 1958

The conclusion of GRAS status of the use of AMF as a component of the fat blend in exempt infant formula for term infants requiring a calorically dense formula and/or fluid restriction is based upon scientific procedures.

Part 6. Narrative

Approach for Assessing Safety

AMF is a food with a long history of safe consumption. As detailed in this notification, AMF is intended for use as a component of the fat blend in exempt infant formula. Like the vegetable oils commonly used in infant formulas, AMF is composed almost entirely of fatty acids, and while some constituents present in AMF are not present in the oils typically used in infant formulas, a review of available data and information demonstrates that the constituents in AMF are present in human milk in comparable or higher concentrations, and under the intended conditions of use, exposures to the constituents by infants consuming infant formula with AMF or human milk fall within a similar range. A series of literature searches was conducted to identify information pertinent to the safety review and included searches of PubMed, the U.S. Food and Drug Administration (FDA), European Food Safety Authority (EFSA), JECFA, Codex, and general searches of the Internet (see Appendix E for PubMed search terms).

Nutritional Role of Lipids in Infant Formula

Human milk is recognized as the gold standard for infant feeding though infant formulas are a Lipids are the predominant source of energy for infants, accounting for approximately 45-55% of total energy intake in human milk and formula (Delphanque et al. 2015). These lipids are present predominately (>95%) in the form of triglycerides, i.e., three fatty acids esterifed to a glycerol backbone. The source of fat in infant formulas is typically a blend of vegetable oils, although fat sources may also include fats such as dairy fat, single cell oils, fish oils, egg phospholipids, and structured lipids designed to mimic the fatty acid profile and absorption of human milk, which is recognized as the gold standard for infant feeding (Green Corkins and Shurley, 2016; Delphanque et al. 2015).

Fat and Essential Fatty Acid Requirements of Infant Formula

Nutrient requirements for infant formula in the United States include limits on the level of protein, fat, and the essential fatty acid linoleic acid, and concentrations of micronutrients (21 CFR §107.100) (Table 8). The regulations specify that infant formulas provide between 3.3 and 6.0 g total fat per 100 kcal and that fat accounts for 30 to 54% of the energy in formula. Additionally, infant formula must contain a minimum of 300 mg linoleic acid per 100 kcal and a minimum of 2.7% of calories. These limits on energy and fat are also applicable to exempt infant formulas unless the infant formula is not generally available at the retail level (i.e.,

accessible through medical prescription for dietary management of specific diseases) and the formulation meets the necessary quality factors (21 CFR §107.50). Infant formula nutrient requirements do not specify limits on other fatty acids or cholesterol, nor do the requirements specify which fat sources may or may not be used.

Since establishment of most nutrient requirements in infant formula in the U.S. as detailed by FDA (21 CFR §107.100), the Life Sciences Research Office (LSRO) Expert Panel (1998) reviewed the available evidence and recommended that infant formulas for term infants provide 4.4 to 6.4 g fat per 100 kcal, 8 to 35% of total fatty acids as linoleic acid, 1.75 to 4.0% fat as α-linolenic acid, and a ratio of linoleic acid to α-linolenic acid of at least 6:1 and not more than 16:1. The recommendation to include specifications for α-linolenic acid resulted from evidence indicating that α-linolenic acid is a precursor for the formation of n-3 long chain polyunsaturated fatty acids, including docosahexaenoic acid (DHA) (Table 8). Codex and European Commission standards for infant formula include similar though not identical specifications for total fat, essential fatty acids, and lauric and myristic fatty acid content, while both standards limit *trans* fatty acids to no more than 3% of total fatty acids (Table 8). The Codex specifications for infant formula prohibit the use of hydrogenated fats and oils and EC standards prohibit the use of cottonseed oil and sesame seed oil. These international regulations and the FDA do not provide further guidance on the suitability of specific fats and oils for use in infant formula that would prohibit use of AMF in infant formula.

Table 8. Fat and Fatty Acid Requirements in Infant Formula for Term Infants

Reference	**Limits on Total Fat per 100 kcal**	**Limits on Linoleic Acid (LA)**	**Limits on α-Linolenic Acid (ALA)**	**Limits on ratio of LA:ALA**	**Lauric + myristic acids**	***Trans* fat**
21 CFR §107.100 Regulation for US, term infant formula	3.3-6.0 g (30-54% of calories)	minimum of 300 mg/100 kcal (minimum of 2.7% of calories)	-	-	-	-
LSRO 1999 Recommendations from LSRO for FDA, term infant formula	4.4-6.4 g	8-35% of total fatty acids (350-2240 mg/100 kcal)	1.75-4% of total fatty acids (77-256 mg/100 kcal)	6:1 to 16:1	-	do not use hydrogenated oils; limit substance to the extent possible
Codex Stan 72-1981, rev 2007 International	4.4-6.0 g	minimum of 300 mg/100 kcal;	minimum of 50 mg/100	5:1 to 15:1	≤20% of total fatty acids	≤3% of total fatty acids

Reference	Limits on Total Fat per 100 kcal	Limits on Linoleic Acid (LA)	Limits on α-Linolenic Acid (ALA)	Limits on ratio of LA:ALA	Lauric + myristic acids	*Trans* fat
standard for standard formula and formula for special medical purposes [a]		guidance upper level of 1400 mg/100 kcal	kcal; maximum not specified			
Current: Directive 2006/141/EC [b] EC for standard formula; also for special medical purposes [c]	4.4-6.0 g	300-1200 mg/100 kcal	≥ 50 mg/100 kcal	5:1 to 15:1	≤20% of total fat content, separately or as a whole	≤3% of total fatty acids
Forthcoming: Commission Delegated Regulation (EU) 2016/127; Regulation (EU) No 609/2013 [d] EC Standard and for special medical purposes	4.4-6.0 g	500-1200 mg/100 kcal	50-100 mg/100 kcal	-	-	≤3% of total fatty acids

[a] Unless modified to meet special nutritional requirements arising from the disease(s), disorder(s) or medical condition(s) for whose dietary management the product is specifically formulated, labelled and presented.
[b] In effect to 21 February 2020; then repealed by Delegated Regulation (EU) 2016/127.
[c] Unless modified to meet special medically-determined nutritional requirements.
[d] In effect from 22 February 2020 (replaces Directive 2006/141/EC).

Digestion, Absorption and Excretion of Fat by the Infant

Lipids are the predominant source of energy for infants, accounting for approximately 45-55% of total energy intake in human milk and formula (Delphanque et al., 2015), and these lipids are present predominately (>95%) in the form of triglycerides. Triglycerides contain a glycerol backbone to which three fatty acids are esterified. The location of the fatty acid on the glycerol backbone is referred to by stereospecific numbering, with the end positions identified as sn-1 and sn-3 (the α positions), and the middle position identified as sn-2 (the β position).

In human milk, approximately 98% of the fat is present in form of triglycerides (Innis, 2011), with saturated and unsaturated fatty acids esterified to a glycerol backbone. Triglycerides in human milk and infant formula differ in the distribution of long-chain saturated fatty acids. The

predominant saturated fatty acid in human milk is palmitic acid (16:0) and approximately 70% or more of the palmitic acid is esterified in the sn-2 position (Innis 2011). In contrast, only 5-20% of the palmitic acid in vegetable oils is esterified in the sn-2 position (Innis 2011), while milk fat is more similar to human milk in that approximately 40% of palmitic acid is in the sn-2 position (Berger et al., 2000).

Digestion of triglycerides in infants begins with the secretion of gastric lipase from gastric mucosal cells. The lipase hydrolyzes fatty acids from the sn-3 position of the triglyceride, leaving sn-1,2 diacylglycerols (Innis, 2011). Pancreatic colipase-dependent lipase released from the pancreatic acinar cells then hydrolyzes the sn-1,3 ester linkages, resulting in a sn-2 monoacylglycerol and unesterified fatty acids products (Innis 2011). Additional lipases released from the pancreas (carboxyl ester lipase and pancreatic lipase related protein 2), lipase secreted from the mammary gland (milk bile salt-stimulated lipase), and salivary lipases may also contribute to digestion of lipids (Innis 2011; Hamosh 1996; Zou et al., 2016). The triglyceride digestion products cross the apical membranes of the enterocytes and are reassembled into triglycerides, packaged into chylomicrons, and secreted into circulation (Innis, 2011). Fatty acids of shorter length including butyric, lauric and myristic acids, are absorbed through the gastric wall, enter the portal vein, and are subsequently transported to the liver where they are oxidized and used as an energy source (Bugaut, 1987; Jensen and Jensen, 1992). Absorption of shorter chain fatty acids is dependent on molecular weight, with higher absorption for lower weight fatty acids.

Results from a study using a static two-phase *in vitro* digestion model to mimic digestion in the gastric and duodenal phases of digestion demonstrate that the total fatty acid release of vegetable and bovine fat-based infant formula is similar to human milk (Hageman et al. 2019(a)). The mature human milk samples in this experiment came from four healthy women collected 3 to 8 months following delivery, while the infant formulas were powder-based formulas with fat blends containing vegetable fat (palm, palm kernel, rapeseed, and sunflower oil) or a combination of vegetable and bovine fat (67% bovine milk fat and 33% of rapeseed, sunflower, and coconut oil). The energy content and macronutrient composition of the two formulas was similar. After the gastric phase of *in vitro* digestion, lipolysis of the milk triglycerides was similar between both formulas and the human milk; however, the percent of free fatty acids as a percent of total esterified fatty acids was significantly higher for both formulas compared to the human milk samples. After the duodenal phase of digestion, a greater percentage of initial triglycerides remained intact among the human milk samples compared to the vegetable fat formula but was not significantly different from the vegetable and bovine fat-based formulas; this difference was observed after 90 min of digestion and at no other time point. During the early stages of duodenal digestion (35-45 minutes), the percent of free fatty acids as percent of total esterified fatty acids from the vegetable and bovine fat-based formula had a significantly

lower release of fatty acids compared to the vegetable-based formula. The vegetable and fat based formula was also significantly lower than the human milk sample at 45 minutes into digestion only. However, total fatty acid release at the end of the duodenal digestion was similar across all samples. The release of individual fatty acids was also significantly different between both infant formulas and the human milk. Release of C4:0 was significantly higher in the vegetable and bovine fat-based formula while release of C11:0 was significantly lower compared to the human milk. Release of C18:1, C18:2, and C18:3 were significantly higher in the human milk compared to the vegetable and bovine milk samples, while C18:2 and C18:3 were significantly higher in the human milk compared to the vegetable fat formulas. Release of C18 fatty acids was not significantly different between the two formulas. Both infant formulas had a greater release of long chain saturated fatty acids compared to human milk, which could have implications for calcium absorption because of the potential for formation of long chain saturated fatty acids/calcium complexes.

AMF contributes fatty acids primarily in the form of palmitic acid, oleic acid, myristic acid, and short-chain fatty acids. A healthy full-term infant has a functional digestive system at birth, though digestive enzymes may be present at a lower level compared to levels in older infants (reviewed in Zou et al., 2016). The nutrient dense formula in which AMF is intended to be used as a component of the fat blend is a high-energy formulation intended for use in term infants with a functional or partially functional gastrointestinal tract in the absence of comorbidities affecting metabolism. Term infants consuming the calorically dense formula with AMF would reasonably digest and metabolize triglycerides as do other term infants consuming human milk or standard infant formula.

Fats Commonly Used in Infant Formula and their Fatty Acid Profiles

Infant formulas typically contain a blend of vegetable oils, potentially in combination with other oils and fats such as dairy fat to achieve a desired fatty acid profile that typically mimics the composition and absorption of key fatty acids in human milk. Commonly used vegetable oils in infant formula currently available in the U.S. include soy, high oleic safflower, high oleic sunflower, palm olein, palm, and coconut (Green Corkins and Shurley, 2016).

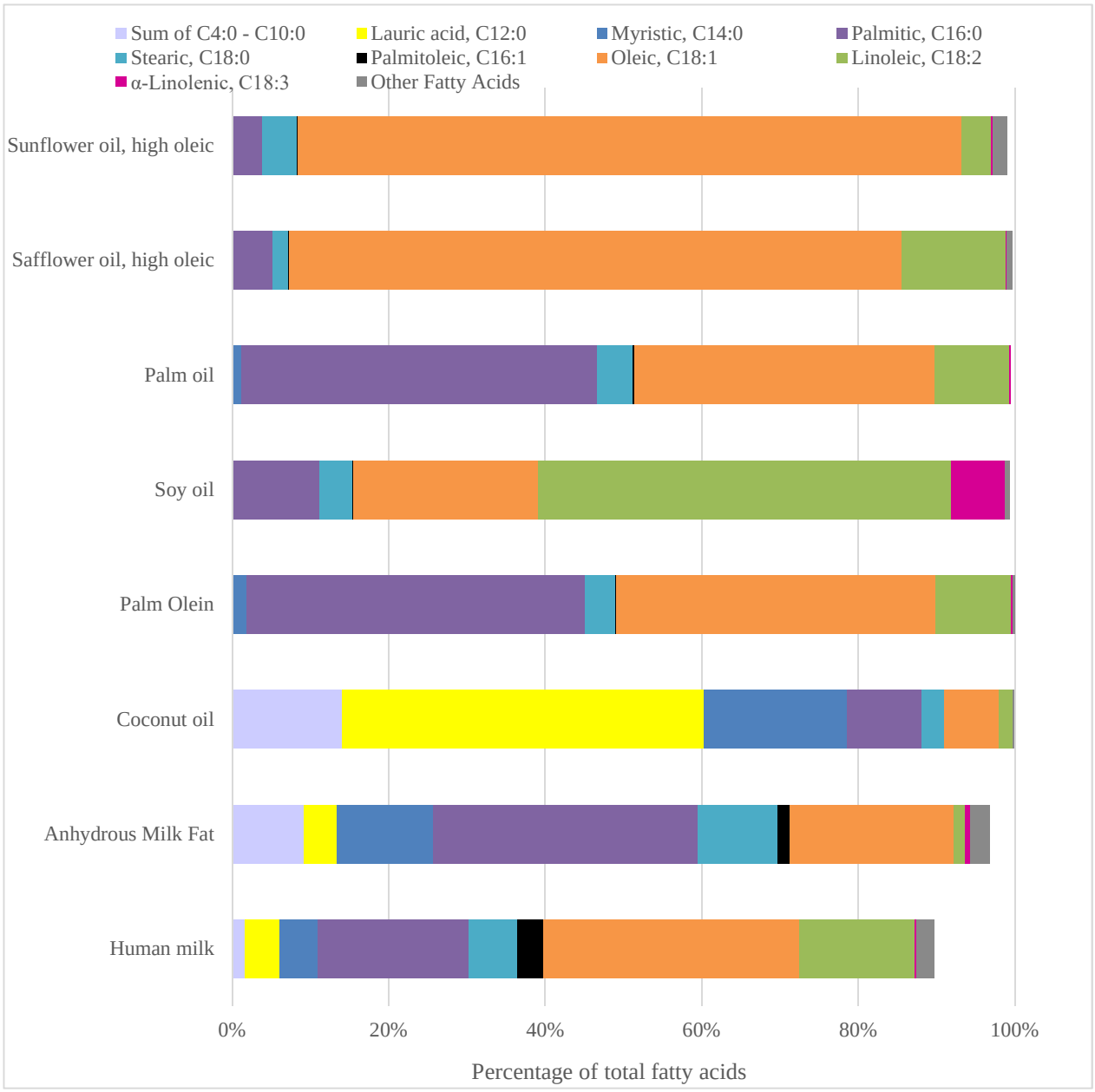
Milk and milk products including AMF have a long history of use in the U.S. food supply, including consumption by toddlers and use for infants. Historically infants not provided human milk may have consumed evaporated milk or sweetened condensed milk, although this practice largely disappeared in the 1970s with the introduction of vegetable oil-based formulas (Innis, 2011; Jensen and Jensen, 1992). The use of vegetable oils in infant formulas rather than milk fat provided higher concentrations of unsaturated fatty acids, avoided a potential source of dioxins,

resolved concerns around the odor of regurgitated butterfat and perceptions of constipation resulting from feeding evaporated milk, and helped control cost (Hageman et al., 2019b).

The current literature indicates that milk fat components including milk fat and/or the milk fat globule membrane (MFGM) can be found in infant formulas used in numerous global markets. For example, a review of infant formulas in Brazil reports that milk fat accounts for 20% of the lipid component in routine infant formulas and 2-3% of the fat content in formulas for specific infant populations (Mendoca et al., 2017). Formulas in China commonly contain cow's milk or goats' milk blended with vegetable oils to better mimic the fatty acid composition of human milk (Sun et al., 2016; Sun et al., 2018).

The concentration of key fatty acids in AMF and vegetable oils commonly used in infant formula are summarized in Figure 3, with human milk included for reference. The fatty acid profile of human milk is distinct from the profile of AMF and the vegetable oils commonly used in infant formula, thus supporting the practice of blending various fat sources to achieve the desired profile in infant formula. Relative to many vegetable oils, AMF is a concentrated source of palmitic acid, stearic acid, palmitoleic acid, and short and medium chain fatty acids, which are among the predominant fatty acids in human milk.

Figure 2. Typical Fatty Acid Profile in Human Milk and Fats Used in Infant Formula Fat Blends



Notes:
Sum of C4:0 - C10:0 for human milk does not include C4:0, C6:0 fatty acids.
Palmitoleic acid content is the sum of undifferentiated C16:1; Oleic acid content is the sum of undifferentiated C18:1; Linoleic acid content is the sum of undifferentiated C18:2. Alpha-linolenic acid content unavailable for palm and sunflower oil; value assumed to be sum of undifferentiated 18:3 for these oils.
Sources: USDA National Nutrient Database for Standard Reference for Soybean (04669), Safflower (04511), Sunflower (04584), and Coconut (04047) Oil; Human milk: Yuhas et al., 2006; Palm Olein: Tarmizi et al., 2007; Anhydrous Milk Fat: see Table 1.

Components in Milkfat Not Found in Vegetable Oils

As previously noted, the lipids in cow's milk are present predominantly in the form of globules, and these globules are surrounded by the MFGM resulting from secretion of the epithelial cells of the mammary gland (Hueron-Luron et al., 2018). The MFGM consists of phospholipids including glycerolphospholipids and sphingolipids, enzymes, proteins, glycolipids, total and partial glycerides, free fatty acids and cholesterol (Avalli and Contarini, 2005; Cavaletto et al., 2008; Contarini and Povolo, 2013). The MFGM is disrupted in the production of AMF and lipid in the form of triglycerides is separated from the MFGM and its constituents as well as other components in milk, resulting in the AMF product that typically contains a minimum of 99.8% total solids in the form of triglycerides and little or no components from the MFGM (Huppertz and Kelly, 2006; Illingworth et al., 2009). AMF is a source of fatty acids present in vegetable oils, though as shown in Figure 3, the relative proportions of specific fatty acids differs between milk fat and common vegetable oils.

In addition to differences in the percent contribution of specific fatty acids, milk fat is a source of some components not found in vegetable fats. In cow's milk, approximately 45% (w/w) of fatty acids are synthesized *de novo* and the remainder are from dietary sources (MacGibbon and Taylor, 2006; Moore and Christie, 1979). Fatty acids synthesized *de novo* include short- and medium-chain fatty acids, with short-chain fatty acids ranging from 3 to 7 carbons thus including butyrate (C:4), and medium-chain fatty acids ranging in chain length from 8 to 13 carbons (FAO, 2010). These straight-chain and even-numbered fatty acids and branched- and odd-chain fatty acids are synthesized in the mammary glands or in ruminal bacteria using precursors that arise from microbial fermentation in the rumen (Jenkins, 1993). Fatty acids in cow's milk arising from ruminant biohydrogenation also include *trans* fatty acids and conjugated linolenic acid.

