SCG
Spherix Consulting Group

March 28, 2024

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
CPK-2 Building, Room 2092
5001 Campus Drive, HFS-225
College Park, MD 20740

Dear GRAS Filing Team:

Enclosed please find a CD containing the "GRAS Determination for the Use of Pasteurized Whole Bovine Milk (WBM) in Non-Exempt Cow's Milk-Based Infant Formula", Form 3667, and all corresponding references. The data and information that serve as the basis for this GRAS Notice is available for review and copying at reasonable times at the office of Dietrich Conze, Managing Partner, Spherix Consulting Group, Inc., 751 Rockville Pike, Unit 30-B, Rockville, MD 20852, Telephone: 240-367-6089; email: dconze@spherixgroup.com or will be sent to FDA upon request.

We thank you for taking the time to review this GRAS Notice. Should you have additional questions, please let us know.

Sincerely,

[redacted]

Dietrich B. Conze, PhD
Managing Partner

Enclosure:

CD containing Form 3667, cover letter, GRAS Determination for the Use of Pasteurized Whole Bovine Milk (WBM) in Non-Exempt Cow's Milk-Based Infant Formula, and all references

GRAS Determination for the Use of Pasteurized Whole Bovine Milk (WBM) in Non-Exempt Cow's Milk-Based Infant Formula

Prepared for:

Crossway Foods, Ltd.
Unit 2017 Orchard Avenue,
Citywest Business Campus,
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Prepared by:

Spherix Consulting Group, Inc.
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March 26, 2024

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LIST OF ABBREVIATIONS

AE: Adverse Event

AP: Alkaline Phosphatase

BS EN: British Standards Implementations of English Language Version of European Standards

BS: British Standard

bw: Body Weight

CCP: Critical Control Point

CFR: United States Code of Federal Regulations

cfu: Colony Forming Unit

CI: Confidence Interval

DIAAS: Digestible Indispensable Amino Acid Score

EC: European Commission

EDI: Estimated Daily Intake

EN: European Union

FAO/WHO: Food and Agriculture Organization/World Health Organization

FDA: United States Food and Drug Administration

FFDCA: United States Federal Food, Drug, and Cosmetic Act

FOIA: Freedom of Information Act

g: Gram

GC-FID: Gas Chromatography-Flame ionization Detection.

GRAS: Generally Recognized As Safe

GRN: GRAS Notice

HPLC: High Performance Liquid Chromatography

IOM: Institute of Medicine

ISO: International Organization for Standardization

IU: International Unit

IUGR: Intrauterine Growth Restricted

IUPAC: International Union of Pure and Applied Chemistry

JAOAC: Journal of Association of Official Agricultural Chemists

kcal: Kilocalories

kg: Kilogram

KNC: Kendal Nutricare

LOD: Limit of Detection

LSRO: Life Sciences Research Office

MFGM: Milk Fat Globule Membrane

mg: Milligram

MS: Mass Spectrometry

N/A: Not Available

N: Nitrogen

NBW: Normal Birth Weight

ND: Not Detected

NHANES: National Health and Nutrition Examination Survey

NP: Not Provided

OPL: Oleic-Palmitic-Linoleic

OPO: Oleic-Palmitic-Oleic

PA: Phosphatidic Acid

PC: Phosphatidylcholine

PDCAAS: Protein Digestibility Amino Acid Score

PE: Phosphatidylethanolamine

PER: Protein Efficiency Ratio

PG: Phosphatidylglycerol

PI: Phosphatidylinositol

PL: Phospholipid

PP: Per Protocol

PS: Phosphatidylserine

QCP: Quality Control Point

RP: Reference Product

SD: Standard Deviation.

SM: Sphingomyelins

TP: Kendamil Infant Formula

µg: Microgram

UPLC/Q-TOF-MS: Ultra-high-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry

USDA: United States Department of Agriculture

WBM: Whole Bovine Milk (also Pasteurized Liquid Whole Bovine Milk)

WWEIA: What We Eat in America

I. SIGNED STATEMENT OF THE CONCLUSION OF GENERALLY RECOGNIZED AS SAFE (GRAS) AND CERTIFICATION OF CONFORMITY TO 21 CFR §170.205-170.260

A. SUBMISSION OF GRAS NOTICE

Crossway Foods is hereby submitting a GRAS Determination in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

Crossway Foods, Ltd.
Unit 2017 Orchard Avenue,
Citywest Business Campus,
Dublin D24 AXR0,
Ireland

C. COMMON OR USUAL NAME

Pasteurized Liquid Whole Bovine Milk (WBM)

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

This notification does not contain any trade secret or confidential information.