Vegetable oils typically used in infant formulas are not a source of short-chain fatty acids (i.e., butyrate), branched- and odd-chain fatty acids, *trans* fatty acids, and conjugated linolenic acid naturally present in milk fat. Infant formulas containing milk fat in the fat blend therefore provide fat components that are not present in formula made solely with vegetable oils. Infant formulas may contain other milk-derived ingredients, however, and milk fat components may be present in low concentrations in infant formula as a residue from other these milk-derived ingredients. Infant formulas made with vegetable oils and milk-derived ingredients such as skimmed milk are estimated to contain as much as 4% residual milk fat (Berger et al., 2000), thus exposing infants to components naturally present in dairy fat.

Milk fat and other bovine-derived fats (e.g., beef fat) are a typical component of the diet, and lactating women consuming milk fat and beef fat may transfer components from these foods to

their infants via human milk. Breastfeeding infants therefore are routinely exposed to milk- and beef-derived components including the components in milk fat not found in vegetable oils, namely short-chain fatty acids (i.e., butyrate), branched- and odd-chain fatty acids, *trans* fatty acids, and conjugated linolenic acid.

Butyric Acid

Butyric acid (C4:0) is a saturated, short-chain fatty acid (SCFA) found primarily in dairy products. Approximately 30% of triglycerides in cow's milk contain butyric acid, with butyric acid typically in the sn-3 position and two long-chain fatty acids in the sn-1 and sn-2 positions (Berger et al., 2000). Butyric acid is also a by-product of dietary carbohydrate fermentation by the gut intestinal microbiota (Mu and Hoy, 2004) and is absorbed in the colon (Cummings, 1995).

Fatty acids of shorter length including butyric acid are rapidly released from triglycerides by lipases. The SCFA are absorbed into enterocytes via passive diffusion and then enter into portal circulation and are subsequently transported to the liver where they are oxidized and used as an energy source (Bugaut, 1987; Jensen and Jensen, 1992; Wang et al., 2013). SCFAs in milk fat therefore may provide an easily metabolizable energy source for the infant (Berger et al., 2000).

The typical concentration of butyric acid in AMF is 2.46% of total fatty acids. Assuming AMF will compose 7% of the fat used in infant formula, butyric acid will account for up to 0.17% of the total fatty acid content in infant formula. Butyric acid is not detected in all analyses of human milk, though concentrations in the range of 0.0009 to 0.76% of total fatty acids have been reported (Appendix F). The proposed concentration of butyric acid in the infant formula fat blend therefore is within the range of concentrations of the component reported in the fatty acid component of human milk. Accounting for a potentially higher energy intake of the calorically dense formula on a bodyweight basis (i.e., a factor of 1.5), the infant's exposure to butyric acid from the intended use in infant formula is within the range of butyric acid intake estimated from human milk. The presence of butyric acid from the intended use of AMF in infant formula does not present a safety concern.

Trans-fatty Acids

Trans fatty acids (TFAs) are unsaturated fatty acids with at least one double bond in a *trans* configuration. These fatty acids which are produced by biohydrogenation in ruminants (including cows) are referred to as natural or ruminant *trans* fats. Vaccenic acid, an isomer of oleic acid, is also produced in the rumen and is the principal ruminant *trans* fatty acid;

concentrations in cow's milk range from 2 to 6% of total fatty acids (MacGibbon and Taylor, 2006). Vaccenic acid can be converted to rumenic acid, the *cis*-9,*trans*-11 isomer of conjugated linoleic acid (CLA) (Bauman and Lock, 2006). *Trans*-palmitoleic acid, C16:1n-7t (also known as 16:1t9, or elaidic acid), is produced by ruminant biohydrogenation and accounts for approximately 0.06% (w/w) of the total milk fatty acid content in dairy fat (Chouinard et al., 1998).

TFA may also be produced in the hydrogenation of oils. The TFA produced in this manner are referred to as artificial or industrial *trans* fats. Hydrogenated oils have not been used in infant formula and *trans* fats have recently been eliminated from use in the U.S., while other countries such as Canada have called on the food industry to voluntarily reduce concentrations of industrial trans fats in processed foods.

The mean concentration of TFA in AMF is 3.07% of total fatty acids. Assuming 7% of the fat in infant formula is present as AMF and all fat is in the form of fatty acids, the TFA content in formula from AMF will be 0.21% of the total fatty acid content. Guidelines for infant formula composition from CODEX and the European Commission limit the concentration of *trans* fatty acids to no more than 3% of total fatty acids. The estimated concentration of TFA in infant formula from the proposed use of AMF is therefore below the limits set by international bodies.

For breastfeeding infants, the composition of human milk reflects the mother's diet, including the presence of low levels of ruminant-derived TFA due to the consumption of a typical and balanced diet. Current dietary guidance acknowledges that small concentrations of natural *trans* fatty acids are present in dairy products and meat and notes that these foods can be an important source of nutrients and do not need to be eliminated (DHHS/USDA, 2015). The concentration of ruminant-derived TFA in recent samples of human milk from women in Canada after elimination of industrial TFA from the food supply is estimated at approximately 1.9% of fatty acids (Appendix F). The proposed concentration of *trans* fatty acids in the infant formula fat blend therefore is well below the range of concentrations reported in the fatty acid component of human milk. Accounting for a potentially higher energy intake of the calorically dense formula on a bodyweight basis (i.e., a factor of 1.5), the infant's exposure to ruminant *trans* fatty acids from the intended use in infant formula is below the range of *trans* fatty acids intake estimated from human milk. The presence of ruminant *trans* fatty acids from the intended use of AMF in infant formula does not present a safety concern.

Conjugated Linoleic Acid

Dairy fat contains over 20 different isomers of CLA. Rumenic acid, 9c, 11t-18:2, is the principal CLA isomer in cow's milk fat, accounting for approximately 80–90% of the total milk CLA content (Parodi, 1977). The concentration of CLA in whole milk is estimated at 4.49 mg/g lipid (Lin et al., 1995). Because *trans*-palmitoleic acid, vaccenic acid, and CLA originate from the biohydrogenation of dietary unsaturated fatty acids by gut microbes in ruminant animals, meat derived from ruminants is also a dietary source of these fatty acids. CLA may also be endogenously synthesized from vaccenic acid by mothers (Mosley et al., 2006).

The mean concentration of CLA in AMF is 0.47% of total fatty acids. Assuming AMF will compose 7% of the fat used in infant formula, CLA will account for 0.03% of the total fatty acid content in infant formula. The concentration of CLA in human milk is estimated to be in the range of 0.07 to 0.49% of fatty acids (Appendix F). The proposed concentration of CLA in the infant formula fat blend therefore is within or potentially below the range of concentrations reported in the fatty acid component of human milk. Accounting for a potentially higher energy intake of the calorically dense formula on a bodyweight basis (i.e., a factor of 1.5), the infant's exposure to CLA from the intended use in infant formula is within or below the range of CLA intake estimated from human milk. The presence of CLA from the intended use of AMF in infant formula does not present a safety concern.

Odd-Chain Fatty Acids

Odd-chain fatty acids are primarily saturated fatty acids and a product of rumen microbial fermentation, thus they are present in dairy fat and meat from ruminants. The odd-chain fatty acid C15:0 accounts for approximately 0.89-1.10% of total fatty acids while C17:0 accounts for approximately 0.52-0.72% of total fatty acids (O'Donnell-Megaro et al., 2011; Hageman et al., 2019(a)). In humans, blood levels of the odd-chain fatty acids C15:0 and C17:0 have served as biomarkers of dairy intake though concentrations of the fatty acids may also reflect endogenous production (Jenkins et al., 2017; Pfeuffer and Jaudszus, 2016). In breastfeeding infants, serum concentrations of odd chain fatty acids are positively correlated with concentration in mother's milk (Hellmuth et al., 2018). The metabolic roles of the odd-chain fatty acids of C15:0 and C17:0 may include functioning as a substrate for the synthesis of odd-numbered glycosphingolipids in the brain and gut, providing a source of intermediates for the citric acid cycle, and removing excess propionic acid from circulation (Pfeuffer and Jaudszus, 2016).

The concentration of odd-chain fatty acids in AMF is 1.16% for C15:0 and 0.51% for C17:0 of total fatty acids. Assuming AMF will compose 7% of the fat used in infant formula, these odd-chain fatty acids will account for 0.08 and 0.04% of the total fatty acid content in infant formula,

respectively. The concentration of C15:0 in human milk is estimated to be in the range of 0.08 to 0.50% of fatty acids and the concentration of C17:0 in human milk is estimated to be in the range of 0.19 to 0.41% of fatty acids (Appendix F). The proposed concentrations of odd chain fatty acids in the infant formula fat blend therefore are within or potentially below the ranges of concentrations reported in the fatty acid component of human milk. Accounting for a potentially higher energy intake of the calorically dense formula on a bodyweight basis (i.e., a factor of 1.5), the infant's exposure to odd chain fatty acids from the intended use in infant formula is within the range of odd chain fatty acid intake estimated from human milk. The presence of odd chain fatty acids from the intended use of AMF in infant formula does not present a safety concern.

Branched-Chain Fatty Acids

Branched-chain fatty acids are primarily saturated fatty acids, with branched-chain fatty acids containing one (mono-methyl) or more (di- or multi-methyl) methyl branch(s). Branched-chain fatty acids terminating in an isopropyl group are iso-branched fatty acids while fatty acids terminating in an isobutyl group are referred to as anteiso. The main branched-chain fatty acids are isomers of tetradecanoic acid (iso C14:0), pentadecanoic acid (iso and anteiso C15:0), hexadecanoic acid (iso C16:0), and heptadecanoic acid (iso and anteiso C17:0) (Vlaeminick et al., 2005). As a product of rumen microbial fermentation, they are present in dairy fat and meat from rumimants. Branched-chain fatty acids are reported to be present in milk and a variety of other dairy products at concentrations in the range of 1 to 2 percent of fatty acids by weight (Ran-Ressler et al., 2011; Ran-Ressler et al., 2014).

Infants are exposed to branched chain fatty acids *in utero*. Branched-chain fatty acids are present in the vernix caseosa, the coating on the skin of the fetus, constituting 10-20% of the vernix dry weight. The vernix is consumed as amniotic fluid by the fetus *in utero*, with an intake of approximately 3.4 to 8.5 mg branched-chain fatty acid per day for a total of approximately 102 to 255 mg during the last month of gestation (Ran-Ressler et al., 2008). It is hypothesized that consumption of branched-chain fatty acid from the vernix is involved in the colonization of the infant gut microbiota and are metabolized.

The branched-chain fatty acid content of transitional and mature human milk is approximately 0.13 to 0.58% of total fatty acid content (Appendix F). Assuming AMF will compose 7% of the fat used in the proposed formula and the branched-chain fatty acid content of the AMF is 2.05% of fatty acids as reported for milk fat (Ran-Ressler et al., 2011), the branched-chain fatty acid content of the formula will be 0.14% of the total fatty acid content. The proposed concentration of branched-chain fatty acids in the infant formula fat blend therefore is within the range of concentrations reported in the fatty acid component of human milk. Accounting for a potentially

higher energy intake of the calorically dense formula on a bodyweight basis (i.e., a factor of 1.5), the infant's exposure to branched-chain fatty acids from the intended use in infant formula is within the range of branched-chain fatty acid intake estimated from human milk. The presence of branched-chain fatty acids from the intended use of AMF in infant formula does not present a safety concern.

Cholesterol

Cholesterol is a sterol present in all tissues that is a key component of cell membranes and functions as a precursor for hormones and bile acids. Tissues are capable of synthesizing adequate levels of cholesterol to meet metabolic and structural needs, therefore there is not a dietary requirement for this component. Infant formulas contain low concentrations of cholesterol although human milk is a source of cholesterol for infants. Breastfeeding infants have higher plasma cholesterol concentrations than infants consuming infant formula and infants consuming infant formula have a higher rate of cholesterol synthesis. These effects are believed to be transient, as plasma cholesterol concentrations and rates of cholesterol synthesis are not evident after the introduction of weaning foods and effects of cholesterol on growth and development have not been observed (IOM 2005).

In the 2005 review of dietary recommendations for energy and macronutrients, the IOM reported that typical concentration of cholesterol in human milk was 100 to 200 mg per liter while the concentration of cholesterol in infant formula was typically 10 to 30 mg per liter. Total cholesterol content of human milk also is reported to range between 90 and 150 per liter (Koletzko, 2016).

The typical concentration of cholesterol in AMF is 236 mg per 100 g. Assuming fat accounts for 50% of the energy in the infant formula and the infant formula provides 100 kcal per 100 mL, each 100 mL of infant formula will deliver 5.56 g of fat. AMF will comprise 7% of the fat in fat blend, therefore each 100 mL of infant formula will contain 0.39 g of AMF. At a typical concentration of 236 mg per 100 g AMF (Table 1), AMF will provide approximately 0.92 mg of cholesterol per 100 mL formula, or roughly 9 mg per liter. The concentration of cholesterol in human milk is reported to be in the range of 90 to 200 mg per liter (IOM, 2005; Koletzko, 2016). The proposed concentration of cholesterol in the infant formula therefore is well below the range of concentrations reported in human milk. Accounting for a potentially higher energy intake of the calorically dense formula on a bodyweight basis (i.e., a factor of 1.5), the infant's exposure to cholesterol from the intended use in infant formula is below the range of cholesterol intake from human milk. The presence of cholesterol from the intended use of AMF in infant formula does not present a safety concern.

Vitamins

AMF is a source of naturally occurring vitamin A, vitamin E and vitamin K, all of which are fat soluble vitamins required in infant formula. Assuming 50% of calories are provided as fat in infant formula and AMF accounts for a maximum of 7.0% of the fat blend, each 100 kcal of infant formula will contain no more than 0.39 g AMF. The typical concentration of vitamin A in AMF is 879 μg per 100 g, the typical concentration of vitamin E in AMF is 1.97 mg per 100 g, and the typical concentration of vitamin K in AMF is 9 μg per 100 g (Table 1), thus each 100 kcal of infant formula will provide approximately 3.5 μg vitamin A (11.7 IU assuming vitamin A as retinol), 0.01 mg vitamin E (0.01 IU), and 0.04 μg vitamin K from AMF.

Infant formula in the U.S. is required to provide 250 to 750 IU vitamin A, a minimum of 0.7 IU vitamin E, and a minimum of 4 μg vitamin K per 100 kcal (21 CFR §107.100). The intended use of AMF in infant formula will provide low levels of these vitamins relative to the required levels.

The Institute of Medicine (IOM) established Tolerable Upper Intake Levels (ULs) for vitamin A, with a UL of 600 μg per day for infants and children 1-3 years of age (IOM, 2001). ULs were also established for vitamin E, though a UL for infant populations was not determinable due to lack of data on adverse effects and concern regarding lack of ability to handle excess amounts (IOM 2000). The UL for vitamin E among children ages 1-3 years, the youngest age group for which a UL was established for this nutrient, is 200 mg vitamin E in any form of supplemental α-tocopherol. The IOM has not established for vitamin K for any age group and identification of a UL was concluded to be not determinable (IOM 2001).

The naturally occurring levels of vitamins A, E and K in AMF are low relative to required levels of these vitamins in infant formula, and applicable ULs for infants and young children, and therefore not a safety concern.

Clinical Studies of Infants Consuming Milk Fat

Milk fat has been used as a component of the fat blend in formulas used in clinical trials, and results from these trials provide evidence to assess the suitability of milk fat in infant formulas. A search of the published literature for clinical studies in which milk fat was provided as a component of infant formula was conducted via PubMed using search terms (dairy OR milk) AND (fat OR lipid) in combination with infants and formula, with limits for papers in the English language. The primary search was conducted in March 2019 and supplemental searches were most recently completed in June 2019 (see Appendix E). Titles and abstracts were screened for interventions providing milk fat.

Infant Studies

Three clinical trials in which infants consumed formula containing milk fat as a component of the fat blend were identified in the published literature (Breij et al., 2019; Gianni et al., 2018(a) and (b); De Souza et al., 2018 and Leite et al., 2013) and results of an additional unpublished study were provided by the notifier (Schouten, 2013). Two of the published trials monitored growth and tolerance of the formulas, with one enrolling infants within the first 3 weeks of life and monitoring growth for 4 months in infants randomized to consume formula with an unspecified proportion of milk fat in the fat blend compared to a vegetable oil based formula (Gianni et al., 2018(a) and (b)), and one enrolling infants within the first 5 weeks of life and monitoring infant to 17 weeks of age in infants randomized to consume formula with dairy fat accounting for 48% of the fat blend or a vegetable oil based formula (Breij et al., 2019). The third published study was a crossover study with two periods of intervention (2 weeks each) when infants were between the ages of 2 and 5 months. Results of these clinical interventions provide supportive evidence that milk fat as a component of the fat blend supports growth and is suitably tolerated by infants. These studies are briefly summarized in Table 9 and additional details as presented below.

Table 9. Clinical studies of infants consuming formula with dairy fat

Reference	Infant Population at Enrollment	Duration of Intervention	Study Formulas	Key Outcomes
Breij et al., 2019	Parallel study 223 healthy infants ≤35 days Completers: 81 in control group, 87 in test group, 69 in breast fed group	Enrollment to age 17 weeks	Test: 48% dairy lipid; blend with plant oils; larger diameter lipid droplets with milk phospholipid coating; increased sn-2 palmitic acid content Control: plant oils formula	-No difference in gains of weight, length, or head circumference between test and control formula. -Lower daily mean formula intake in test group at weeks 13 and 17 compared with control formula; difference in weight adjusted formula intake not significantly different. -More frequent stool frequency in test group at week 13, increased diarrhea incidence at weeks 5, 8 and 13, and increased occurrence of regurgitation at weeks 5, 13 and 17; no effect on vomiting. -No difference AEs/SAEs. -No effect on plasma vitamin A or vitamin E. Author's conclusion: "supports adequate growth and is well

Reference	Infant Population at Enrollment	Duration of Intervention	Study Formulas	Key Outcomes
				tolerated and safe for use in infants."
Gianni et al., 2018a; Gianni et al., 2018b	Parallel study 88 healthy infants ≤3 weeks Completers: 23-24/group among formula fed infants; 19 in breast fed infants	4 months	Test: dairy fat (% dairy not specified); blend with plant oils Controls: plant oils or plant oils with ARA and DHA	-No difference in gains of weight, length, or head circumference or fat mass among formulas. -No difference in gastrointestinal parameters, gastrointestinal symptoms, or infant behavior. -Increased total omega-3 fatty acid levels in whole blood and RBC membranes compared to plant oil control; no effect compared to plant oils with ARA and DHA. Author's conclusion: "formula containing dairy lipid provides adequate nutrition for normal growth in healthy term infants and is as well tolerated as control formulas containing only plant oils as lipid sources."
De Souza et al., 2018; Leite et al., 2013	Crossover study 33 infants age 68 - 159 ± 3 days during each intervention; metabolic testing in 17 males	2 weeks	Test: 2.8% milk fat with plant oils with ARA and DHA Control: plant oils with ARA and DHA	-No effect on formula intake and adverse effects. -Increased stool frequency and percentage of formed stools with consumption of the formula containing milk fat and palm olein during the metabolic observation; no difference during tolerance period
Schouten, 2013 [unpublished]	Single arm trial; open label 50 healthy term infants	6 weeks	49% milk fat by weight in fat blend	-Based on data from a historical control group of Asian infants, no difference in the severity and occurrence of gastrointestinal symptoms was observed.

Abbreviations: AE – adverse events; ARA – arachidonic acid; DHA – docosahexaenoic acid; RBC – red blood cells; SAE – serious adverse events

Breij and colleagues (2019) examined the effects of a formula containing a mixture of dairy lipids and vegetable fats delivered in large (3-5 μm diameter) milk phospholipid-coated liquid

droplets compared to an isoenergetic standard vegetable oil-based formula with typical lipid droplets (0.5 μm diameter). In the test formula, the dairy lipids, including milk phospholipids, accounted for 48% of the fat blend with the balance of lipids (52%) comprised of vegetable oil. The concentration of sn-2 palmitic acid was also 3 times the concentration in the control formula. The test and control formulas therefore differed on three aspects, namely size of the lipid droplets, the coating on the lipid droplets, and the source of lipid. Based on the patent referenced for production of the test formula, the test formula was assumed to contain dairy lipids from AMF and/or butter oil. Healthy infants up to 35 days of age were recruited and a total of 223 infants were randomized to the test formula (115 randomized, 87 completed, 91 in per protocol population) or the control formula (108 randomized, 81 completed, 83 in per-protocol population); infants consumed the assigned formula until 17 weeks of age. A group of 88 infants consuming human milk served as a reference control. The primary outcome of the intervention was daily weight gain; additional growth parameters of length and head circumference were monitored, as well as adverse events (AEs), tolerance, stool characteristics, and plasma vitamin levels.

The dropout rate between formula groups did not differ and there were no differences in the reason for early termination. There were no differences in daily weight gain between the two formula groups in the total population of infants or the subset enrolled within the first 14 days of life. Among the per-protocol population, weight-for-age, length-for-age, and head circumference-for-age z-scores did not differ between the formula groups at weeks 5, 8, 3, and 17 of life; weight-for-length at weeks 13 and 17 of age was lower in the test group compared with the control group although all values were within ± 0.5 of the z-score bandwidth. At 13 and 17 weeks of age, median daily formula intake was lower among infants consuming the formula with large-diameter milk phospholipid-coated liquid droplets and dairy fat compared to infants consuming the formula with vegetable oil; however, formula intake on a body weight basis did not differ. At age 13 weeks, stool frequency was reported to be more frequent among infants consuming the test formula containing dairy lipids compared to infants consuming formula with vegetable oil; no differences were reported at weeks 5, 8, or 17 of life. The incidence of 3 or more watery stools in a day was higher among infants consuming the formula with large-diameter milk phospholipid-coated liquid droplets and dairy fat compared to infants consuming formula with vegetable oil as reported at weeks 5, 8 and 13 (but not 17) of age. Although statistical comparisons between the formula groups and the breastfed reference control group were not conducted, the incidence of watery stool among infants consuming modified infant formula with dairy fat (23-28%) appears comparable to the incidence among breastfed infants (21-31%); the authors speculated that the stool softening effect of the test formula may be attributed to a higher proportion of sn-2 palmitate. Mild or moderate regurgitation was reported among a higher percentage of infants consuming the test formula than the control formula at weeks 5, 13, and 17 of age. The occurrence of vomiting and diaper rash did not differ between

formula groups. Plasma concentrations of vitamins A and E did not differ between the two formula groups. Based on a review of AEs and serious adverse events (SAEs), the investigators concluded that the occurrence of any (S)AEs during the study presented no safety concerns.

Given the differences between the test and control formulas in both lipid structure and lipid source, findings from this study cannot be attributed to either aspect in isolation. The results nonetheless provide supportive evidence for dairy fat in infant formula to support growth. Infants consuming formula with large-diameter milk phospholipid-coated liquid droplets and dairy fat accounting for 48% of total fat had an increased prevalence of regurgitation and increased prevalence of watery stools compared to a standard formula. Stool characteristics of infants consuming the test formula were generally more consistent with tolerance parameters observed in the breastfed infants, and thus suggest suitable tolerability. The authors concluded that the formula "with large, milk phospholipid-coated droplet containing dairy lipids supports adequate growth and is well tolerated and safe for use in infants."

Gianni and colleagues investigated the impact of infant formula containing dairy lipids and plant oils on measures of growth and gastrointestinal tolerance in a sample of 88 infants in Italy (Gianni et al., 2018(a)). In this double-blind randomized controlled trial, healthy term infants less than 3 weeks of age were randomly allocated to one of three groups and provided with a specific infant formula (manufactured by Milumel, Lactialis, France) for consumption for 4 months. The three infant powder formula groups included: (1) formula containing a mixture of dairy fat with plant oils (completed by 23 infants), (2) formula containing only plant oils (completed by 24 infants), and (3) formula containing plant oils supplemented with the long chain polyunsaturated fatty acids ARA and DHA (completed by 23 infants). A group of breast-fed infants was also included as a reference group; 29 infants began the trial of which 19 completed the study. The dairy fat component was described as cream and the proportion of total fat provided by dairy fat in the formula was not specified (Gianni et al., 2018(b)). The primary objective of the study was to compare the effect of infant formula containing dairy fat on erythrocyte membrane concentrations of omega-3 fatty acids; secondary outcomes included effects on growth and body composition which were monitored at baseline and after 2 and 4 months of formula intake, and gastrointestinal tolerance which was monitored from 2-day diaries completed by parents after 1 and 3 months of formula intake.

Rates of discontinuation were 23% in both the dairy fat and plant oils formula group and the plant oils with ARA and DHA formula group and 14% in the control formula group (plant oils). No differences in daily intake among the formula groups were observed, with mean daily intake of formula greater than 600 ml per day at 1 month and greater than 700 ml per day at 3 months of intake. Gains of weight, length, head circumference, and fat mass were not different between

the formula-fed groups at 2 and 4 months; all infants were reported to have anthropometric measures within the normal range for growth standards There were no significant differences among the three formula-fed groups in gastrointestinal parameters (daily stool frequency, stool consistency, stool color), gastrointestinal symptoms (abdominal pain, flatulence, regurgitation, vomiting), or in infant behavior (general behavior, sleeping disturbances). The study authors concluded that "formula containing dairy lipid provides adequate nutrition for normal growth in healthy term infants and is as well tolerated as control formulas containing only plant oils as lipid sources."

In the same study population, Gianni and colleagues also investigated the effects of the infant formulas on omega-3 fatty acid content in red blood cells (RBC) (Gianni et al., 2018b). Fatty acids in RBC phosphatidylethanolamine of the RBC membrane and fatty acids in whole blood were evaluated after 4 months of formula intake. Total omega-3 fatty acid levels in whole blood and RBC phosphatidylethanolamine were significantly higher in infants fed the formula containing a mix of dairy lipids and plant oils than the plant oil formula alone, but similar to those fed the formula from plant oils supplemented with long chain polyunsaturated fatty acids and to the breast-fed infants. DHA levels in the RBC membrane were significantly higher with intake of formula with dairy lipids and plant oils compared to the plant oil formula but significantly lower than levels observed among breast-fed infants and infants consuming the polyunsaturated fatty acid supplemented formula. However, DHA in the whole blood was not different between infants consuming the dairy lipids and plant oil formula compared to the infants consuming plant oil formula or breast-fed infants, but values were lower than among infants consuming the polyunsaturated fatty acids supplement formula. Levels of docosapentaenoic acid (DPA) in RBC phosphatidylethanolamine were highest among infants consuming the dairy lipid containing formula compared to all other formula-fed and breast-fed infants.

Findings from this study suggest that dairy lipids in infant formulas could stimulate the conversion of omega-3 fatty acid precursors into long-chain derivatives. Although growth and tolerance were secondary outcomes, results from the study provide evidence that formula with dairy fat comprising a component of the fat blend supports normal growth in healthy term infants over the first four months of life and the formula is well tolerated.

De Souza and colleagues (2018) and Leite and colleagues (2013) investigated the effects of infant formulas containing varying fat compositions on absorption of fat, fatty acid acids, and calcium balance among healthy infants in a crossover design. This study was conducted with commercially available formula in Brazil. Infants were randomized to consume one of two formulas: one formula was a powder containing a fat blend comprised of 2.8% milk fat, 44%

palm olein oil, 21.7% palm kernel oil, 18.5% canola oil, 10.9% corn oil, and 2.1% of a mixture of DHA, ARA, and soy lecithin; the other powder formula contained a fat blend comprised of 41.4% high oleic sunflower oil, 29.6% coconut oil, and 27.6% soy oil, and 1.4% of a mixture of DHA and ARA. Each formula was consumed for 14 days in the sample of 33 infants ages 68 ± 3 days at enrollment to day 159 followed by a 4-day metabolic testing period in 17 male participants. Formula intake and adverse event incidence were not significantly different between formula treatment groups. During the 4-day metabolic period, stool frequency was significantly higher and mean stool consistency score was significantly lower (indicating an increased percentage of formed stools) with consumption of the formula containing milk fat and palm olein as the predominant fat, although differences between the groups were not significant during the tolerance period (Leite et al. 2013). Fecal fat excretion was significantly higher and total fat, total fatty acid, and calcium absorption was significantly lower among infants consuming the formula containing predominantly palm olein oil with milk fat. The investigators suggested that the higher fecal fat excretion was likely attributed to the high palm olein oil content of the formula. Infant formulas containing palm olein as a predominant source of fat have been demonstrated to reduce absorption of fat and calcium and decrease bone mineralization compared to formulas without palm olein (Koo et al., 2006).

Energy dense infant formula containing milk fat has been the test article in an unpublished, open-label multi-center, single arm trial (Schouten 2013). Fifty healthy term infants (delivered 37-42 weeks' gestation) consumed a cow's milk based infant formula providing 100 kcal per 100 mL for a period of 6 weeks. Milk fat accounted for 49% by weight of the modified fat blend in the formula. Throughout the study, investigators monitored gastrointestinal tolerance, anthropometrics, and adverse events associated with the formula treatment. The investigators reported a "low number" of adverse events possibly attributed to the formula. Based on data from a historical control group of Asian infants, no difference in the severity and occurrence of gastrointestinal symptoms was observed. Results from this study provide corroborative support for the suitability of milk fat as a component in the fat blend of infant formula.

Earlier studies with milk fat as a component of the fat blend in infant formula provide evidence that the infant lipoprotein response to dietary fat is generally consistent with expected responses. Among the early studies identified with milk fat was a trial examining the effects of evaporated milk (a common source of infant feeding more than 50 years ago) compared to corn oil on blood cholesterol levels (Golawin and Pomeranze, 1962). Infants consuming infant formula containing corn oil daily for 12 weeks had lower serum cholesterol levels than infants fed evaporated milk or breast-fed infants; serum cholesterol levels among corn oil fed infants rose to levels comparable to those of infants consumed evaporated milk group following introduction of complementary foods at 16 weeks.

Animal Studies

Numerous studies in animals have examined the effect of an infant formula containing milk fat on efficacy parameters such as digestion, concentrations of DHA and ARA, immunity and microbiota composition (Aidoud et al., 2018; Delplanque et al., 2013; Dinel et al., 2016(a); Dinel et al., 2016(b); Drouin et al., 2018; Du et al., 2012; Le Huërou-Luron et al., 2018(b); Lemaire et al., 2018). Findings from these studies suggest that milk fat as a component of infant formula may support infant health (Appendix G).

GRAS Criteria

The regulatory framework for determining whether the use of a substance in food for animals can be considered GRAS in accordance with section 201(s) of the Federal Food, Drug, and Cosmetic Act ("the Act"), is set forth at 21 CFR §170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data information.

In the preamble to the final rule for GRAS notifications, FDA stated that a GRAS conclusion, based on scientific procedures may be supported by scientific data (such as human, animal, analytical or other scientific studies), information, methods and principles, published or unpublished, appropriate to establish the safety of a substance under the conditions of intended use (FDA, 2016). The safety standard requires that there be a reasonable certainty of no harm under the conditions of intended use of the substance. To be eligible for a GRAS conclusion based on scientific procedures, there must be evidence of a consensus among qualified experts that the proposed use is safe and the pivotal data and information supporting the safety of the ingredient's intended use must be publicly available.

Safety Assessment

Milk and milk products including AMF have a long history of use in the U.S. food supply, including consumption by toddlers and use of milk-derived ingredients in infant formula. Historically, infants not provided human milk may have consumed evaporated milk or sweetened condensed milk, although this practice largely disappeared in the with the introduction of vegetable oil-based formulas in the 1970s. The safety of the proposed use of AMF in infant formula has been established through consideration of the manufacture of the substance to ensure a food-grade product, the physiological nature of the substance (i.e., a fat), and consideration of constituents in AMF in the context of typical components of the infant diet.

As previously noted, specifications are in place to ensure that the AMF meets limits appropriate for a food ingredient regarding potential contaminants of concern, including metals (e.g., lead, arsenic, cadmium, mercury), aflatoxins, PCB compounds, dioxins, and PAHs. Data demonstrate that these specifications are met.

Fat is an important component for infant health. The contribution of fat to total energy intake of human milk or formula is approximately 48 to 50% (IOM, 2005; Martin et al., 2016). The intended use of AMF (i.e., milk fat) as a component of the fat blend in infant formula contributes to the range of fatty acids important for infant nutrition. As shown in Table 7, the maximum intended use of AMF in calorically dense infant formula is 7.0% of fat by weight. Assuming fat accounts for 50% of the energy, the estimated intake of AMF is 0.39 g per 100 kcal. Assuming typical and high intake of the formula is within the range of 120 to 175 kcal/kg bw/day, intake of AMF is in the range of 0.47 to 0.68 g/kg bw/day.

The use of AMF in infant formula is a source of components naturally present in milk fat that are not present in vegetable oils, including short-chain fatty acids (butyrate), branched- and odd-chain fatty acids, ruminant-derived *trans* fatty acids, conjugated linolenic acid, and cholesterol. Butyric acid, branched- and odd-chain fatty acids, ruminant-derived *trans* fatty acids, conjugated linolenic acid, and cholesterol are, however, present in human milk, which is recognized as the gold standard for human infant nutrition.

As summarized in Table 10, the available literature demonstrates that the concentrations of butyric acid, branched- and odd-chain fatty acids, *trans*-fatty acids, conjugated linolenic acid, and cholesterol in the lipid component of human milk are comparable to or higher than the estimated concentrations in the fat component of infant formula based on the composition of AMF and the maximum intended use of AMF in the fat blend.

Table 10. Summary of concentrations of milk fat components in the calorically dense infant formula and human milk

Component in Milk/Formula	Mean Concentration in AMF	Concentration in Calorically Dense Infant Formula[a] (% of fat blend)	Range in Human Milk[b] (% of total fatty acids)
AMF	100	7.0	
Butyric Acid	2.46	0.17	0.0009 - 0.76
Trans Fatty Acids	3.07	0.21	1.9
Conjugated Linoleic Acid	0.47	0.03	0.07 - 0.49
Odd-Chain Fatty Acids			
C15:0	1.16	0.08	0.08 - 0.50
C17:0	0.51	0.04	0.19 - 0.41
Branched-Chain Fatty Acids	0.13	0.14	0.13 - 0.58
Cholesterol	236 mg per 100 g	0.92 mg per 100 mL	9 - 20 mg per 100 mL

[a] Assumes AMF accounts for 7% of the fat blend by weight and fat accounts for 50% of kcal in formula
[b] See Appendix F for human milk data sources.

Accounting for a potentially higher energy intake of the calorically dense formula on a bodyweight basis (i.e., a factor of 1.5), the infant's exposure to these milk fat components resulting from the intended use of AMF in infant formula is also within the range of intake estimated from consumption of human milk.

The intended use of AMF in infant formula therefore provides intake of these fat components that are below or within typical ranges of intake by infants and does not present a safety concern. AMF also is a source of naturally occurring vitamins A, E, and K. The naturally occurring levels of these nutrients are low relative to required levels in infant formula and therefore not a safety concern.

Results from published clinical interventions in which infant consumed formula with dairy lipids provided as cream or AMF in fat globules surrounded by a MFGM and unpublished data in which infants consumed formula with dairy lipids as AMF provide supportive evidence that milk fat as a component of the fat blend in infant formula supports growth and is suitably tolerated by infants. Data from animal studies also provide evidence that partial replacement of oils in infant formula with dairy lipids may help support concentrations of important fatty acids.

Conclusion Regarding Safety and General Recognition of Safety

General recognition of safety through scientific procedures requires common knowledge throughout the scientific community knowledgeable about the safety of food ingredients that

there is a reasonable certainty that a substance is not harmful under the intended conditions of use in foods. The aforementioned regulatory and scientific reviews related to the consumption and safety of AMF as a component of the fat blend in exempt infant formula are published in the peer-reviewed scientific literature and, therefore, are generally available and generally known among the community of qualified food ingredient safety experts. There is broad-based and widely disseminated knowledge concerning milk fat and use of oils in the fat blend of infant formula. The data and publicly available information supporting the safety of the proposed maximum intended use of AMF in exempt infant formula for term infants requiring a calorically dense formula and/or fluid restriction is 7.0 percent by weight of the fat blend as detailed in this document are not only widely known and disseminated but are also commonly accepted among qualified food safety experts.

Discussion of Information Inconsistent with GRAS Determination

No information has been identified that would be inconsistent with a finding that the proposed use of AMF in exempt infant formula, meeting appropriate specifications specified herein and used according to cGMP, is safe and GRAS.

Part 7. List of Supporting Data and Information in GRAS Notice

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Appendices

Appendix A. Analytical Data on AMF

NLDNR

Eurofins CLF
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Professor-Wagner-Straße 11
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Eurofins CLF Specialised Nutrition Testing Services GmbH · Professor-Wagner-Straße 11 · D-61381 Friedrichsdorf

To: [redacted]

Cc:

Person in charge Evelyn Núñez B.

Report date 25.03.2019

Analytical Report No 19-3086-01-DAN-1

AMF_FC

Sample Information:

02-TAS-DAN

		CLF order no.	CLF-02993-19
		Analytical order no.	WREQ56380
CLF sample code	19-3086-01	**Client order no.**	4503110882
Client sample code	120011632		
Local article no.	0	**Date analysis order**	08.03.2019
Concern article no.		**Date sample receipt**	11.03.2019
PDS number		**Analysis started**	12.03.2019
Material class	RS-Oils and fats	**Analysis finished**	25.03.2019
Batch	BBoWTLS	**Country**	
Supplier		**Production date**	
Consistence	liquid	**Expiry date**	
Sample amount	2x500 ml	**Delivery date**	
Packaging			
Customer Remarks			

Notification

The results relate exclusively to the above mentioned sample.
The test report shall not be reproduced except in full, without written approval of CLF

Results

Typical Analysis

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_0929	Moisture and volatile matter content		0,07	0,01	g/100g		
N01_10ME	Fat		100	0,1	g/100g		

Method description

Code	Description
N01_10ME	Fat in foodstuffs by Soxleth extraction acc. ASU L 13.05-3:2002-05, mod.***
SUB_0929	Moisture and volatile matter content in fat & oil by gravimetry acc. ASU L 13.00-16, external**

Minerals and Trace Elements

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N06_10ME	Iron		0,06	0,02	mg/100g		
N06_10ME	Copper		<20	20	µg/100g		
N06_13ME	Molybdenum		<1,0	1	µg/100g		
N06_13ME	Chromium		4,1	1	µg/100g		
N06_14ME	Selenium		<2,0	2	µg/100g		

Method description

Code	Description
N06_10ME	Minerals and trace elements in foodstuffs (ICP-OES) acc. DIN EN 13805:2002-06, mod. & prDIN EN 16943:2016-01, mod.
N06_13ME	Chromium and Molybdenum in foodstuffs (ICP-MS) acc. DIN EN 13805:2002-06, mod.
N06_14ME	Selenium in dietetic foodstuffs & raw materials for their production (ICP-MS) acc. DIN EN 13805:2002-06, mod.