E. INTENDED USE

Pasteurized Liquid Whole Bovine Milk is intended to be used as an ingredient in non-exempt, cow's milk-based infant formula at the maximum level of 153 g WBM/100 g infant formula powder.

F. BASIS FOR CONCLUSION OF GRAS STATUS

Crossway Foods' conclusion of GRAS status for the intended use of WBM in non-exempt, cow's milk-based infant formula is based on scientific procedures in accordance with 21 CFR § 170.30(a) and (b.

G. PREMARKET APPROVAL

The intended use of WBM in non-exempt, cow's milk-based infant formula is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Crossway Foods has concluded that such use is GRAS through scientific procedures.

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS Determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, PhD, DABT, President, Spherix Consulting Group, Inc., at 751 Rockville Pike, Unit 30-B, Rockville, MD 20852. Telephone: 301-775-9476; Email: ckruger@spherixgroup.com, or be sent to FDA upon request.

I. FREEDOM OF INFORMATION ACT (FOIA)

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA.

J. CERTIFICATION STATEMENT

On behalf of Crossway Foods, I hereby certify that to the best of my knowledge, this GRAS Notice is a complete, representative and balanced submission that includes both favorable and unfavorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of WBM in non-exempt, cow's milk-based infant formula.

Signature of Authorized Representative

26th March, 2024
Date

Name: Mairead Ni Chuinn,

Title: Director, Crossway Foods, Ltd.

II. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

A. COMMON OR USUAL NAME

Pasteurized Liquid Whole Bovine Milk (WBM)

B. IDENTITY

Per Title 21 of the United States Code of Federal Regulations (CFR) §131.110, milk is the lacteal secretion, practically free from colostrum, obtained by completely milking one or more healthy cows. Milk that is in final package form for beverage use shall have been pasteurized or ultrapasteurized, and shall contain not less than 8.25 % milk solids not fat and not less than 3.25 % milkfat. Milk may have been adjusted by separating part of the milkfat therefrom, or by adding thereto cream, concentrated milk, dry whole milk, skim milk, concentrated skim milk, or non-fat dry milk. Milk may be homogenized.

The subject of this GRAS Determination is pasteurized liquid whole bovine milk (WBM), sourced from organic and non-organic cows (*Bos taurus*), that contains approximately 9.2 % milk solids not fat and 3.8% milk fat. The milk is not adjusted or homogenized and no optional ingredients are added (See Chapter 2, Section E. Manufacturing Process). Thus, WBM meets the definition of milk specified in 21 CFR §131.110.

C. SOURCE

All raw milk (organic and non-organic) used to manufacture WBM complies with Regulation (EC) No 853/2004 and is sourced from qualified suppliers that adhere to Good Agricultural Practices. Additional information regarding the quality of the raw milk used to produce WBM is provided in Chapter 2, Section E.1. Quality.

D. COMPOSITION

The composition of whole milk is well-known and reference values for its components are available in United States Department of Agriculture's FoodData Central, which is a database that provides reference values for commonly consumed foods. To confirm that WBM is similar to whole milk, the average level and range of levels of the proximates, fatty acids, amino acids, and selected micronutrients from three representative lots of non-organic WBM were compared to the reference values for whole milk obtained from FoodData Central. Although the levels of some of the components in non-organic WBM differ from their reference values, all of differences were less than two-fold (Tables 1, 2, 3, and 4). Importantly, as summarized on page

14 of GRN 001041, which established the GRAS status of the use of dry whole milk in infant formula, bovine milk is subject to natural variation due to cow breed, the environment, management of the milking process, animal health, physiology, and nutrition (Linn, 1988). Moreover, the reference values for whole milk provided in FoodData Central are point estimates that do not reflect natural variability. Published studies also indicate that it is not possible to distinguish organic from non-organic milk due to the compounding effects of animal genetics, health, breed, diet, management and the environment on milk composition (reviewed in Schwendel et al., 2015; Linehan et al., 2024). Thus, considering the lack of more than two-fold differences in the components between non-organic WBM and whole milk, and compositional equivalence of organic and non-organic milk, it is reasonable to conclude that the nutritional quality of WBM (organic and non-organic) is comparable to whole milk.

To confirm that it does not contain appreciable levels of heavy metals, the levels of lead, arsenic, mercury, and cadmium were quantified in three representative lots of non-organic WBM. Except for levels of arsenic, which ranged from not detected to 0.003 ppm, the levels of lead, cadmium and mercury were below the limit of detection (Table 5). Assuming that WBM contains approximately 13 g solids/100 g liquid (Table 1; 100 g/100g (total moisture) - 87.0 g/100 g (average moisture level)), the resulting levels of lead, arsenic, mercury, and cadmium are similar those found in the dry whole milk ingredients that are GRAS for use in infant formula (Table 5; see Table 2 of GRN 000980, page 10; see Table 7 of GRN 001041, page 20).

Table 1. Proximates in Pasteurized Liquid Whole Milk (WBM) and Whole Milk

Component (Unit)	WBM Sample Average[a]	WBM Sample Range[a]	USDA Reference Values for Whole Milk[b]
Moisture (g/100 g)	87.0	86.6-87.4	88.1
Crude Protein (Nx6.38) (g/100 g)	3.6	3.2-4.5	3.28
Fat (g/100 g)	3.8	3.5-4.0	3.2
Carbohydrate (g/100 g)[c]	4.6	3.6-5.2	4.67
Ash (g/100 g)	0.8	0.7-0.9	0.8[d]

Abbreviations: N – nitrogen; USDA – United States Department of Agriculture; g – gram.
[a]Based on the results from three non-consecutive batches of non-organic milk.
[b]Standard macronutrient values for whole fluid milk (FDC ID: 2340762) (USDA 2022); https://fdc.nal.usda.gov/fdc-app.html#/food-details/1097512/nutrients, accessed on 12-5-2023.
[c]Calculated based on the results from 3 non-consecutive batches using the following equation: Carbohydrate = 100 – (moisture + protein + fat + ash)
[d]Ash value was not reported for milk, whole (FDC ID: 2340762) (USDA, 2022). Therefore, the ash content was taken from Milk, whole, 3.25% milkfat, with added vitamin D (FDC ID: 746782) (USDA, 2019).

Table 2. Fatty Acid Composition in Pasteurized Liquid Whole Milk (WBM) and Whole Milk[c]

Component (Unit)	WBM Sample Average[a]	WBM Sample Range[a]	USDA Reference Values For Whole Milk[b]
10:0 Capric (mg/100 g)	110	90-120	84
12:0 Lauric (mg/100 g)	130	116-139	97
14:0 Myristic (mg/100 g)	380	340-410	303
16:0 Palmitic (mg/100 g)	1050	960-1120	857
18:0 Stearic (mg/100 g)	360	350-370	309
16:1 Palmitoleic (mg/100 g)	56	52-61	47
18:1 Oleic (mg/100 g)	720	700-730	694
18:2 Linoleic (mg/100 g)	64	48-82	115
18:3 Alpha Linolenic (mg/100 g)	17	13-20	12
Saturated Fatty Acids (Acid Form) (mg/100 g)	2450	2250-2600	1860
Monounsaturated Fatty Acids (Acid Form) (mg/100 g)	870	840-900	688
Polyunsaturated Fatty Acids (Acid Form) (mg/100 g)	100	80-120	108
Trans Fatty Acids (Acid Form) (mg/100 g)	86	78-93	NP
Total Fatty Acids (mg/100 g)	3020	2070-3660	2636

Abbreviations: NP – not provided; USDA – United States Department of Agriculture; g – gram; mg – milligram.

[a]Based on the results from three non-consecutive batches of non-organic milk.; all fatty acids were quantified using gas chromatography with a flame ionization detector.

[b]Standard fatty acids values for whole fluid milk (FDC ID: 2340762) (USDA, 2022); https://fdc.nal.usda.gov/fdc-app.html#/food-details/1097512/nutrients, accessed on 12-5-2023.