Fat soluble Vitamins

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N03_07ME	Vitamin K1 (phyllochinon)		n.e.	2	µg/100g		
SUB_1016	Vitamin A (retinol)		894	21	µg/100g		
SUB_1016	Vitamin A (retinol) in IU (calc.)		?9800	700	IU/kg		
SUB_1017	Vitamin E (d,l-alpha-tocopherol)		1,95	0,5	mg/100g		
SUB_1017	Vitamin E (beta-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (delta-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (gamma-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (sum-tocopherol)		1,95		mg/100g		
SUB_1017	Vitamin E equiv. (d-alpha-tocopherol)		-		mg/100g		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_1017	Vitamin E (d-alpha-tocopherol)		-		IU/100g		

Method description

Code	Description
N03_07ME	Vitamin K1 in foodstuffs (HPLC-FD) acc. DIN EN 14148:2003-10, mod.
SUB_1016	Vitamin A by LC-DAD acc. EN 12823-1 2014, external*
SUB_1017	Vitamin E by LC-FLD acc. EN 12822:2014, external*

Fatty Acids

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C4:0, Butyric acid		2,43	0,02	%		
N07_05ME	C6:0, Caproic acid		2,11	0,02	%		
N07_05ME	C8:0, Caprylic acid		1,35	0,02	%		
N07_05ME	C10:0, Capric acid		3,28	0,02	%		
N07_05ME	C12:0, Lauric acid		4,31	0,02	%		
N07_05ME	C14:0, Myristic acid		12,29	0,02	%		
N07_05ME	C14:1w5, Myristoleic acid		1,16	0,02	%		
N07_05ME	C15:0, Pentadecanoic acid		1,15	0,02	%		
N07_05ME	C15:1w5, Pentadecenoic acid		<0,02	0,02	%		
N07_05ME	C16:0, Palmitic acid		33,98	0,02	%		
N07_05ME	C16:1w7, Palmitoleic acid		1,62	0,02	%		
N07_05ME	C17:0, Margaric acid		0,51	0,02	%		
N07_05ME	C17:1w7, Heptadecenoic acid		<0,02	0,02	%		
N07_05ME	C18:0, Stearic acid		10,10	0,02	%		
N07_05ME	C18:t1w9, trans-Oleic acid		0,39	0,02	%		
N07_05ME	C18:t1w7, trans-Vaccenic acid		1,08	0,02	%		
N07_05ME	C18:t1, trans isomers (except of trans-Oleic- and trans-Vaccenic acid)		1,04	0,02	%		
N07_05ME	C18:1w9, Oleic acid		19,01	0,02	%		
N07_05ME	C18:1w7, Vaccenic acid		0,42	0,02	%		
N07_05ME	C18:t2, trans isomers		0,47	0,02	%		
N07_05ME	C18:2w6, Linoleic acid (LA)		1,44	0,02	%		
N07_05ME	C20:0, Arachidic acid		0,15	0,02	%		
N07_05ME	C18:3w6, g-Linolenic acid (GLA)		0,03	0,02	%		
N07_05ME	C18:t3, trans isomers		<0,02	0,02	%		
N07_05ME	C20:1w9, Eicosenoic acid		0,05	0,02	%		
N07_05ME	C18:3w3, a-Linolenic acid (ALA)		0,56	0,02	%		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C18:2conj., conjugated Linoleic acid (CLA)		0,46	0,02	%		
N07_05ME	C21:0, Heineicosanoic acid		<0,02	0,02	%		
N07_05ME	C18:4w3, Stearidonic acid		<0,02	0,02	%		
N07_05ME	C20:2w6, Eicosadienoic acid		0,02	0,02	%		
N07_05ME	C22:0, Behenic acid		0,06	0,02	%		
N07_05ME	C20:3w6, Eicosatrienoic acid		0,09	0,02	%		
N07_05ME	C22:1w9, Erucic acid		<0,02	0,02	%		
N07_05ME	C20:3w3, Eicosatrienoic acid		0,02	0,02	%		
N07_05ME	C20:4w6, Arachidonic acid (AA)		0,10	0,02	%		
N07_05ME	C22:2w6, Docosadienoic acid		0,11	0,02	%		
N07_05ME	C20:5w3, Eicosapentaenoic acid (EPA)		0,07	0,02	%		
N07_05ME	C24:0, Lignoceric acid		0,05	0,02	%		
N07_05ME	C24:1w9, Nervonic acid		<0,02	0,02	%		
N07_05ME	C22:4w6, Docosatetraenoic acid		<0,02	0,02	%		
N07_05ME	C22:5w6, Docosapentaenoic acid		<0,02	0,02	%		
N07_05ME	C22:5w3, Docosapentaenoic acid		0,11	0,02	%		
N07_05ME	C22:6w3, Docosahexaenoic acid (DHA)		<0,02	0,02	%		
N07_05ME	Non identified fatty acids		<0,02		%		
N07_05ME	Sum saturated fatty acids (SFA)		71,77		%		
N07_05ME	Sum unsaturated fatty acids (UFA)		28,25		%		
N07_05ME	Sum monounsaturated fatty acids (MUFA)		24,77		%		
N07_05ME	Sum polyunsaturated fatty acids (PUFA)		3,48		%		
N07_05ME	Sum trans fatty acids		2,98		%		
N07_05ME	Sum w3-fatty acids LCP		0,20		%		
N07_05ME	Sum w6-fatty acids LCP		0,32		%		
N07_05ME	Ratio w6 LCP / w3 LCP		1,60				
N07_05ME	Sum C18:2w6 + C18:3w3		2,00		%		
N07_05ME	Sum C18:3w3 + C18:3w6		0,59		%		
N07_05ME	Sum C20:4w6 + C22:6w3		0,10		%		
N07_05ME	Ratio C18:2w6 / C18:3w3		2,57				
N07_05ME	Ratio C20:4w6 / C22:6w3		-				
N07_05ME	Ratio C22:6w3 / C20:5w3		-				
N07_05ME	ratio PUFA / SFA		0,05				
N07_05ME	Ratio SFA / UFA		2,54				
N07_05ME	C12:0, Lauric acid iP		4,09		g/100g		
N07_05ME	C14:0, Myristic acid iP		11,68		g/100g		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C18:1w9, Oleic acid iP		18,06		g/100g		
N07_05ME	C18:2w6, Linoleic acid (LA) iP		1,37		g/100g		
N07_05ME	C18:3w3, a-Linolenic acid (ALA) iP		0,53		g/100g		
N07_05ME	C20:4w6, Arachidonic acid (AA) iP		95,00		mg/100g		
N07_05ME	C20:5w3, Eicosapentaenoic acid (EPA) iP		66,50		mg/100g		
N07_05ME	C22:6w3, Docosahexaenoic acid (DHA) iP		<19,00		mg/100g		
N07_05ME	Sum saturated fatty acids (SFA) iP		68,18		g/100g		
N07_05ME	Sum monounsaturated fatty acids (MUFA) iP		23,53		g/100g		
N07_05ME	Sum polyunsaturated fatty acids (PUFA) iP		3,31		g/100g		
N07_05ME	Ratio C12+C14iP / TFA iP		0,17				
N07_05ME	Sum saturated + unsaturated fatty acids (TFA)		95,02		g/100g		
N07_05ME	Sum C12 + C14 iP		15,77		g/100g		
N07_05ME	Sum trans fatty acids iP		2,83		g/100g		

Method description

Code	Description
N07_05ME	Fatty acid composition [g/100g fatty acids] of foodstuffs (GC-FID), acc. AOAC 2012.13:2012

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_0365	Cholesterol		236	1	mg/100g		
SUB_1242	Peroxide Value		0,7	0,1	meq O2/kg		

Method description

Code	Description
SUB_0365	Cholesterol by GC-FID acc. ASU L05.00-16 mod., external*
SUB_1242	Peroxide value in oils, acc. DGF C-VI 6a - Part 1, external*

Results

Mycotoxins

Aflatoxin M1

R02_03ME Determination of aflatoxin M1 in milk and milk based products using HPLC after immunoaffinity column clean-up acc. DIN EN ISO 14501:2006-02, mod.

Parameter	Result	LOQ	Unit
Aflatoxin M1	<5^	10	ng/kg

Toxical Elements

Mercury

R03_02ME Determination of mercury in foodstuffs by AAS hydride technique, acc. DIN EN 13805:2002-06, mod. & DIN EN 13806:2002-11, mod.

Parameter	Result	LOQ	Unit
Mercury	<1	1	µg/kg

Aluminium, < 2ppm

R03_04ME Determination of aluminum in foodstuffs by solid phase AAS acc. internal method (version 2)

Parameter	Result	LOQ	Unit
Aluminium	<100	100	µg/kg

Lead

R03_01ME Determination of lead in foodstuffs by solid phase AAS acc. internal method (version 5)

Parameter	Result	LOQ	Unit
Lead	7	5	µg/kg

Tin, external*

SUB_0172 Determination of tin, external*

Parameter	Result	LOQ	Unit
Tin	<0,2	0,2	mg/kg

Antimony, external*

SUB_0175 Determination of antimony, external*

Parameter	Result	LOQ	Unit
Antimony	<0,05	0,05	mg/kg

Nickel, external*

SUB_0177 Determination of nickel (HTC/AAS, ICP-OES, ICP-MS), external*

Parameter	Result	LOQ	Unit
Nickel	<0,1	0,1	mg/kg

Cadmium (Solid phase AAS)

R03_01ME Determination of cadmium in foodstuffs by solid phase AAS acc. internal method (version 5)

Parameter	Result	LOQ	Unit
Cadmium	<1	1	µg/kg

Total arsenic in foodstuffs (ICP-MS)

R03_09ME Total arsenic in foodstuffs (ICP-MS) acc. internal method (version 1)

Parameter	Result	LOQ	Unit
Arsenic total	<15	15	µg/kg

Inorganic arsenic (ICP-OES)

R03_10ME Determination of inorganic arsenic in cereals by ICP-OES acc. ASU L15.06-02:2013-01, mod.

Parameter	Result	LOQ	Unit
Arsenic inorganic	<30	30	µg/kg

Environmental Contaminants

Dioxins (17) + PCB (12+6) in oils + fats (except fish oil), external *

SUB_0392 Determination of PCDD/PCDF and dl-PCBs (Dioxins) in fats and oils by GC-HRMS acc. EU VO 589/2014, external*

Parameter	Result	LOQ	Unit
Dioxin-like PCBs WHO(2005)-dl-PCB TEQ (upper bound)	0,241		pg/g
Dioxins and Furans WHO(2005)-PCDD/F TEQ (upper bound)	0,298		pg/g
Sum WHO(2005)-PCDD/F + dl-PCBs TEQ (upper bound)	0,539		pg/g
non dioxine like PCBs (ndl PCBs), sum of	1,88		ng/g

Radioactivity

Radioactivity Cs-134/137, external*

SUB_0165 Determination of Radioactivity by Gamma spectrometer acc. internal method, external*

Parameter	Result	LOQ	Unit
Activity Cs 134	<10^		Bq/kg
Activity Cs 137	<10^		Bq/kg
Reference date for activities	14.03.2019		

Results

Microbiological Parameters

Code	Parameter	Result	LOQ	Unit
M01_02ME	Total Viable Count 30°C, aerobe	<100	100	cfu/g
M01_02ME	Total Viable Count 55°C, aerobe	<100	100	cfu/g
M01_02ME	Anaerobic plate count 30°C	<100	100	cfu/g
M01_02ME	Anaerobic plate count 55°C	<100	100	cfu/g
M01_03ME	Bacillus cereus	<10	10	cfu/g
M01_05ME	Staphylococcus aureus per 1g	<10	10	cfu/g
M01_06ME	Yeast	<10	10	cfu/g
M01_06ME	Mould	<10	10	cfu/g
M01_07ME	Enterobacteriaceae - Detection per 1g	negative		per g
M01_14ME	Salmonella - Detection per 25g	negative		per 25g
M01_26ME	Sulphite Reducing Clostridia (SRC)	<1	1	cfu/g
M01_30ME	Cronobacter spp. - Detection per 300g	negative		per 300g
M01_35ME	Thermophilic Thermoresistant Sporeformers, Aerobic	<1	1	cfu/g
M01_35ME	Thermophilic Thermoresistant Sporeformers, Anaerobe	<1	1	cfu/g

Method description

Code	Description
M01_02ME	Total viable count (30°C and 55°C) acc. ISO 4833-1:2013-09, mod.
M01_03ME	Bacillus cereus - Enumeration acc. ISO 7932:2004-06, mod.
M01_05ME	Staphylococcus aureus (coagulase positive Staphylococci) - Enumeration acc. ISO 6888-1:2003-12, mod.
M01_06ME	Yeasts and Moulds - Enumeration acc. ISO 6611:2003-12, mod.
M01_07ME	Enterobacteriaceae - Detection acc. DIN ISO 21528-1:2009-12, mod.
M01_14ME	Salmonella - Conventional Method acc. DIN EN ISO 6579:2007-10, mod.
M01_26ME	Horizontal Method for the enumeration of sulphite reducing clostridia growing under anaerobic conditions acc. ISO 15213:2003-05, mod.
M01_30ME	Cronobacter spp. detection acc. ISO/TS 22964:2006-02, mod.
M01_35ME	Thermophilic thermoresistant sporeformers (TTS, 10 min, 80 °C) - Enumeration acc. internal method (version 3)

Legend results:

LOQ	: Limit Of Quantitation: Up from this level quantitative results are available
^	: Limit Of Detection
LOD	: Limit Of Detection
-	: Result cannot be calculated
n.d.	: Not detectable
n.e.	: Not evaluable
n.a.	: Not analysed
Dev.	: Deviation from target value
OOS?	: Out of specification?
*	: Not part of CLF's accreditation; performed by approved subcontractor
**	: Part of CLF's accreditation; performed by approved subcontractor
***	: Not part of CLF's accreditation
SUB	: Subcontractor (Original analytical report from subcontractor available on demand)

Legend method descriptions:

ASU	: Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (Official Collection of Methods of Analysis according to German Food and Feed Act)
DIN	: Deutsches Institut für Normung e.V. (German Institute for Standardization)
EN	: European Norm
ISO	: International Organization for Standardization
AOAC	: Association of Official Analytical Chemists
NEN	: Nederlands Normalisatie-instituut (Dutch Institute for Standardization)
NF	: Norme française (Norm by French Institute for Standardization)
USP	: United States Pharmacopeia
mod.	: modified (Details about modifications see annex to CLF's ISO 17025 accreditation certificate)

Remarks: 19-3086-01
Aluminium, Cadmium, Lead: Result confirmed by 2-fold determination.

Vitamin K1: analysis is not possible due to matrix issues.

Bianca Puff
- Food Chemist -
Manager Business Development & Customer Relations

Evelyn Núñez B.
- Food Chemist -
Analytical Service Manager

Notification

This analytical report is created automatically and is valid without any signature.

NLDNR

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To: [redacted]

Cc:

Person in charge Evelyn Núñez B.

Report date 25.03.2019

Analytical Report No 19-3086-02-DAN-1

AMF_FC

Sample Information:

02-TAS-DAN

		CLF order no.	CLF-02993-19
		Analytical order no.	WREQ56380
CLF sample code	19-3086-02	**Client order no.**	4503110882
Client sample code	120011632		
Local article no.	0	**Date analysis order**	08.03.2019
Concern article no.		**Date sample receipt**	11.03.2019
PDS number		**Analysis started**	12.03.2019
Material class	RS-Oils and fats	**Analysis finished**	25.03.2019
Batch	BBoWT5I	**Country**	
Supplier		**Production date**	
Consistence	liquid	**Expiry date**	
Sample amount	2x500 ml	**Delivery date**	
Packaging			
Customer Remarks			

Notification

The results relate exclusively to the above mentioned sample.
The test report shall not be reproduced except in full, without written approval of CLF

Results

Typical Analysis

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_0929	Moisture and volatile matter content		0,06	0,01	g/100g		
N01_10ME	Fat		100	0,1	g/100g		

Method description

Code	Description
N01_10ME	Fat in foodstuffs by Soxleth extraction acc. ASU L 13.05-3:2002-05, mod.***
SUB_0929	Moisture and volatile matter content in fat & oil by gravimetry acc. ASU L 13.00-16, external**

Minerals and Trace Elements

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N06_10ME	Iron		<0,02	0,02	mg/100g		
N06_10ME	Copper		<20	20	µg/100g		
N06_13ME	Molybdenum		<1,0	1	µg/100g		
N06_13ME	Chromium		1,9	1	µg/100g		
N06_14ME	Selenium		<2,0	2	µg/100g		

Method description

Code	Description
N06_10ME	Minerals and trace elements in foodstuffs (ICP-OES) acc. DIN EN 13805:2002-06, mod. & prDIN EN 16943:2016-01, mod.
N06_13ME	Chromium and Molybdenum in foodstuffs (ICP-MS) acc. DIN EN 13805:2002-06, mod.
N06_14ME	Selenium in dietetic foodstuffs & raw materials for their production (ICP-MS) acc. DIN EN 13805:2002-06, mod.