Table 3. Amino Acid Composition in Pasteurized Liquid Whole Milk (WBM) and Whole Milk

Component (Unit)	WBM Sample Average[a]	WBM Sample Range[a]	USDA Reference Values for Whole Milk[b]
Alanine (g/100 g)	0.11	0.108-0.113	0.11
Arginine (g/100 g)	0.113	0.108-0.119	0.127
Aspartic Acid (g/100 g)	0.263	0.256-0.275	0.279
Cysteine (g/100 g)	0.028[c]	0.027-0.030[c]	0.037
Glutamic Acid (g/100 g)	0.719	0.696-0.741	0.788
Glycine (g/100 g)	0.064	0.062-0.066	0.069
Histidine (g/100 g)	0.091	0.088-0.094	0.097
Isoleucine (g/100 g)	0.167	0.164-0.172	0.173
Leucine (g/100 g)	0.328	0.321-0.336	0.333
Lysine (g/100 g)	0.296	0.285-0.303	0.298
Methionine (g/100 g)	0.081	0.076-0.085	0.09
Phenylalanine (g/100 g)	0.162	0.160-0.164	0.161
Proline (g/100 g)	0.336	0.323-0.345	0.333
Serine (g/100 g)	0.191	0.185-0.199	0.188
Threonine (g/100 g)	0.152	0.148-0.158	0.154
Tryptophan (g/100 g)[a]	0.0478	0.0474-0.0484	0.043
Tyrosine (g/100 g)	0.156	0.152-0.159	0.162
Valine (g/100 g)	0.208	0.204-0.213	0.207

Abbreviations: USDA – United States Department of Agriculture; g – gram.

[a]Based on the results from three non-consecutive batches of non-organic milk. Except for tryptophan all amino acids were quantified using ion chromatography and an ultraviolet light detector. The levels of tryptophan were quantified using liquid chromatography and a fluorescence detector.

[b]Standard amino acid values for Milk, whole, 3.25% milkfat, with added vitamin D (USDA, 2019). Amino acid values were not reported for milk, whole (FDC ID: 2340762) (USDA, 2022). Therefore, the amino acid content was taken from Milk, whole, 3.25% milkfat, with added vitamin D (FDC ID: 746782) (USDA, 2019).

[c]Includes cysteine and cystine.

Table 4. Selected Micronutrient Composition in Pasteurized Liquid Whole Milk (WBM) and Whole Milk

Component (Unit)	WBM Sample Average[a]	WBM Sample Range[a]	USDA Reference Values for Whole Milk[b]
Vitamin A (IU/100 g)[d]	127.9	98.7-155.3	106.7
Vitamin D (IU/100 g)[e]	<4 (Vitamin D3)	<4 (Vitamin D3)	44 (Vitamin D2+D3)[c]
Iron (mg/100 g) [f]	<0.1	<0.1	0
Iodine (µg/100 g)[g]	14	11.6-15.4	NP
Selenium (µg/100 g)[g]	2.33	1.3-3.5	1.9
Sodium (mg/100 g) [f]	34	21-45	38
Potassium (mg/100 g)[h]	163	140 - 200	150
Chloride (mg/100 g)[i]	100	100	NP
Cholesterol (mg/100 g)	9.5	2.4-13.5	12

Abbreviations: NP – not provided; USDA – United States Department of Agriculture; IU- international unit; mg – milligram; µg – microgram; JAOAC - Journal of Association of Official Agricultural Chemists
[a]Based on the results from three non-consecutive batches of non-organic milk.
[b]Standard macronutrient values for whole fluid milk (FDC ID: 2340762) (USDA, 2022); https://fdc.nal.usda.gov/fdc-app.html#/food-details/1097512/nutrients, accessed on 12-5-2023; iodine levels not provided.
[c]Calculated using the following equation: 1.1 µg/100 g X 40 IU/µg; Vitamin D2+D3; high vitamin D value due to fortification of milk with vitamin D.
[d]Quantified using liquid chromatography and diode array detector.
[e]Quantified using high performance liquid chromatography.
[f]Quantified using inductively coupled plasma-optical emission spectrometry.
[g]Quantified using inductive coupled plasma-mass spectrometry.
[h]Quantified using inductively coupled plasma-optical emission spectrometry.
[i]Quantified using a method based on (Brammell, 1974).
[j]Quantified using a method based on (al-Hasani et al., 1993).