Fat soluble Vitamins

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N03_07ME	Vitamin K1 (phyllochinon)		n.e.	2	µg/100g		
SUB_1016	Vitamin A (retinol)		834	21	µg/100g		
SUB_1016	Vitamin A (retinol) in IU (calc.)		?7800	700	IU/kg		
SUB_1017	Vitamin E (d,l-alpha-tocopherol)		1,96	0,5	mg/100g		
SUB_1017	Vitamin E (beta-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (delta-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (gamma-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (sum-tocopherol)		1,96		mg/100g		
SUB_1017	Vitamin E equiv. (d-alpha-tocopherol)		-		mg/100g		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_1017	Vitamin E (d-alpha-tocopherol)		-		IU/100g		

Method description

Code	Description
N03_07ME	Vitamin K1 in foodstuffs (HPLC-FD) acc. DIN EN 14148:2003-10, mod.
SUB_1016	Vitamin A by LC-DAD acc. EN 12823-1 2014, external*
SUB_1017	Vitamin E by LC-FLD acc. EN 12822:2014, external*

Fatty Acids

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C4:0, Butyric acid		2,50	0,02	%		
N07_05ME	C6:0, Caproic acid		2,09	0,02	%		
N07_05ME	C8:0, Caprylic acid		1,34	0,02	%		
N07_05ME	C10:0, Capric acid		3,28	0,02	%		
N07_05ME	C12:0, Lauric acid		4,24	0,02	%		
N07_05ME	C14:0, Myristic acid		12,25	0,02	%		
N07_05ME	C14:1w5, Myristoleic acid		1,16	0,02	%		
N07_05ME	C15:0, Pentadecanoic acid		1,16	0,02	%		
N07_05ME	C15:1w5, Pentadecenoic acid		<0,02	0,02	%		
N07_05ME	C16:0, Palmitic acid		33,84	0,02	%		
N07_05ME	C16:1w7, Palmitoleic acid		1,62	0,02	%		
N07_05ME	C17:0, Margaric acid		0,51	0,02	%		
N07_05ME	C17:1w7, Heptadecenoic acid		<0,02	0,02	%		
N07_05ME	C18:0, Stearic acid		10,18	0,02	%		
N07_05ME	C18:t1w9, trans-Oleic acid		0,39	0,02	%		
N07_05ME	C18:t1w7, trans-Vaccenic acid		1,14	0,02	%		
N07_05ME	C18:t1, trans isomers (except of trans-Oleic- and trans-Vaccenic acid)		1,07	0,02	%		
N07_05ME	C18:1w9, Oleic acid		18,96	0,02	%		
N07_05ME	C18:1w7, Vaccenic acid		0,42	0,02	%		
N07_05ME	C18:t2, trans isomers		0,50	0,02	%		
N07_05ME	C18:2w6, Linoleic acid (LA)		1,46	0,02	%		
N07_05ME	C20:0, Arachidic acid		0,16	0,02	%		
N07_05ME	C18:3w6, g-Linolenic acid (GLA)		0,03	0,02	%		
N07_05ME	C18:t3, trans isomers		<0,02	0,02	%		
N07_05ME	C20:1w9, Eicosenoic acid		0,06	0,02	%		
N07_05ME	C18:3w3, a-Linolenic acid (ALA)		0,58	0,02	%		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C18:2conj., conjugated Linoleic acid (CLA)		0,47	0,02	%		
N07_05ME	C21:0, Heineicosanoic acid		<0,02	0,02	%		
N07_05ME	C18:4w3, Stearidonic acid		<0,02	0,02	%		
N07_05ME	C20:2w6, Eicosadienoic acid		<0,02	0,02	%		
N07_05ME	C22:0, Behenic acid		0,06	0,02	%		
N07_05ME	C20:3w6, Eicosatrienoic acid		0,09	0,02	%		
N07_05ME	C22:1w9, Erucic acid		<0,02	0,02	%		
N07_05ME	C20:3w3, Eicosatrienoic acid		<0,02	0,02	%		
N07_05ME	C20:4w6, Arachidonic acid (AA)		0,09	0,02	%		
N07_05ME	C22:2w6, Docosadienoic acid		0,12	0,02	%		
N07_05ME	C20:5w3, Eicosapentaenoic acid (EPA)		0,08	0,02	%		
N07_05ME	C24:0, Lignoceric acid		0,05	0,02	%		
N07_05ME	C24:1w9, Nervonic acid		<0,02	0,02	%		
N07_05ME	C22:4w6, Docosatetraenoic acid		<0,02	0,02	%		
N07_05ME	C22:5w6, Docosapentaenoic acid		<0,02	0,02	%		
N07_05ME	C22:5w3, Docosapentaenoic acid		0,10	0,02	%		
N07_05ME	C22:6w3, Docosahexaenoic acid (DHA)		<0,02	0,02	%		
N07_05ME	Non identified fatty acids		<0,02		%		
N07_05ME	Sum saturated fatty acids (SFA)		71,66		%		
N07_05ME	Sum unsaturated fatty acids (UFA)		28,34		%		
N07_05ME	Sum monounsaturated fatty acids (MUFA)		24,82		%		
N07_05ME	Sum polyunsaturated fatty acids (PUFA)		3,52		%		
N07_05ME	Sum trans fatty acids		3,10		%		
N07_05ME	Sum w3-fatty acids LCP		0,18		%		
N07_05ME	Sum w6-fatty acids LCP		0,30		%		
N07_05ME	Ratio w6 LCP / w3 LCP		1,67				
N07_05ME	Sum C18:2w6 + C18:3w3		2,04		%		
N07_05ME	Sum C18:3w3 + C18:3w6		0,61		%		
N07_05ME	Sum C20:4w6 + C22:6w3		0,09		%		
N07_05ME	Ratio C18:2w6 / C18:3w3		2,52				
N07_05ME	Ratio C20:4w6 / C22:6w3		-				
N07_05ME	Ratio C22:6w3 / C20:5w3		-				
N07_05ME	ratio PUFA / SFA		0,05				
N07_05ME	Ratio SFA / UFA		2,53				
N07_05ME	C12:0, Lauric acid iP		4,03		g/100g		
N07_05ME	C14:0, Myristic acid iP		11,64		g/100g		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C18:1w9, Oleic acid iP		18,01		g/100g		
N07_05ME	C18:2w6, Linoleic acid (LA) iP		1,39		g/100g		
N07_05ME	C18:3w3, a-Linolenic acid (ALA) iP		0,55		g/100g		
N07_05ME	C20:4w6, Arachidonic acid (AA) iP		85,50		mg/100g		
N07_05ME	C20:5w3, Eicosapentaenoic acid (EPA) iP		76,00		mg/100g		
N07_05ME	C22:6w3, Docosahexaenoic acid (DHA) iP		<19,00		mg/100g		
N07_05ME	Sum saturated fatty acids (SFA) iP		68,08		g/100g		
N07_05ME	Sum monounsaturated fatty acids (MUFA) iP		23,58		g/100g		
N07_05ME	Sum polyunsaturated fatty acids (PUFA) iP		3,34		g/100g		
N07_05ME	Ratio C12+C14iP / TFA iP		0,16				
N07_05ME	Sum saturated + unsaturated fatty acids (TFA)		95,00		g/100g		
N07_05ME	Sum C12 + C14 iP		15,67		g/100g		
N07_05ME	Sum trans fatty acids iP		2,95		g/100g		

Method description

Code	Description
N07_05ME	Fatty acid composition [g/100g fatty acids] of foodstuffs (GC-FID), acc. AOAC 2012.13:2012

Fat characteristics

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_0365	Cholesterol		241	1	mg/100g		
SUB_1242	Peroxide Value		0,8	0,1	meq O2/kg		

Method description

Code	Description
SUB_0365	Cholesterol by GC-FID acc. ASU L05.00-16 mod., external*
SUB_1242	Peroxide value in oils, acc. DGF C-VI 6a - Part 1, external*

Results

Mycotoxins

Aflatoxin M1

R02_03ME Determination of aflatoxin M1 in milk and milk based products using HPLC after immunoaffinity column clean-up acc. DIN EN ISO 14501:2006-02, mod.

Parameter	Result	LOQ	Unit
Aflatoxin M1	<5^	10	ng/kg

Toxical Elements

Mercury

R03_02ME Determination of mercury in foodstuffs by AAS hydride technique, acc. DIN EN 13805:2002-06, mod. & DIN EN 13806:2002-11, mod.

Parameter	Result	LOQ	Unit
Mercury	<1	1	µg/kg

Aluminium, < 2ppm

R03_04ME Determination of aluminum in foodstuffs by solid phase AAS acc. internal method (version 2)

Parameter	Result	LOQ	Unit
Aluminium	<100	100	µg/kg

Lead

R03_01ME Determination of lead in foodstuffs by solid phase AAS acc. internal method (version 5)

Parameter	Result	LOQ	Unit
Lead	6	5	µg/kg

Tin, external*

SUB_0172 Determination of tin, external*

Parameter	Result	LOQ	Unit
Tin	<0,20	0,2	mg/kg

Antimony, external*

SUB_0175 Determination of antimony, external*

Parameter	Result	LOQ	Unit
Antimony	<0,05	0,05	mg/kg

Nickel, external*

SUB_0177 Determination of nickel (HTC/AAS, ICP-OES, ICP-MS), external*

Parameter	Result	LOQ	Unit
Nickel	<0,1	0,1	mg/kg

Cadmium (Solid phase AAS)

R03_01ME Determination of cadmium in foodstuffs by solid phase AAS acc. internal method (version 5)

Parameter	Result	LOQ	Unit
Cadmium	<1	1	µg/kg

Total arsenic in foodstuffs (ICP-MS)

R03_09ME Total arsenic in foodstuffs (ICP-MS) acc. internal method (version 1)

Parameter	Result	LOQ	Unit
Arsenic total	<15	15	µg/kg

Inorganic arsenic (ICP-OES)

R03_10ME Determination of inorganic arsenic in cereals by ICP-OES acc. ASU L15.06-02:2013-01, mod.

Parameter	Result	LOQ	Unit
Arsenic inorganic	<30	30	µg/kg

Environmental Contaminants

Dioxins (17) + PCB (12+6) in oils + fats (except fish oil), external *

SUB_0392 Determination of PCDD/PCDF and dl-PCBs (Dioxins) in fats and oils by GC-HRMS acc. EU VO 589/2014, external*

Parameter	Result	LOQ	Unit
Dioxin-like PCBs WHO(2005)-dl-PCB TEQ (upper bound)	0,247		pg/g
Dioxins and Furans WHO(2005)-PCDD/F TEQ (upper bound)	0,325		pg/g
Sum WHO(2005)-PCDD/F + dl-PCBs TEQ (upper bound)	0,573		pg/g
non dioxine like PCBs (ndl PCBs), sum of	1,99		ng/g

Radioactivity

Radioactivity Cs-134/137, external*

SUB_0165 Determination of Radioactivity by Gamma spectrometer acc. internal method, external*

Parameter	Result	LOQ	Unit
Activity Cs 134	<10^		Bq/kg
Activity Cs 137	<10^		Bq/kg
Reference date for activities	14.03.2019		

Results

Microbiological Parameters

Code	Parameter	Result	LOQ	Unit
M01_02ME	Total Viable Count 30°C, aerobe	<100	100	cfu/g
M01_02ME	Total Viable Count 55°C, aerobe	<100	100	cfu/g
M01_02ME	Anaerobic plate count 30°C	<100	100	cfu/g
M01_02ME	Anaerobic plate count 55°C	<100	100	cfu/g
M01_03ME	Bacillus cereus	<10	10	cfu/g
M01_05ME	Staphylococcus aureus per 1g	<10	10	cfu/g
M01_06ME	Yeast	<10	10	cfu/g
M01_06ME	Mould	<10	10	cfu/g
M01_07ME	Enterobacteriaceae - Detection per 1g	negative		per g
M01_14ME	Salmonella - Detection per 25g	negative		per 25g
M01_26ME	Sulphite Reducing Clostridia (SRC)	<1	1	cfu/g
M01_30ME	Cronobacter spp. - Detection per 300g	negative		per 300g
M01_35ME	Thermophilic Thermoresistant Sporeformers, Aerobic	<1	1	cfu/g
M01_35ME	Thermophilic Thermoresistant Sporeformers, Anaerobe	<1	1	cfu/g

Method description

Code	Description
M01_02ME	Total viable count (30°C and 55°C) acc. ISO 4833-1:2013-09, mod.
M01_03ME	Bacillus cereus - Enumeration acc. ISO 7932:2004-06, mod.
M01_05ME	Staphylococcus aureus (coagulase positive Staphylococci) - Enumeration acc. ISO 6888-1:2003-12, mod.
M01_06ME	Yeasts and Moulds - Enumeration acc. ISO 6611:2003-12, mod.
M01_07ME	Enterobacteriaceae - Detection acc. DIN ISO 21528-1:2009-12, mod.
M01_14ME	Salmonella - Conventional Method acc. DIN EN ISO 6579:2007-10, mod.
M01_26ME	Horizontal Method for the enumeration of sulphite reducing clostridia growing under anaerobic conditions acc. ISO 15213:2003-05, mod.
M01_30ME	Cronobacter spp. detection acc. ISO/TS 22964:2006-02, mod.
M01_35ME	Thermophilic thermoresistant sporeformers (TTS, 10 min, 80 °C) - Enumeration acc. internal method (version 3)

Legend results:

LOQ	: Limit Of Quantitation: Up from this level quantitative results are available
^	: Limit Of Detection
LOD	: Limit Of Detection
-	: Result cannot be calculated
n.d.	: Not detectable
n.e.	: Not evaluable
n.a.	: Not analysed
Dev.	: Deviation from target value
OOS?	: Out of specification?
*	: Not part of CLF's accreditation; performed by approved subcontractor
**	: Part of CLF's accreditation; performed by approved subcontractor
***	: Not part of CLF's accreditation
SUB	: Subcontractor (Original analytical report from subcontractor available on demand)

Legend method descriptions:

ASU	: Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (Official Collection of Methods of Analysis according to German Food and Feed Act)
DIN	: Deutsches Institut für Normung e.V. (German Institute for Standardization)
EN	: European Norm
ISO	: International Organization for Standardization
AOAC	: Association of Official Analytical Chemists
NEN	: Nederlands Normalisatie-instituut (Dutch Institute for Standardization)
NF	: Norme française (Norm by French Institute for Standardization)
USP	: United States Pharmacopeia
mod.	: modified (Details about modifications see annex to CLF's ISO 17025 accreditation certificate)

Remarks: 19-3086-02
Aluminium, Cadmium, Lead: Result confirmed by 2-fold determination.

Vitamin K1: analysis is not possible due to matrix issues.

Bianca Puff
- Food Chemist -
Manager Business Development & Customer Relations

Evelyn Núñez B.
- Food Chemist -
Analytical Service Manager

Notification

This analytical report is created automatically and is valid without any signature.

NLDNR

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To: [redacted]

Cc:

Person in charge Evelyn Núñez B.

Report date 25.03.2019

Analytical Report No 19-3086-03-DAN-1

AMF_FC

Sample Information:

02-TAS-DAN

		CLF order no.	CLF-02993-19
		Analytical order no.	WREQ56380
CLF sample code	19-3086-03	**Client order no.**	4503110882
Client sample code	120011632		
Local article no.	0	**Date analysis order**	08.03.2019
Concern article no.		**Date sample receipt**	11.03.2019
PDS number		**Analysis started**	12.03.2019
Material class	RS-Oils and fats	**Analysis finished**	25.03.2019
Batch	BBoWBZ7	**Country**	
Supplier		**Production date**	
Consistence	liquid	**Expiry date**	
Sample amount	2x500 ml	**Delivery date**	
Packaging			
Customer Remarks			

Notification

The results relate exclusively to the above mentioned sample.
The test report shall not be reproduced except in full, without written approval of CLF

Results

Typical Analysis

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_0929	Moisture and volatile matter content		0,07	0,01	g/100g		
N01_10ME	Fat		100	0,1	g/100g		

Method description

Code	Description
N01_10ME	Fat in foodstuffs by Soxleth extraction acc. ASU L 13.05-3:2002-05, mod.***
SUB_0929	Moisture and volatile matter content in fat & oil by gravimetry acc. ASU L 13.00-16, external**

Minerals and Trace Elements

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N06_10ME	Iron		0,05	0,02	mg/100g		
N06_10ME	Copper		<20	20	µg/100g		
N06_13ME	Molybdenum		<1,0	1	µg/100g		
N06_13ME	Chromium		2,5	1	µg/100g		
N06_14ME	Selenium		<2,0	2	µg/100g		

Method description

Code	Description
N06_10ME	Minerals and trace elements in foodstuffs (ICP-OES) acc. DIN EN 13805:2002-06, mod. & prDIN EN 16943:2016-01, mod.
N06_13ME	Chromium and Molybdenum in foodstuffs (ICP-MS) acc. DIN EN 13805:2002-06, mod.
N06_14ME	Selenium in dietetic foodstuffs & raw materials for their production (ICP-MS) acc. DIN EN 13805:2002-06, mod.

Fat soluble Vitamins

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N03_07ME	Vitamin K1 (phyllochinon)		n.e.	2	µg/100g		
SUB_1016	Vitamin A (retinol)		909	21	µg/100g		
SUB_1016	Vitamin A (retinol) in IU (calc.)		30300	700	IU/kg		
SUB_1017	Vitamin E (d,l-alpha-tocopherol)		2,00	0,5	mg/100g		
SUB_1017	Vitamin E (beta-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (delta-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (gamma-tocopherol)		<0,5	0,5	mg/100g		
SUB_1017	Vitamin E (sum-tocopherol)		2,00		mg/100g		
SUB_1017	Vitamin E equiv. (d-alpha-tocopherol)		-		mg/100g		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_1017	Vitamin E (d-alpha-tocopherol)		-		IU/100g		

Method description

Code	Description
N03_07ME	Vitamin K1 in foodstuffs (HPLC-FD) acc. DIN EN 14148:2003-10, mod.
SUB_1016	Vitamin A by LC-DAD acc. EN 12823-1 2014, external*
SUB_1017	Vitamin E by LC-FLD acc. EN 12822:2014, external*

Fatty Acids

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C4:0, Butyric acid		2,44	0,02	%		
N07_05ME	C6:0, Caproic acid		2,12	0,02	%		
N07_05ME	C8:0, Caprylic acid		1,35	0,02	%		
N07_05ME	C10:0, Capric acid		3,27	0,02	%		
N07_05ME	C12:0, Lauric acid		4,23	0,02	%		
N07_05ME	C14:0, Myristic acid		12,16	0,02	%		
N07_05ME	C14:1w5, Myristoleic acid		1,16	0,02	%		
N07_05ME	C15:0, Pentadecanoic acid		1,16	0,02	%		
N07_05ME	C15:1w5, Pentadecenoic acid		<0,02	0,02	%		
N07_05ME	C16:0, Palmitic acid		33,73	0,02	%		
N07_05ME	C16:1w7, Palmitoleic acid		1,63	0,02	%		
N07_05ME	C17:0, Margaric acid		0,51	0,02	%		
N07_05ME	C17:1w7, Heptadecenoic acid		<0,02	0,02	%		
N07_05ME	C18:0, Stearic acid		10,15	0,02	%		
N07_05ME	C18:t1w9, trans-Oleic acid		0,38	0,02	%		
N07_05ME	C18:t1w7, trans-Vaccenic acid		1,16	0,02	%		
N07_05ME	C18:t1, trans isomers (except of trans-Oleic- and trans-Vaccenic acid)		1,07	0,02	%		
N07_05ME	C18:1w9, Oleic acid		19,19	0,02	%		
N07_05ME	C18:1w7, Vaccenic acid		0,42	0,02	%		
N07_05ME	C18:t2, trans isomers		0,51	0,02	%		
N07_05ME	C18:2w6, Linoleic acid (LA)		1,45	0,02	%		
N07_05ME	C20:0, Arachidic acid		0,16	0,02	%		
N07_05ME	C18:3w6, g-Linolenic acid (GLA)		0,03	0,02	%		
N07_05ME	C18:t3, trans isomers		<0,02	0,02	%		
N07_05ME	C20:1w9, Eicosenoic acid		0,04	0,02	%		
N07_05ME	C18:3w3, a-Linolenic acid (ALA)		0,57	0,02	%		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C18:2conj., conjugated Linoleic acid (CLA)		0,47	0,02	%		
N07_05ME	C21:0, Heineicosanoic acid		<0,02	0,02	%		
N07_05ME	C18:4w3, Stearidonic acid		<0,02	0,02	%		
N07_05ME	C20:2w6, Eicosadienoic acid		0,02	0,02	%		
N07_05ME	C22:0, Behenic acid		0,06	0,02	%		
N07_05ME	C20:3w6, Eicosatrienoic acid		0,09	0,02	%		
N07_05ME	C22:1w9, Erucic acid		<0,02	0,02	%		
N07_05ME	C20:3w3, Eicosatrienoic acid		0,04	0,02	%		
N07_05ME	C20:4w6, Arachidonic acid (AA)		0,10	0,02	%		
N07_05ME	C22:2w6, Docosadienoic acid		0,12	0,02	%		
N07_05ME	C20:5w3, Eicosapentaenoic acid (EPA)		0,08	0,02	%		
N07_05ME	C24:0, Lignoceric acid		0,05	0,02	%		
N07_05ME	C24:1w9, Nervonic acid		<0,02	0,02	%		
N07_05ME	C22:4w6, Docosatetraenoic acid		<0,02	0,02	%		
N07_05ME	C22:5w6, Docosapentaenoic acid		<0,02	0,02	%		
N07_05ME	C22:5w3, Docosapentaenoic acid		0,10	0,02	%		
N07_05ME	C22:6w3, Docosahexaenoic acid (DHA)		<0,02	0,02	%		
N07_05ME	Non identified fatty acids		<0,02		%		
N07_05ME	Sum saturated fatty acids (SFA)		71,39		%		
N07_05ME	Sum unsaturated fatty acids (UFA)		28,63		%		
N07_05ME	Sum monounsaturated fatty acids (MUFA)		25,05		%		
N07_05ME	Sum polyunsaturated fatty acids (PUFA)		3,58		%		
N07_05ME	Sum trans fatty acids		3,12		%		
N07_05ME	Sum w3-fatty acids LCP		0,22		%		
N07_05ME	Sum w6-fatty acids LCP		0,33		%		
N07_05ME	Ratio w6 LCP / w3 LCP		1,50				
N07_05ME	Sum C18:2w6 + C18:3w3		2,02		%		
N07_05ME	Sum C18:3w3 + C18:3w6		0,60		%		
N07_05ME	Sum C20:4w6 + C22:6w3		0,10		%		
N07_05ME	Ratio C18:2w6 / C18:3w3		2,54				
N07_05ME	Ratio C20:4w6 / C22:6w3		-				
N07_05ME	Ratio C22:6w3 / C20:5w3		-				
N07_05ME	ratio PUFA / SFA		0,05				
N07_05ME	Ratio SFA / UFA		2,49				
N07_05ME	C12:0, Lauric acid iP		4,02		g/100g		
N07_05ME	C14:0, Myristic acid iP		11,55		g/100g		

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
N07_05ME	C18:1w9, Oleic acid iP		18,23		g/100g		
N07_05ME	C18:2w6, Linoleic acid (LA) iP		1,38		g/100g		
N07_05ME	C18:3w3, a-Linolenic acid (ALA) iP		0,54		g/100g		
N07_05ME	C20:4w6, Arachidonic acid (AA) iP		95,00		mg/100g		
N07_05ME	C20:5w3, Eicosapentaenoic acid (EPA) iP		76,00		mg/100g		
N07_05ME	C22:6w3, Docosahexaenoic acid (DHA) iP		<19,00		mg/100g		
N07_05ME	Sum saturated fatty acids (SFA) iP		67,82		g/100g		
N07_05ME	Sum monounsaturated fatty acids (MUFA) iP		23,80		g/100g		
N07_05ME	Sum polyunsaturated fatty acids (PUFA) iP		3,40		g/100g		
N07_05ME	Ratio C12+C14iP / TFA iP		0,16				
N07_05ME	Sum saturated + unsaturated fatty acids (TFA)		95,02		g/100g		
N07_05ME	Sum C12 + C14 iP		15,57		g/100g		
N07_05ME	Sum trans fatty acids iP		2,96		g/100g		

Method description

Code	Description
N07_05ME	Fatty acid composition [g/100g fatty acids] of foodstuffs (GC-FID), acc. AOAC 2012.13:2012

Fat characteristics

Code	Parameter	Exp. Value	Result	LOQ	Unit	Dev. [%]	OOS?
SUB_0365	Cholesterol		232	1	mg/100g		
SUB_1242	Peroxide Value		0,9	0,1	meq O2/kg		

Method description

Code	Description
SUB_0365	Cholesterol by GC-FID acc. ASU L05.00-16 mod., external*
SUB_1242	Peroxide value in oils, acc. DGF C-VI 6a - Part 1, external*

Results

Mycotoxins

Aflatoxin M1

R02_03ME Determination of aflatoxin M1 in milk and milk based products using HPLC after immunoaffinity column clean-up acc. DIN EN ISO 14501:2006-02, mod.

Parameter	Result	LOQ	Unit
Aflatoxin M1	<5^	10	ng/kg

Toxical Elements

Mercury

R03_02ME Determination of mercury in foodstuffs by AAS hydride technique, acc. DIN EN 13805:2002-06, mod. & DIN EN 13806:2002-11, mod.

Parameter	Result	LOQ	Unit
Mercury	<1	1	µg/kg

Aluminium, < 2ppm

R03_04ME Determination of aluminum in foodstuffs by solid phase AAS acc. internal method (version 2)

Parameter	Result	LOQ	Unit
Aluminium	<100	100	µg/kg

Lead

R03_01ME Determination of lead in foodstuffs by solid phase AAS acc. internal method (version 5)

Parameter	Result	LOQ	Unit
Lead	6	5	µg/kg

Tin, external*

SUB_0172 Determination of tin, external*

Parameter	Result	LOQ	Unit
Tin	<0,2	0,2	mg/kg

Antimony, external*

SUB_0175 Determination of antimony, external*

Parameter	Result	LOQ	Unit
Antimony	<0,05	0,05	mg/kg

Nickel, external*

SUB_0177 Determination of nickel (HTC/AAS, ICP-OES, ICP-MS), external*

Parameter	Result	LOQ	Unit
Nickel	<0,1	0,1	mg/kg

Cadmium (Solid phase AAS)

R03_01ME Determination of cadmium in foodstuffs by solid phase AAS acc. internal method (version 5)

Parameter	Result	LOQ	Unit
Cadmium	<1	1	µg/kg

Total arsenic in foodstuffs (ICP-MS)

R03_09ME Total arsenic in foodstuffs (ICP-MS) acc. internal method (version 1)

Parameter	Result	LOQ	Unit
Arsenic total	<15	15	µg/kg

Inorganic arsenic (ICP-OES)

R03_10ME Determination of inorganic arsenic in cereals by ICP-OES acc. ASU L15.06-02:2013-01, mod.

Parameter	Result	LOQ	Unit
Arsenic inorganic	<30	30	µg/kg

Environmental Contaminants

Dioxins (17) + PCB (12+6) in oils + fats (except fish oil), external *

SUB_0392 Determination of PCDD/PCDF and dl-PCBs (Dioxins) in fats and oils by GC-HRMS acc. EU VO 589/2014, external*

Parameter	Result	LOQ	Unit
Dioxin-like PCBs WHO(2005)-dl-PCB TEQ (upper bound)	0,241		pg/g
Dioxins and Furans WHO(2005)-PCDD/F TEQ (upper bound)	0,327		pg/g
Sum WHO(2005)-PCDD/F + dl-PCBs TEQ (upper bound)	0,569		pg/g
non dioxine like PCBs (ndl PCBs), sum of	2,02		ng/g

Radioactivity

Radioactivity Cs-134/137, external*

SUB_0165 Determination of Radioactivity by Gamma spectrometer acc. internal method, external*

Parameter	Result	LOQ	Unit
Activity Cs 134	<10^		Bq/kg
Activity Cs 137	<10^		Bq/kg
Reference date for activities	14.03.2019		

Results

Microbiological Parameters

Code	Parameter	Result	LOQ	Unit
M01_02ME	Total Viable Count 30°C, aerobe	<100	100	cfu/g
M01_02ME	Total Viable Count 55°C, aerobe	<100	100	cfu/g
M01_02ME	Anaerobic plate count 30°C	<100	100	cfu/g
M01_02ME	Anaerobic plate count 55°C	<100	100	cfu/g
M01_03ME	Bacillus cereus	<10	10	cfu/g
M01_05ME	Staphylococcus aureus per 1g	<10	10	cfu/g
M01_06ME	Yeast	<10	10	cfu/g
M01_06ME	Mould	<10	10	cfu/g
M01_07ME	Enterobacteriaceae - Detection per 1g	negative		per g
M01_14ME	Salmonella - Detection per 25g	negative		per 25g
M01_26ME	Sulphite Reducing Clostridia (SRC)	<1	1	cfu/g
M01_30ME	Cronobacter spp. - Detection per 300g	negative		per 300g
M01_35ME	Thermophilic Thermoresistant Sporeformers, Aerobic	<1	1	cfu/g
M01_35ME	Thermophilic Thermoresistant Sporeformers, Anaerobe	<1	1	cfu/g

Method description

Code	Description
M01_02ME	Total viable count (30°C and 55°C) acc. ISO 4833-1:2013-09, mod.
M01_03ME	Bacillus cereus - Enumeration acc. ISO 7932:2004-06, mod.
M01_05ME	Staphylococcus aureus (coagulase positive Staphylococci) - Enumeration acc. ISO 6888-1:2003-12, mod.
M01_06ME	Yeasts and Moulds - Enumeration acc. ISO 6611:2003-12, mod.
M01_07ME	Enterobacteriaceae - Detection acc. DIN ISO 21528-1:2009-12, mod.
M01_14ME	Salmonella - Conventional Method acc. DIN EN ISO 6579:2007-10, mod.
M01_26ME	Horizontal Method for the enumeration of sulphite reducing clostridia growing under anaerobic conditions acc. ISO 15213:2003-05, mod.
M01_30ME	Cronobacter spp. detection acc. ISO/TS 22964:2006-02, mod.
M01_35ME	Thermophilic thermoresistant sporeformers (TTS, 10 min, 80 °C) - Enumeration acc. internal method (version 3)

Legend results:

LOQ	: Limit Of Quantitation: Up from this level quantitative results are available
^	: Limit Of Detection
LOD	: Limit Of Detection
-	: Result cannot be calculated
n.d.	: Not detectable
n.e.	: Not evaluable
n.a.	: Not analysed
Dev.	: Deviation from target value
OOS?	: Out of specification?
*	: Not part of CLF's accreditation; performed by approved subcontractor
**	: Part of CLF's accreditation; performed by approved subcontractor
***	: Not part of CLF's accreditation
SUB	: Subcontractor (Original analytical report from subcontractor available on demand)

Legend method descriptions:

ASU	: Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (Official Collection of Methods of Analysis according to German Food and Feed Act)
DIN	: Deutsches Institut für Normung e.V. (German Institute for Standardization)
EN	: European Norm
ISO	: International Organization for Standardization
AOAC	: Association of Official Analytical Chemists
NEN	: Nederlands Normalisatie-instituut (Dutch Institute for Standardization)
NF	: Norme française (Norm by French Institute for Standardization)
USP	: United States Pharmacopeia
mod.	: modified (Details about modifications see annex to CLF's ISO 17025 accreditation certificate)

Remarks: 19-3086-03
Aluminium, Cadmium, Lead: Result confirmed by 2-fold determination.

Vitamin K1: analysis is not possible due to matrix issues.

Bianca Puff
- Food Chemist -
Manager Business Development & Customer Relations

Evelyn Núñez B.
- Food Chemist -
Analytical Service Manager

Notification

This analytical report is created automatically and is valid without any signature.

Appendix B. Statement of Quality Assurance

[redacted]

[redacted]

[redacted]

Subject: [redacted] Milk Fat Food Grade

We, [redacted] herewith inform you that we are committed to manufacture products safely and sustainably, and to meet applicable regulatory and statutory requirements through implementation of our quality and food safety management processes.

[redacted] manufacturing sites have management systems in place that have been certified according FSSC. These include standard operation procedures to ensure and document that the materials used in commercial production are of a suitable grade to be used in food.

Furthermore, the raw material for [redacted] Milk Fat is pasteurized cream originating from cow's milk. [redacted]

The [redacted] Milk Fat will be manufactured under conditions suitable for human consumption, in accordance to EU food law requirements and EU regulations on hygiene for foodstuffs as well as Food GMPs. Also no additives are used in the production of [redacted] Milk Fat.
During filling the product is flushed with nitrogen as a preservative. This nitrogen meets application requirements.

We trust to have informed you sufficiently.

[redacted]

Appendix C. Certificates of Analysis on 5 Lots of AMF

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

Order information	
Customer	[redacted]

Product information	
Product description	Anhydrous Milk Fat IFT Tank truck
Lot number	BB0XW1H
Production date	05-06-2019

Chemical and physical analysis				
Parameter	Unit	Result	Standard	Method
Fat *	%	99.9	Min. 99.8	Calculated
Moisture *	%	0.1	Max. 0.1	IDF 23
FFA (as oleic acid) *	%	0.2	Max. 0.3	IDF 6
Peroxide value *	Meq O_2/kg	0.2	Max. 0.3	IDF 74

Microbiological analysis				
Parameter	Unit	Result	Standard	Method
Total plate count (30ºC)	CFU/g	<500	<500	ISO 4833
Total plate count (55ºC)	CFU/g	<2500	<2500	ISO 4833
Enterobacteriaceae	/g	Absent	Absent	ISO 21528-2
Yeast & moulds *	CFU/g	<10	<10	ISO 6611
Staphylococcus Aureus *	/g	Absent	Absent	ISO 6888-3
Salmonella	/250g	Absent	Absent	ISO 6579
Thermophilic aerobic and anaerobic spores *	CFU/g	<100	<100	
Sulphite Reducing Clostridia	CFU/g	<1	<1	Weenk
Bacillus Cereus	CFU/g	<10	<10	ISO 7932

Remarks
Results of parameters marked with * are based on monitoring program. Shelf life: Out of direct sunlight: 21 days at 45 °C when stored under nitrogen (During filling the product is flushed with nitrogen E 941)

Signing	
Date	14-06-2019
Name	H. Pel

[redacted]

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

Order information	
Customer	[redacted]

Product information	
Product description	Anhydrous Milk Fat IFT Tank truck
Lot number	BB0Z9ZN
Production date	18-07-2019

Chemical and physical analysis				
Parameter	Unit	Result	Standard	Method
Fat *	%	99.9	Min. 99.8	Calculated
Moisture *	%	0.1	Max. 0.1	IDF 23
FFA (as oleic acid) *	%	0.2	Max. 0.3	IDF 6
Peroxide value *	Meq O_2/kg	0.2	Max. 0.3	IDF 74

Microbiological analysis				
Parameter	Unit	Result	Standard	Method
Total plate count (30ºC)	CFU/g	<500	<500	ISO 4833
Total plate count (55ºC)	CFU/g	<2500	<2500	ISO 4833
Enterobacteriaceae	/g	Absent	Absent	ISO 21528-2
Yeast & moulds *	CFU/g	<10	<10	ISO 6611
Staphylococcus Aureus *	/g	Absent	Absent	ISO 6888-3
Salmonella	/250g	Absent	Absent	ISO 6579
Thermophilic aerobic and anaerobic spores *	CFU/g	<100	<100	
Sulphite Reducing Clostridia	CFU/g	<1	<1	Weenk
Bacillus Cereus	CFU/g	<10	<10	ISO 7932

Remarks
Results of parameters marked with * are based on monitoring program. Shelf life: Out of direct sunlight: 21 days at 45 °C when stored under nitrogen (During filling the product is flushed with nitrogen E 941)

Signing	
Date	25-07-2019
Name	W de Haan

[redacted]

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

Order information	
Customer	[redacted]

Product information	
Product description	Anhydrous Milk Fat IFT Tank truck
Lot number	BB0XJBS
Production date	09-05-2019

Chemical and physical analysis				
Parameter	Unit	Result	Standard	Method
Fat *	%	99.9	Min. 99.8	Calculated
Moisture *	%	0.1	Max. 0.1	IDF 23
FFA (as oleic acid) *	%	0.2	Max. 0.3	IDF 6
Peroxide value *	Meq O_2/kg	0.2	Max. 0.3	IDF 74

Microbiological analysis				
Parameter	Unit	Result	Standard	Method
Total plate count (30ºC)	CFU/g	<500	<500	ISO 4833
Total plate count (55ºC)	CFU/g	<2500	<2500	ISO 4833
Enterobacteriaceae	/g	Absent	Absent	ISO 21528-2
Yeast & moulds *	CFU/g	<10	<10	ISO 6611
Staphylococcus Aureus *	/g	Absent	Absent	ISO 6888-3
Salmonella	/250g	Absent	Absent	ISO 6579
Thermophilic aerobic and anaerobic spores *	CFU/g	<100	<100	
Sulphite Reducing Clostridia	CFU/g	<1	<1	Weenk
Bacillus Cereus	CFU/g	<10	<10	ISO 7932

Remarks
Results of parameters marked with * are based on monitoring program. Shelf life: Out of direct sunlight: 21 days at 45 °C when stored under nitrogen (During filling the product is flushed with nitrogen E 941)

Signing	
Date	29-08-19
Name	E. Modderman

[redacted]

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

Order information	
Customer	[redacted]

Product information	
Product description	Anhydrous Milk Fat IFT Tank truck
Lot number	BB0Z7VD
Production date	08-07-2019

Chemical and physical analysis				
Parameter	Unit	Result	Standard	Method
Fat *	%	99.9	Min. 99.8	Calculated
Moisture *	%	0.1	Max. 0.1	IDF 23
FFA (as oleic acid) *	%	0.2	Max. 0.3	IDF 6
Peroxide value *	Meq O_2/kg	0.2	Max. 0.3	IDF 74

Microbiological analysis				
Parameter	Unit	Result	Standard	Method
Total plate count (30ºC)	CFU/g	<500	<500	ISO 4833
Total plate count (55ºC)	CFU/g	<2500	<2500	ISO 4833
Enterobacteriaceae	/g	Absent	Absent	ISO 21528-2
Yeast & moulds *	CFU/g	<10	<10	ISO 6611
Staphylococcus Aureus *	/g	Absent	Absent	ISO 6888-3
Salmonella	/250g	Absent	Absent	ISO 6579
Thermophilic aerobic and anaerobic spores *	CFU/g	<100	<100	
Sulphite Reducing Clostridia	CFU/g	<1	<1	Weenk
Bacillus Cereus	CFU/g	<10	<10	ISO 7932

Remarks
Results of parameters marked with * are based on monitoring program. Shelf life: Out of direct sunlight: 21 days at 45 °C when stored under nitrogen (During filling the product is flushed with nitrogen E 941)

Signing	
Date	29-08-2019
Name	E. Modderman

[redacted]

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

Order information	
Customer	[redacted]

Product information	
Product description	Anhydrous Milk Fat IFT Tank truck
Lot number	BB0ZLR2
Production date	19-08-2019

Chemical and physical analysis				
Parameter	Unit	Result	Standard	Method
Fat *	%	99.9	Min. 99.8	Calculated
Moisture *	%	0.1	Max. 0.1	IDF 23
FFA (as oleic acid) *	%	0.2	Max. 0.3	IDF 6
Peroxide value *	Meq O_2/kg	0.2	Max. 0.3	IDF 74

Microbiological analysis				
Parameter	Unit	Result	Standard	Method
Total plate count (30ºC)	CFU/g	<500	<500	ISO 4833
Total plate count (55ºC)	CFU/g	<2500	<2500	ISO 4833
Enterobacteriaceae	/g	Absent	Absent	ISO 21528-2
Yeast & moulds *	CFU/g	<10	<10	ISO 6611
Staphylococcus Aureus *	/g	Absent	Absent	ISO 6888-3
Salmonella	/250g	Absent	Absent	ISO 6579
Thermophilic aerobic and anaerobic spores *	CFU/g	<100	<100	
Sulphite Reducing Clostridia	CFU/g	<1	<1	Weenk
Bacillus Cereus	CFU/g	<10	<10	ISO 7932

Remarks
Results of parameters marked with * are based on monitoring program. Shelf life: Out of direct sunlight: 21 days at 45 °C when stored under nitrogen (During filling the product is flushed with nitrogen E 941)

Signing	
Date	29-08-2019
Name	E. Modderman

[redacted]

Appendix D. Monitoring for Potential Contaminants

[redacted]

[redacted]

[redacted]

Subject: [redacted] Milk Fat Bulk monitoring

We, [redacted] inform you that we monitor our product [redacted] Milk Fat Bulk (Milk Fat 99.8%, art. 896919) amongst others for the following parameters, which is based on our FoQus standard and agreed specifications:

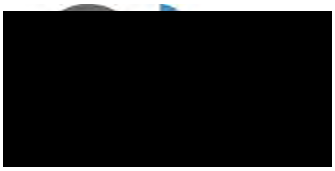
Table 1. Results of monitoring for contaminants and quality factors in [redacted] Milk Fat Bulk

Parameter	Limit	No of Samples	Results: Median	Results: Maximum	Time frame	Frequency of Sampling	Method of Analysis	LOD
E. coli	absent in 1 g	N=8	<10 cfu/1g	<10 cfu/1g	2019	Monitoring	FC-method, LMX 25h, Coli ID 24h	<10
L. monocytogenes	absent in 25 g	N=15	Negative	Negative	2019	Monitoring	FC-method equivalent to ISO 11290-1	
Iron		N=2	<0.8mg/kg	<0.8mg/kg	2019	Monitoring	FC-method using AOAC 984.27	<0.8
Copper		N=2	<0.1mg/kg	<0.1mg/kg	2019	Monitoring	FC-method using AOAC 984.27	<0.1

We trust to have informed you sufficiently.