Table 5. Heavy Metal Content of Pasteurized Liquid Whole Milk (WBM)

Heavy Metal	WBM Sample Range	Calculated Maximum Amount found in WBM solids[e]	Product Specifications for the Subject of GRN 000980[f]	Product Specifications for the Subject of GRN 001041[g]
Lead (ppm)[a,b]	ND	0.038	≤ 0.05	≤ 0.05
Arsenic (ppm)[a,c]	ND-0.003	0.023	≤ 0.5	≤ 0.1
Cadmium (ppm)[a,d]	ND	0.008	≤ 0.05	≤ 0.05
Mercury (ppm)[a,b]	ND	0.038	≤ 0.05	≤ 0.05

[a]Quantified using ICP-MS - Inductive Coupled Plasma-Mass Spectrometry; Based on the results from three non-consecutive batches of non-organic milk.
[b]Limit of detection (LOD) = 0.005 ppm
[c]LOD= 0.002 ppm
[d]LOD= 0.001 ppm
[e]Calculated using the following equation: (analytical data or LOD mg/1000 g)(100 g WBM/13 g milk solids)(1000).
[f]See Table 2 of GRN 000980, page 10.
[g]see Table 7 of GRN 001041, page 20.

E. MANUFACTURING PROCESS

1. Quality

Pasteurized Liquid Whole Bovine Milk is produced according to current Good Manufacturing Practices (cGMPs) using processing techniques that are standard in the dairy industry. All procured milk complies with Regulation (EC) No 853/2004 and is obtained from qualified suppliers that adhere to Good Agricultural Practices. All food contact materials meet the European hygienic standards specified by Regulation (EC) No 1935/2004 and/or comply with the conditions of use specified in CFR §177.2600. Each batch of incoming raw milk is qualified for use in the manufacture of infant formula upon arrival at the manufacturing facility by confirming that the temperature of the milk is not more than 5˚C, and the fat, protein, and total solids content, and titratable acidity comply with defined acceptance criteria (See Chapter 1, Section F.1. Product Specifications). The milk also cannot contain antibiotics. Importantly, due to the limits on the amount of time raw milk can be stored per the Grade "A" Pasteurized Milk Ordinance (i.e., not more than 72 hr), testing for compliance with specifications for heavy metals and microbes cannot be conducted prior to its use in infant formula. Therefore, the milk from all new suppliers of raw milk is qualified for use by comparing the levels of iron, iodine, sodium, potassium and chloride to established qualification specifications and conducting screens for the presence of heavy metals, pesticides, biocides, veterinary drugs, dioxins, and polychlorinated biphenyls. Additionally, the raw milk from all qualified suppliers is tested for the levels of ash, cholesterol, vitamin A, vitamin D3, and the presence of heavy metals, nitrates, nitrites, melamine, and aflatoxin M1 on a yearly basis.

2. Production

All raw milk used to manufacture WBM is delivered to the production facility by certified dairy carriers equipped with tankers that maintain a temperature not higher than 5˚C.

Upon arrival to the manufacturing facility, all raw milk is tested to confirm that it meets the product specifications outlined in Table 6 and immediately processed (Figure 1). The milk is filtered through a 1 mm stainless steel filter, pasteurized in accordance with the conditions specified in the Grade "A" Pasteurized Milk Ordinance (i.e., temperatures greater than or equal to 72˚C for durations greater than or equal to 15 sec), chilled, transferred to stainless-steel containers, and stored at not more than 10°C for not more than 72 hours before being used for the production of infant formula.

There are three quality control points (QCPs) and two critical control points (CCPs) in the production process. The QCPs include testing for pH and titratable acidity in the raw milk, filtering the milk to remove foreign bodies, and confirming that the storage temperature is not more than 10˚C for not more than 72 hours. The first CCP is the antibiotic testing that occurs upon receipt of the raw milk. The test is a broad-spectrum test conducted for due diligence

purposes to confirm the absence of the residual β-lactam-containing antibiotics. If any antibiotic is detected, the batch of raw milk is discarded.

The second CCP occurs at the pasteurization step, which entails monitoring the temperature of the pasteurization plant and conducting an alkaline phosphatase (AP) test to confirm that the raw milk has been pasteurized.

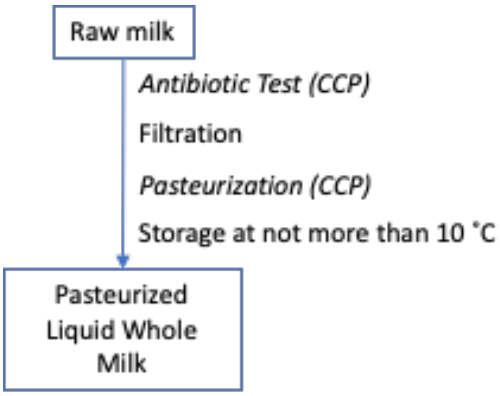

Figure 1. Flowchart of Pasteurized Liquid Whole Milk

Upon arrival at the Kendal Nutricare facility, all raw milk is tested to confirm the absence of antibiotics (CCP). Upon passing the critical control point, the raw milk is filtered, pasteurized (CCP), and stored at a temperature of not more than 10˚C. CCP: Critical Control Point.

F. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Specifications

Due to the limited storage time for liquid milk, per the "Grade A" Pasteurized Milk Ordinance (≤ 72 hr), testing of WBM for compliance with an established set of product specifications that ensure that the levels of the nutrients, heavy metals and microbes are below specified limits is not feasible due the time needed for shipping and testing the samples. Therefore, strict quality assurance and control practices have been established that govern the sourcing, transport, and receipt of all raw milk. Additionally, to ensure a consistent food-grade ingredient, each lot of raw milk is tested in-line and qualified for use against product specifications that ensure that the raw material, and by extension WBM, contains minimum levels of fat and protein, suitable levels of titratable acidity and solids, and no antibiotics. All methods used for qualifying each batch against the product specifications are validated and fit-for-use. Data from five non-consecutive lots of the raw milk show that it reproducibly meets all product specifications, demonstrating adequate control of the manufacturing process (Table 6).

Table 6. Raw Milk Product Specifications and Batch Data

Parameter (unit)	Method	Specification	Lot Analysis Date					
			Non-organic milk			Organic milk		
			01/09/2023	06/05/2023	08/15/2023	01/06/2023	06/03/2023	08/18/2023
Fat (%)	Fourier transform infrared spectroscopy[a]	≥ 3.2	4.19	4.19	4.35	3.99	4.27	4.19
Protein (%)	Fourier transform infrared spectroscopy[a]	≥ 2.9	3.39	3.28	3.42	3.28	3.40	3.25
Total Solids (%)	Fourier transform infrared spectroscopy[a]	11.5-15.0	13.27	13.09	12.99	12.73	13.13	12.87
Titratable acidity (Dornic degrees)	Fourier transform infrared spectroscopy[a]	12-18	16.2	16.2	16.0	15.7	15.6	15.6
Antibiotics[b]	Immunoreceptor assay utilizing ROSA® (Rapid One Step Assay) lateral flow technology[a]	Negative	Negative	Negative	Negative	Negative	Negative	Negative

[a]Validated.
[b]Limit of Detection: amoxicillin = 3 parts per billion (ppb); ampicillin = 3 ppb; cefacetrile = 30 ppb; cefalexin; cefalexin = 500 ppb; cefalonium = 8 ppb; cefazolin = 30 ppb; cefoperazone = 1 ppb; cefquinome = 15 ppb; ceftiofur and metabolite = 50 ppb; cloxacillin = 20 ppb; dicloxacillin = 15 ppb oxacillin = 10 ppb; penicillin G = 2 ppb.

2. Quality Attributes

To further demonstrate control of the production process and quality of WBM, the levels of aflatoxin M1 and a variety of microbes were quantitated in three lots of WBM (Tables 7 and 8). Aflatoxin M1 was not detected in any of the lots. Except for the total viable count and the aerobic thermophilic spores, all remaining microbiological parameters in all lots tested were either not detected or below the limit of quantitation.

Table 7. Aflatoxin Composition of Pasteurized Liquid Whole Bovine Milk

Parameter (Unit)	Method	Lot Number		
		KTM08/006	KTM08/007	KTM09/008
Aflatoxin M1 (µg/kg)	Reverse phase HPLC with MS/MS detection[b]	<0.01 µg/kg[a]	<0.005 µg/L[a]	<0.005 µg/L[a]

Abbreviations: n/a- not available; HPLC - High Performance Liquid Chromatography; MS - Mass Spectrometry;
[a]Limit of detection; LODs differ among the batches due to the use of different laboratories for the analysis.
[b]Validated.
N.B. 'KT codes' are unique laboratory sample codes. Samples were taken from non-consecutive batches.