[redacted]

[redacted]



CONTAMINANTS AND RESIDUES IN DUTCH FARM MILK AND DAIRY PRODUCTS

RESULTS OF THE MONITORING PROGRAM OF THE DUTCH DAIRY INDUSTRY IN 2017

Component	Unit	Limit	2017			2016			2012 up to and including 2016
			No. of samples	Median value [1]	Max. value	No. of samples	Median value [1]	Max. value	Median value [1]
Aflatoxin M1									
Farm milk, composite sample [2]	µg/kg milk	0,05	480	<0,010	<0,010	480	<0,010	<0,010	<0,010
Heavy metals									
Farm milk, composite sample [3]			20			20			
Cadmium	µg/kg milk	-		<0,5	<0,5		<0,5	<0,5	<0,5
Lead	µg/kg milk	20		<5	<5		<5	<5	<5
Mercury	µg/kg milk	-		<0,2	<0,2		<0,2	<0,2	<0,2
Arsenic	µg/kg milk	-		<2	<2		<2	<2	<2
Radioactive substances									
Farm milk, composite sample [4]			12			12			
^{134}Cs and ^{137}Cs	Bq/kg milk	370		<10	<10		<10	<10	<10
Organochlorine pesticides									
Farm milk, composite sample [5]			271			180			
HCB	mg/kg fat	0,25/0,125 [6]		<0,01	<0,01		<0,01	<0,01	<0,01
α-HCH	mg/kg fat	0,10		<0,01	<0,01		<0,01	<0,01	<0,01
β-HCH	mg/kg fat	0,075		<0,01	<0,01		<0,01	<0,01	<0,01
γ-HCH (lindane)	mg/kg fat	0,025		<0,01	<0,01		<0,01	<0,01	<0,01
Heptachlor	mg/kg fat	0,10		<0,03	<0,03				
(sum of heptachlor, cis-heptachlor epoxide, trans-heptachlor epoxide)							<0,01	<0,01	<0,01
Chlordane	mg/kg fat	0,05		<0,03	<0,03				
(sum of cis-chlordan, trans-chlordan, oxychlordan)							<0,01	<0,01	<0,01
DDT	mg/kg fat	1,00		<0,04	<0,04				
(sum of p,p'-DDE							<0,01	<0,01	<0,01
p,p'-DDD (TDE)							<0,01	<0,01	<0,01
p,p'-DDT							<0,01	<0,01	<0,01
o,p'-DDT)									
Aldrin and dieldrin	mg/kg fat	0,15		<0,02	<0,02		<0,03	<0,03	<0,03 [7]
ß- Endosulfan	mg/kg fat	1,25		<0,025	<0,025				
Endrin	mg/kg fat	0,02		<0,01	<0,01				
Chloroform									
Butter	mg/kg butter	[8]	24	0,02	0,04	24	0,02	0,03	0,02
PCB-compounds									
Farm milk, composite sample [5]			271			180			
Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 [9]	ng/g fat	40		12,0	12,0		12,0	12,0	12,0
Dioxins and dioxin-like PCB's									
Farm milk, composite sample [10]			72			72			
Total dioxins (sum dioxins and furans)	pg TEQ/g fat	2,5		0,23	0,32		0,25	0,32	0,26
Total dioxins and dioxin-like PCB's	pg TEQ/g fat	5,5		0,41	0,64		0,50	0,58	0,53
Polyaromatic hydrocarbons									
Farm milk, composite sample [11]			20			20			
Benzo(a)pyrene	µg/kg fat	2,0		<0,10	<0,10		<0,10	0,10	<0,10
Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene	µg/kg fat	10,0		<0,40	<0,40		<0,40	0,45	<0,40

Component	Unit	Limit	2017			2016			2012 up to and including 2016
			No. of samples	Median value [1]	Max. value	No. of samples	Median value [1]	Max. value	Median value [1]
Melamine and cyanuric acid									
Farm milk, composite sample [4]			36			36			
Melamine	mg/kg milk	2,5		<0,010	<0,010		<0,010	<0,010	<0,010
Cyanuric acid	mg/kg milk	-		<0,010	0,026 [16]		<0,010	<0,010	<0,010
Veterinary drugs									
Anthelmintics									
Farm milk, composite sample [12]			360			300			
Avermectines									
Abamectin	µg/kg milk	-		<0,05	<0,05		<0,1	<0,1	<0,1
Doramectin	µg/kg milk	[13]		<0,05	<0,05		<0,1	<0,1	<0,1
Eprinomectin	µg/kg milk	20		<4	<4		<10	<10	<10
Ivermectin	µg/kg milk	[13]		<0,05	0,52 [14]		<0,1	<0,1	<0,1
Moxidectine	µg/kg milk	40		<2	10,6		<2	4,1	<2
Benzimidazoles/levamisole/triclabendazoles									
Albendazole	µg/kg milk	100		<3	<3				
(sum of albendazole sulfoxide albendazole sulfone albendazole 2-amino sulfone)							<1	1,1	<1
Flubendazole	µg/kg milk	-		<2	<2				
(sum of flubendazole 2-amino flubendazole)							<1	<1	<1
Levamisole	µg/kg milk	[13]		<1	<1		<1	<1	<1
Mebendazole	µg/kg milk	[13]		<3	<3				
(sum of mebendazole amine mebendazole 5-hydroxymebendazole)							<1	<1	<1
Oxfendazolesulfone	µg/kg milk	10		<3	<3				
(sum of fenbendazole							<1	<1	<1
oxfendazole							<1	<1	<1
oxfendazolesulfone)							<1	<1	<1
Oxibendazole	µg/kg milk	-		<2	<2				
(sum of oxibendazole-amine oxibendazole)							<1	<1	<1
Thiabendazole	µg/kg milk	100		<2	<2				
(sum of thiabendazole 5-hydroxythiabendazole)							<1	1,5	<1
Triclabendazole	µg/kg milk	10		<3	3,0		<3	<3	<10
(sum of triclabendazole triclabendazolesulfone triclabendazolesulfoxide ketotriclabendazole)									
Antibiotics			**% non-compliant [15]**			**% non-compliant [15]**			**% non-compliant [15]**
Farm milk			2,1 x 10^6			2,2 x 10^6			
ß-lactam antibiotics	% non-compliant			0,012			0,013		0,016
Other (non ß-lactam antibiotics)	% non-compliant			<0,001			<0,001		<0,001

1) The median is the middle value in a set of numbers that are arranged by size. This means that 50% of the numbers is below the median and 50% of the numbers is above the median.
2) A composite sample of farm milk was prepared from 4 individual farm milk samples.
3) A composite sample of farm milk was prepared from 15 individual farm milk samples.
4) A composite sample of farm milk was prepared from 50 individual farm milk samples.
5) A composite sample of farm milk was prepared from 8 individual farm milk samples (from 2017) or from 12 individual farm milk samples (till 2017).
6) From 10 May 2017 the regulatory limit for HCB is 0,125 mg/kg fat. Till 10 May 2017 the regulatory limit for HCB was 0,25 mg/kg fat.
7) Median value of 2014 up to and including 2016.
8) In Germany a limit for food of 0,1 mg/kg is applied.
9) Calculated as upper bound concentration according to Regulation (EC) No 1881/2006. Upper bound concentrations are calculated on the assumption that all the values of the different congeners below the limit of quantification are equal to the limit of quantification.
10) A composite sample of farm milk was prepared from 25 individual farm milk samples.
11) A composite sample of farm milk was prepared from 20 individual farm milk samples.
12) A composite sample of farm milk was prepared from 3 individual farm milk samples.
13) Not allowed for administration to animals which produce milk for human consumption.
14) In 3 of the 360 samples ivermectin was detected (0,06, 0,09 and 0,52 µg/kg). Follow-up action was taken.
15) % samples non-compliant with limit in milk payment testing instead of median value.
16) For cyanuric acid there is no regulatory limit. Nevertheless, follow-up action was taken to determine the cause.

Appendix E. PubMed Literature Searches

Date	Search Terms	Citations
7-Jan-19	Search "infant formula" AND (fat or lipid) AND (source or quality) Sort by: Best Match Filters: published in the last 10 years; English	96
29-Mar-19	Search (dairy OR Milk) AND (fat OR lipid) AND infant AND formula Filters: English	1309
31-Mar-19	Search branched chain fatty acids AND (formula OR infant OR "breast milk" OR "human milk")Sort by: Best Match Filters: English	165
28-Apr-19	Search ((human milk) OR (breast milk) OR breastmilk) AND (CLA OR "conjugated linolenic acid")Filters: published in the last 10 years; English	83
1-Jun-19	Search (trans fat OR (trans fat) OR vaccenic OR rumenic OR CLA OR "conjugated linoleic acid") AND (infant OR newborn OR "breast milk" OR "human milk" OR breastmilk OR "breastfed" OR "breast fed") Filters: English	383
3-Jun-19	Search odd chain fatty acid AND (breast milk OR human milk) Filters: English	16

Appendix F. Supportive Data on Concentrations of Components in Human Milk

Human Milk Concentrations of Select Fatty Acids in Milk Fat

Butyric Acid

In studies of the fatty acid composition of human milk, short-chain fatty acids are not always detected, though low concentrations of butyric acid have been reported in some studies. In studies of the composition of mature human milk in which butyric acid was detected and reported, the concentration ranged from 0.009 to 0.76% of total fatty acids, with the highest concentrations reported in human milk collected from women in Italy (0.76%) followed by China (0.6%) (Appendix F - Table 1). In a study of 102 women participating in the Cambridge Baby Growth Study cohort, free butyrate was detected at a concentration of 0 to 3.5 mg per 100 mL in human milk samples with higher peak values of butyrate among women who were exclusively breastfeeding compared with women partially breastfeeding (Prentice et al., 2019). Assuming 4.0 g fat per 100 mL mature human milk (IOM, 2005), the concentration of free butyrate in human milk ranges from approximately 0 to 0.09% of total fatty acids. Overall, the available data indicate that the concentration of butyric acid in human milk is variable and in the range of not detectable to 0.76% of total fatty acids.

Appendix F - Table 1. Butyric acid in Mature Human Milk

Reference	Mother's Location of Residence	Number of Mothers	Butyric Acid Concentration in Human Milk, % of total fatty acids (mean, SD [range])
Glew et al. 2011	US (New Mexico)	19	0.009 ± 0.006
Mosley et al., 2005	US (Arizona)	81	0.01 ± 0.01 [0.00–0.04]
Prentice et al., 2019	United Kingdom	102	0 to 3.5 mg per 100 mL (0 to 0.09%)[a]
Chardigny et al., 1995[b]	France	10	0.03 ± 0.01 [0.01-0.06]
Hageman et al., 2019(a)	The Netherlands	4	<0.1 [ND-<0.01]
Santillo et al., 2018	Italy	5	0.76
Sun et al., 2016	China (Wuxi)	10	ND
Wan et al., 2010	China (Northern)	52	0.6
Le Huërou-Luron et al., 2018(a)	NS	NS	-
Jensen, 1996 [c]	NS	NS	0.19

Abbreviations: ND – not detected; NS – not specified; SD – standard deviation.
[a] Assuming 4.0 g fat per 100 mL mature human milk (IOM, 2005)
[b] Data are normalized compilations from up to 15 papers as reported by the authors.
[c] as cited by Jensen, 1999

Commercial formulas containing milk fat as a component of the fat blend are available in many markets, and these formulas are a source of the specific fatty acids present in milk fat including butyric acid. Analyses of various formulas containing cow's milk or goat's milk indicate mean butyric acid concentrations in the range of approximately 0.3 to 3% of fatty acids (Appendix F -

Table 2). Formulas made with vegetable oils are estimated to contain as much as 4% residual milk fat from milk-derived ingredients such as skimmed milk (Berger et al., 2000), and analyses of these products show that on average butyric acid accounts for up to 0.2% of fatty acids.

Appendix F - Table 2. Butyric acid in Commercial Infant Formulas

Reference	Location	Butyric Acid Concentration in Infant Formula, % of total fatty acids (mean, SD, [range])	
		Milk Formula [a]	Vegetable Oil Formula
Berger, 2000	Switzerland	3.21	0.04
Hageman et al., 2019(a)	The Netherlands	2.6	<0.1
Le Huërou-Luron et al., 2018(a)	NS	-	<0.05%
Prosser et al., 2010	New Zealand	1.2 and 1.9 (mol%) 3.1 ± 1.0 (mol%) [goat]	-
Sun et al., 2016	China	0.85 ± 0.93 [0.18-3.52] 0.33 ± 0.1 [0.21-0.49] [goat]	0.21 ± 0.49 [0-1.91]

Abbreviations: NS – not specified; SD – standard deviation.
[a] Cow's milk unless otherwise specified as goat's milk.

Trans Fatty Acids

The concentration of TFA in human milk has been investigated in several studies (Appendix F - Table 3). Given the removal of industrial TFA from the U.S. food supply that began with the mandatory declaration of TFA on nutrition labeling in 2006, studies of populations of women in North America providing human milk samples since 2006 were prioritized for information on TFA concentrations in human milk. For comparison, concentrations in samples collected prior to 2006 are included to show changes over time.

Friesen and Innis (2006) examined concentrations of TFA and CLA in human milk from women in 2004 to 2006 following the introduction of mandatory labeling of TFA in Canada in 2005 and compared concentrations to levels in milk samples collected from women in 1998. The mean concentration of TFA (% of total fatty acids) was significantly lower in 39 milk samples collected between September 2005 and January 2006 compared to the concentration in 103 samples collected in 1998, prior to the labeling requirement (4.6 ± 0.32% versus 7.1 ± 0.32%). A more recent analysis of human milk samples from lactating women in Canada between 2009 and 2011 indicates that concentrations of TFA continued to decline over time, with samples collected in 2011 containing on average 1.9 ± 0.5% total fatty acids as TFA (Ratnayake et al., 2014). The concentrations of SFA were stable over the period of collection, suggesting that women were not increasing intakes of saturated fats such as butter or beef fat. The intake of

trans fat was estimated at 0.8 g per person per day based on an established association between milk fat concentrations and dietary intake as specified by Craig-Schmidt and colleagues and calculated by the authors (Ratnayake et al., 2014). This intake of TFA is equivalent to 0.3% energy, which is well below the WHO's recommendation for intake of not more than 1% of total energy from TFA (FAO, 2010).

Appendix F - Table 3. Total *trans* Fatty Acid (TFA) in Human Milk

Reference	Mother's Location of Residence	Year	*Trans* Fatty Acid Concentration, % of total fatty acids (mean, SD [range])
Ratnayake et al., 2014	Canada	2011	1.9 ± 0.5 [0.9–3.9]
Ratnayake et al., 2014	Canada	2010	2.2 ± 0.7 [1.0–6.8]
Ratnayake et al., 2014	Canada	2009	2.7 ± 0.9 [1.4–7.2]
Friesen and Innis, 2006	Canada	2006	4.6 ± 0.32
Friesen and Innis, 2006	Canada	2005	5.3 ± 0.49
Glew et al. 2011	US	2005-2009	5.51
Friesen and Innis, 2006	Canada	2004	6.2 ± 0.48
Glew et al. 2008	US	2005-2006	4.01
Friesen and Innis, 2006	Canada	2004	6.2 ± 0.48
Innis and King, 1999	Canada	1998	7.1 ± 0.32
Mosley et al. 2005	US	1996-2002	7.0 ± 2.3 [2.5–13.8]
Chen et al., 1995	Canada	1992	7.19 ± 3.03 [0.1–17.2]
Chappell et al., 1985	Canada	≤1984	2.9
Abbreviations: SD – standard deviation.			

Conjugated Linoleic Acid

The concentration of CLA was examined in human milk samples from women in nine countries, with concentrations ranging from 0.07 to 0.34% of total fatty acids (Yuhas et al., 2006). In another study, CLA content of mature human milk from mothers in Poland consuming dairy products by level of consumption was examined (Martysiak-Zurowska et al., 2018). Dairy consumption by fifty mothers was determined based on data reported in 3 dietary recalls; low dairy consumption was defined by consumption of < 4.2 g dairy/day (n=20) and high dairy consumption was defined by consumption of > 12.7 g dairy/day (n=30). Total CLA content of human milk was significantly higher among mothers with high consumption of dairy products compared to mothers with low consumption; the concentration of CLA in human milk from high and low consumers of dairy products was 0.49% and 0.27 % of total fatty acids, respectively.

Concentrations of individual CLA isomers and total CLA have been measured and reported in human milk and commercial infant formulas (Appendix F - Table 4). Human milk samples

collected during a prospective birth cohort study from 312 mothers in The Netherlands 1 month postpartum were analyzed for CLA content (Mueller et al. 2010). Mothers were subdivided into groups based on dairy consumption (0-10 g, 10-20, 20-40, and 40-76 g dairy/day) and dietary lifestyle (conventional meat/dairy, 50-90% organic meat/dairy, >90% organic meat/dairy) to determine the effect of dietary consumption on milk fatty acid composition. Total CLA (the sum of CLA 9(*cis*),11(*trans*) (i.e., CLA c9,t11) and CLA 9(*trans*),11(*trans*)CLA) (i.e., CLA t9,c11) was 0.25 to 0.38% of total milk fat. While the level of CLA t9,c11 was not significantly different across mothers with different levels of dairy fat consumption or organic dairy/meat, level of CLA c9,t11 increased significantly with dairy fat intake and percent of intake from organic dairy/meat. In a sample of breastfeeding women in Poland, concentrations of CLA c9,t11 and total CLA were higher in milk samples among women consuming dairy products compared to women consuming a diet void of dairy fat, though there was no difference in concentrations of CLA t10,c12 in comparisons by diet (Martysiak-Zurowska et al., 2018).