Table 8. Microbial Composition of Pasteurized Liquid Whole Bovine Milk

Parameter (Unit)	Method	Lot Number			Average	Range
		KTN02/052	KTN02/053	KTN02/054		
Total Viable Count, 2 days 30°C (cfu/g)	BS EN ISO 4833:2013/ Amd1:2022	180	100	60	113.33	60-180
Aerobic thermophilic spores (cfu/g)	Enumeration of Aerobic and Anaerobic Thermophilic spores using PCA (plate count agar) or MPCA (milk plate count agar)	40	30	<10	35	<10-40
Coliforms (cfu/g)	BS EN ISO 4832:2006 AM 2009	<5	<5	<5	n/a	n/a
Yeasts & Mold (cfu/g)	Enumeration of Yeast and Mold using OGYE (otratetracycline glucose yeast extract) agar.	<5	<5	<5	n/a	n/a
Cronobacter sakazakii (/10 g)	ISO 22964:2017	ND	ND	ND	n/a	n/a
Bacillus cereus spores (cfu/g)	Enumeration of Bacillus cereus and Bacillus spp using BCA PEMBA (Bacillus cereus agar PEMBA) agar	<10	<10	<10	n/a	n/a
Enterobacteriaceae (/10 g)	BS EN ISO 21528:2017	ND	ND	ND	n/a	n/a
Coagulase positive Staphylococcus (cfu/g)	BS EN ISO 6888-3:2003	ND	ND	ND	n/a	n/a
Listeria monocytogenes (/25g)	SOLUS Listeria Elisa Method[a]	ND	ND	ND	n/a	n/a
Campylobacter species (cfu/10 g)	BS EN ISO 10272-1:2017/Amd1 2023	ND	ND	ND	n/a	n/a
Salmonella (/25 g)	SOLUS Salmonella ELISA Method[a]	ND	ND	ND	n/a	n/a
Sulphite reducing clostridia (cfu/g)	BS EN ISO 15213:2003	<5	<5	<5	n/a	n/a

Abbreviations: n/a- not applicable; HPLC - High Performance Liquid Chromatography; MS - Mass Spectrometry; ND - Not Detected; cfu - colony forming units; BS – British Standard; ISO – International Organization for Standardization; EN – European Union
[a]Validated.
N.B. 'KT codes' are unique laboratory sample codes. Samples were taken from non-consecutive batches.

G. STABILITY

In accordance with the Grade "A" Pasteurized Milk Ordinance (FDA, 2019), all milk used in the production of the infant formula (raw milk and WBM) is stored at a temperature of not more than 10˚C for not longer than 72 hours.

III. DIETARY EXPOSURE

A. PROPOSED USE AND USE LEVEL

Pasteurized whole bovine milk is intended to be used as a source of protein, fat and carbohydrate in non-exempt cow's milk-based powdered infant formula, and as a substitute for the dry whole milk ingredients that are the subjects of GRNs 000980 and GRN 001041. During formulation of the final infant formula, WBM is wet-blended with a variety infant formula ingredients, such as vegetable oils, lactose, vitamins, minerals and other sources of whey proteins. The product is then spray-dried, and dry-blended with the remaining ingredients to create a powdered infant formula with a nutrient composition that complies with 21 CFR §107.100.

The maximum intended use of WBM in infant formula is 153 g WBM/100 g infant formula powder, which is equivalent to 19.9 g WBM solids/100 grams infant formula considering an average solids content of 13 g solids/100 g WBM (see Table 1; 100 g/100g (total moisture) - 87.0 g/100 g (average moisture level)). Because the infant formula will be reconstituted at a rate of 12.9 g/100 mL water to achieve a final energy density of 67 kcal/100 ml, the maximum amount of WBM solids in reconstituted infant formula is 2.6 g/100 ml or 3.8 g/100 kcal. Pasteurized whole bovine milk will also account for approximately 55% of the total protein, 21% of the total fat, and 12.7% of the total carbohydrates of the infant formula.

B. ESTIMATED DAILY INTAKE

As specified on page 22 of GRN 001041, the estimated exposure to WBM from the ingestion of infant formula can be calculated using data collected in dietary recalls. Because the subject of this GRAS Determination is intended to be used as a substitute for the subject of GRN 001041, the per user estimated intakes of energy from infant formula that were used to calculate the exposure to the dry whole milk that is the subject of GRN 001041 can also be used to calculate the exposure to WBM. Therefore, the per user estimated intake of energy from infant formula is incorporated by reference as presented in Table 8 of GRN 001041 (Table 9).

As stated in GRN 001041, the estimated energy intake from infant formula was highest among infants 3-5 months of age with mean and 90th percentile 2-day average intakes of 539 and 833 kcal/day, respectively. On a body weight basis, the highest energy intake from infant formula was among infants 0-2 months of age with mean and 90th percentile 2-day average intakes of 95 and 146 kcal/kg body