Appendix F - Table 4. Conjugated Linoleic Acid (CLA) in Human Milk and Commercial Formula

CLA	CLA Fatty Acid Concentration, % of total fatty acids (mean or range of means; ± SD)	
	Infant Formula [a]	**Human Milk [b]**
CLA c9,t11	ND – 0.03	0.07 – 0.40
CLA t10,c12	ND – 0.02	ND – 0.06
Total CLA	ND – 0.04	0.07 – 0.49

Abbreviations: ND – not detected; SD – standard deviation.
a Martysiak-Zurowska et al., 2018
b Individual isomers: Yuhas et al., 2006; Individual isomers and total CLA: Martysiak-Zurowska et al., 2018 Total CLA: Mueller et al. 2010.

Odd-Chain Fatty Acids

The concentration of odd-chain fatty acids in AMF, human milk, and milk-based formula are summarized in Appendix F - Table 5.

Appendix F - Table 5. Odd Chain Fatty Acid Concentration in Milk Fat, Human Milk and Commercial Formula

Odd-Chain Fatty Acid	Odd-Chain Fatty Acid Concentration, % of total fatty acids (mean)		
	AMF [a]	**Human Milk [b,c]**	**Commercial Milk Formula [c]**
C13:0	-	0.01 - 0.05	-
C15:0	1.16	0.08 - 0.50	0.6
C17:0	0.51	0.19 - 0.41	0.3

Abbreviations: AMF – anhydrous milk fat.

[a] Analytical data; mean of 3 samples (See Appendix A for analytical data)
[b] Hageman et al., 2019(a)
[c] Yuhas et al., 2006

Branched-Chain Fatty Acids

Branched-chain fatty acids are present in human milk and several studies have examined these concentrations as well as the association between concentrations in human milk and maternal intake of the fatty acids (Aitchison et al., 1977; Dingess et al., 2017; Jie et al., 2018). Total branched-chain fatty acid concentrations in human milk from mothers of term infants as a percentage of total FA content are summarized below and in Appendix F - Table 6.

The branched-chain fatty acid content of human milk was approximately 0.58% of total fatty acid content and dietary branched-chain fatty acid was approximately 0.93% in an analysis of lipid composition of human milk among five mothers 4 to 6 months into lactation (Aitchison et al. 1977).

The branched-chain fatty acid content of human milk from mothers of term infants in the Cincinnati, OH, USA; Shanghai, China; and Mexico City, and Mexico was assessed in a cross-sectional analysis of 359 women (~120 women from each site) at 4-weeks postpartum (Dingess et al. 2017). Milk obtained via an electric breast pump was analyzed for *iso*-14:0, *anteios*-15:0, *iso*-16:0, *anteiso*-17:0, *iso*-18:0, total branched-chain fatty acids, and total branched-chain fatty acid as a percent of total fatty acids. Twenty-four-hour dietary recalls were also collected from 115 women from Cincinnati, OH, to determine the association between dietary intake of branched-chain fatty acids from beef and dairy and human milk branched-chain fatty acid content. The concentration of branched-chain fatty acids was 4.27 ± 0.25, 6.10 ± 0.36, and 7.90 ± 0.41 g/100 mL human milk among women from China, Mexico, and the U.S., respectively. Total branched-chain fatty acid as a percent of total fatty acids ranged from 0.13 to 0.30% from mothers 4-weeks postpartum, with the highest concentrations among women from the U.S (Appendix F - Table 6). Total branched-chain fatty acid content of human milk was not associated with intake of beef and dairy; however, beef intake was associated with human milk *iso*-16:0 content and dairy intake was associated with human milk *iso*-14:0, *anteios*-15:0, and *iso*-16:0 content. Levels of branched-chain fatty acid in transitional milk (11 ± 3 days post-partum) and mature milk (collected at 30 ± 3, 60 ± 3, and 90 ± 3 days post-partum) were not significantly different in a study of women from China (Jie et al. 2018); the branched-chain fatty acid content of human milk from all mothers consisted primarily of branched-chain fatty acid in the sn-2 position (52-65% of all BCFA).

Appendix F - Table 6. Total Branched Chain Fatty Acid (BCFA) Concentration as a Percentage of Total Fatty Acids from Human Milk for Term Infants

Mother's Location of Residence	Duration of Breastfeeding (weeks)	Total BCFA Concentration (% of total fatty acids)	Reference
China	0.6	0.55	Jie et al. 2018
China	1.6	0.37	Jie et al. 2018
Cincinnati, OH, USA	4	0.30	Dingess et al. 2017
Mexico City, Mexico	4	0.24	Dingess et al. 2017
Shanghai, China	4	0.13	Dingess et al. 2017
China	4.3 - 12.9	0.32	Jie et al. 2018
Davis, California, USA	17.4 - 26.1	0.58	Aitchison, 1977

Appendix G. Animal Studies of the Effect of Milk Fat in Infant Formula

Animal Studies of the Effect of Milk Fat in Infant Formula

Studies in animals have examined the effect of an infant formula containing milk fat on efficacy parameters such as digestion, concentrations of DHA and ARA, immunity and microbiota composition (Aidoud et al., 2018; Delplanque et al., 2013; Dinel et al., 2016(a); Dinel et al., 2016(b); Drouin et al., 2018; Du et al., 2012; Le Huërou-Luron et al., 2018(b); Lemaire et al., 2018); these studies are summarized below. Findings from these studies suggest that milk fat as a component of infant formula may support infant health.

The effect of milk fat and milk fat globule membrane (MFGM) fragments in infant formula on gut digestion, mucosal immunity and microbiota composition was evaluated in newborn piglets (Le Huërou-Luron et al., 2018(b)). Three formula blends were developed and fed to three separate groups from post-natal day 3 to day 7 (n=18) or to day 28 (n=24, n=23). The test formula consisted of either a standard infant formula consisting of vegetable lipids (palm and rapeseed oil) stabilized by protein, vegetable lipids (palm and rapeseed oil) stabilized by a mixture of protein and MFGM fragments, or a mixture of 61% milk fat and vegetable lipids (palm, rapeseed, and sunflower oil) stabilized by protein and MFGM fragments. A fourth group of sow's suckling piglets served as a reference group (n=12). The composition of the formula had no impact on piglet growth, but it did affect median jejunum weight and jejunum and ileum mucosal density, which were shown to be significantly higher in piglets fed formulas with MFGM compared to the formula containing vegetable lipids stabilized by protein. The addition of both MFGM fragments and milk fat to formula was found to induce a lower susceptibility to proteolysis. The authors stated that this could have been due to the abundance of saturated fats in the milk lipids which may contribute to changes in interfacial composition. The formula with dairy and MFGM was also rich in short- and medium-chain saturated fatty acids compared to the formulas which contained only vegetable lipids, and short- and medium-chain fatty acids are known to play a role in the modulation of immune responses.

Lemaire and colleagues examined the long-term effects of early consumption of dairy lipids and *Lactobacillus fermentum* on gut microbiota, the host entero-insular axis, and metabolism in a group of 27 male and female Yucatan minipigs (Lemaire et al., 2018). Piglets were given either one of three isoenergetic formulas (manufactured by Lactalis, Retiers, France) containing either plant lipids only (palm, rapeseed, and sunflower oils), a blend of plant lipids and dairy lipids (cream (53.4%), rapeseed, and sunflower oils), or the plant and dairy blend supplemented with *L. fermentum* from post-natal day 3 until post-natal day 28. Compared to a standard infant formula, the experimental formulas contained higher amounts of proteins and lipids and a lower amount of lactose although the ratio of lipids to proteins and the ratio of linoleic acid to α-linolenic acid were kept similar to those found in infant formulas. At post-natal day 28 the piglets were

weaned onto a standard diet and at post-natal 57 they were challenged with a high-energy diet until euthanasia at post-natal 140. Weight gain on post-natal day 28 with intake of the two formulas containing dairy lipids was significantly higher than weight gain observed with intake of the plant lipids only formula. After weaning, there were no significant differences in growth and food intake between the three groups (formula intake between post-natal day 3 and 28 was measured but results were not reported). The composition of the formula had no significant effect on lipid profile, plasma fasting glucose, and insulin concentrations. The results demonstrated that the addition of dairy lipids and the probiotic to infant formulas had an impact on gut microbiota composition. The authors concluded that the addition of dairy lipids and *L. fermentum* in infant formulas would be safe for long-term intake.

A study was conducted in young male rats to investigate the ability of dairy-fat-based diets to modulate the brain fatty acid restoration by comparison to corresponding vegetable saturated-fat-based diets (Delplanque et al., 2013). The investigators evaluated the impact of replacing the palm oil used in standard European infant formula by a dairy fat-based formula in first generation of post-weaning male rats deficient in omega-3, born from ALA-poor dams. After four weeks of breastfeeding, young pups (n=8) were weaned and received a 10% fat (w/w) semi-synthetic diet adjusted to meet the nutritional needs of growing animals for 6 weeks. Two groups received either palm oil or anhydrous butter made from dairy fat blended with rapeseed and sunflower oils to provide identical ALA and LA levels. Another treatment group received a butter diet similar to the diet with anhydrous butter that included an increased proportion of rapeseed oil to provide higher ALA levels and to reduce the LA/ALA ratio. The fourth diet was a pure rapeseed fat diet with the lowest LA/ALA ratio. No long chain PUFAs were added in these diets. At the end of the post-weaning period, body weights did not differ in the groups and food intake was similar across dietary treatments (monitored during the last 2 weeks of the post-weaning period). The main findings of the study were that a dairy-fat-based diet with 1.5% ALA is more efficient than a palm oil blend with the same level of ALA and the same LA/ALA ratio of 10 to increase brain DHA concentrations. It was also observed that a dairy-fat-based diet enriched with 2.3% ALA is even more efficient, which the authors noted could be attributed to both the increased level of dietary ALA and the concomitant decrease in the LA/ALA ratio induced by the selective enrichment of the blend with rapeseed. Additionally, the dairy-fat-based diet enriched with 2.3% ALA was as efficient as the 8.3% ALA rapeseed diet, despite the large amount of ALA provided by rapeseed and its even lower LA/ALA ratio of 2.6. Together, these observations demonstrate that brain DHA levels can be substantially improved by dairy fat based-diets.

The effect of adding DHA and ARA (in the ratio 3:2) to two representative commercial infant formulas on brain activity and brain and eye lipids in Wistar male rat pups was evaluated (Aidoud et al., 2018). The formula lipid composition was either based on a pure plant oil blend,

or dairy fat with a plant oil blend, with or without supplementation with DHA and ARA. Sham gastrostomy was performed at post-natal day 5, and the rats were weaned to solid food between post-natal day 19 and 20. Twenty pups were fed the 4 experimental formulas along with 5 dam-suckled sham-gastrostomy pups and 5 dam-suckled non-surgical external controls. The authors observed that adding DHA and ARA to formulas alters the DHA content and lipidome of nervous tissue in the neonate, making it closer to dam milk-fed controls, and normalizes brain functional activity. The authors also noted that the dairy fat-based formula with no addition of DHA and ARA had the oxylipin profile closest to that of the human milk.

Drouin et al., (2018) also examined the potential impact of a partial incorporation of dairy lipids in the diet compared to diets with a mixture of vegetable oils on the n-3 long chain PUFA content in tissues. In this study, 32 Sprague Dawley rats (n = 8/group) were fed isoenergetic and isolipidic diets from 3 weeks of age for a period of 6 weeks. The four different lipid blends were prepared from a combination of commercially available natural oil sources, including a blend of 50% vegetable oils and 50% dairy lipids (w/w) from butterfat, vegetable oil only, or each test diet supplemented with 0.5% DHA. Lipid blends were made with a LA/ALA ratio of approximately 5. The FA composition of the lipid blends and the oils used in the semi-synthetic diets were designed according to the fatty acid compositions of commercially available European infant formulas. In a second experiment, 32 rats (n = 8/group) were fed vegetable oil or dairy lipid-based diets, not supplemented with DHA from weaning for 3 or 6 weeks. All diets provided the same quantity of total fatty acids (2.3%) of precursor ALA. At the end of both experiments, body weight gains and organ weights did not differ between groups, with the exception of brain weight which was higher in rats fed the dairy lipid diet compared to rats fed the vegetable oil diet. Overall, the dairy lipid diet increased DHA levels in brain and retina at the same levels as the DHA supplemented diets and both the dairy lipid diet and the dairy lipid plus DHA diets increased the concentrations of ALA in the liver and LA in all studied tissues. The status of n-3 docosapentaenoic acid also increased with the dairy lipid diet in the liver, heart, and red blood cells.

Another study evaluated the protective effect of partial replacement of vegetable lipids by dairy lipids supplemented with DHA and ARA on post-natal inflammation and its consequence on memory (Dinel et al., 2016(a)). In this study, diets were given to male mice from the first day of gestation and they were maintained on the same diet until adulthood. The mice were fed diets with vegetable lipids and poor in n-3 PUFA, with vegetable lipids and balanced in n-3/n-6 PUFA, with dairy lipids and balanced n-3/n-6 PUFA n-3/n-6 PUFA, or with dairy lipids enriched in DHA and ARA from the first day of gestation until adulthood. In an additional group, pups deficient in n-3 PUFA on a vegetable lipid diet were switched to a balanced dairy diet from weaning to adulthood. Overall, the data suggest that there is a protective effect of dairy lipids

toward an adverse immune stimulation in early life on memory at adulthood, neuroplasticity, and neuro-inflammation.

In a further study, Dinel and colleagues (2016(b)) evaluated the impact of partial replacement of vegetable oil in infant formula by dairy fat on DHA brain level, neuroplasticity and corticosterone in mice. Adult male and female Swiss mice were fed diets containing 5% of total lipids from different sources: balanced vegetable lipids, vegetable lipids supplemented with DHA (0.2%) and ARA (0.4%), balanced dairy lipids, and dairy lipids supplemented with DHA (0.2%) and ARA (0.4%). Diets were given to mice from the first day of gestation and to the male offspring until adulthood (post-natal day 90). The authors observed that the dairy lipids diet increased DHA and neuroplasticity in the brain of mice at post-natal day 14 and at adulthood compared to vegetable lipids. At post-natal day 14, ARA and DHA supplementation increased DHA in the mice brain in vegetable lipids but not in dairy lipids. DHA and ARA supplementation further improved neurogenesis and decreased corticosterone level in dairy lipids mice at adulthood.

Du et al., (2012) evaluated how different dietary fat matrices improved DHA content in the brain among rats. In this study, the effects of different butter- ALA-enriched blends and palm-ALA regular blends (with or without supplementation with long-chain n-3) on the restoration of the fatty acid profiles of the brain from ALA-deficient post-weaning rats were compared. After weaning for 4 weeks, young pups (n=10 each males and females) from ALA-deficient Wistar dams received a 10% fat (w/w) diet for 6 weeks. Two groups received diets including rapeseed and sunflower oils to provide the same level of ALA (1.5% of fatty acids) and LA (13% to 16% of fatty acids), respectively, blended with palm oil or butter made from summer milk. The third group received a palm diet similar to vegetable oil diet with supplementation of DHA and ARA (0.12% and 0.4% of FA, respectively). The fourth group received a butter diet similar to the butter diet that included an increased proportion of rapeseed oil to provide higher ALA levels (2.3% of fatty acids). At the end of the post-weaning period, the body weights did not differ between the diet treatments and the food intake was similar across dietary treatments. The main finding of the study is that an anhydrous dairy-fat-based diet with 1.5% ALA was more efficient than a palm oil blend with as much ALA and 0.12% added DHA and 0.4% ARA for increasing brain DHA levels in post-weaning rats. In addition, both anhydrous dairy fats optimized brain DHA levels more than pure vegetable fat blends.

Bonnette, Richard

From: Tao, Xin <xin.tao@hoganlovells.com>
Sent: Thursday, January 09, 2020 11:47 AM
To: Bonnette, Richard
Cc: Steinborn, Steven B.
Subject: RE: Your recent submissions to the FDA GRAS Notification program (corn oil, citric acid esters of mono and diglycerides, anhydrous milk fat)
Attachments: AMF_Appendix C. Certificates of Analysis on AMF.PDF; AMF_Appendix D. Monitoring for Potential Contaminants.pdf; CITREM Appendix A_Various information - Citrem N 12 Veg MB (093224) Feb....pdf; CITREM Appendix B-1_PAH, Dioxin, Dioxin-like PCBs, Jan. 2017.pdf; CITREM Appendix B-2_2016,Pesticides -Cover letter + Monitoring report (1....pdf; Corn oil_Appendix A.PDF; Corn oil_Appendix B.PDF; Corn oil_Appendix C.PDF; AMF_Appendix A. Analytical Data on AMF.PDF; AMF_Appendix B. Statement of Quality Assurance.pdf

Dear Richard,

Thank you for your note. Here is to confirm the redactions we made all relate to the confidential supplier and customer information, exempt from disclosure under FOIA, and not related to the safety of the GRAS ingredients. Attached, please find the unredacted versions of these pages. For your ease of reference, we also summarize them with the table below:

Document	Page #	Redacted Info
GRAS AMF Appendix A	1, 10, 19	confidential supplier and customer information
GRAS AMF Appendix B	1	confidential supplier and customer information
GRAS AMF Appendix C	1, 2, 3, 4, 5	confidential supplier and customer information
GRAS AMF Appendix D	1, 2, 3, 4	confidential supplier and customer information
GRAS CITREM Appendix A	1, 2	confidential supplier and customer information
GRAS CITREM Appendix B-1	1	confidential supplier and customer information
GRAS CITREM Appendix B-2	1, 2	confidential supplier and customer information
GRAS Corn Oil Appendix A	1, 2	confidential supplier and customer information
GRAS Corn Oil Appendix B	1, 2, 3, 4, 6, 7	confidential supplier and customer information
GRAS Corn Oil Appendix C	1, 2, 3, 4, 5, 6, 7, 8, 9	confidential supplier and customer information

As the above table indicates, all the information we redacted are exempt from disclosure under the Freedom of Information Act, 5 USC 552 as trade secret or as commercial information that is privileged or confidential. They do not

relate to the safety of the ingredients, and we do not view them as basis for our safety conclusions. We also do not view the redacted information as part of the GRAS notices we submitted to the agency.

We trust this is responsive to your request. Please let us know if you have any questions.

Best regards,
Steve
Xin

Xin Tao
Senior Associate

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From: Bonnette, Richard [mailto:Richard.Bonnette@fda.hhs.gov]
Sent: Friday, January 03, 2020 1:24 PM
To: Steinborn, Steven B.
Cc: Tao, Xin
Subject: Your recent submissions to the FDA GRAS Notification program (corn oil, citric acid esters of mono and diglycerides, anhydrous milk fat)

Dear Mr. Steinborn,
The GRAS submissions for corn oil, citric acid esters of mono and diglycerides, and anhydrous milk fat (all dated November 7, 2019) have completed our prefiling evaluation in the Office of Food Additive Safety. Our pre-filing team here noted that there are minor sections in each of these submissions that are redacted and a non-redacted version was not included. We suspect that these redactions do not obscure safety-relevant information, but will need to see unredacted versions of these sections to make that determination. Can you please provide unredacted versions of these pages that indicate the information that is to be held as exempt from disclosure under FOIA? Also it will be helpful if you provide a brief sentence or two about the nature of the information marked as confidential and why it isn't relevant for safety. You can provide these requested pages by email or by regular mail.

Another option would be to ask us to cease our evaluation of these submissions prior to filing and then resubmit revised versions of these submissions that do not contain redactions.

Let me know if you have any questions.

Regards,
Richard

Richard E. Bonnette, M.S.
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration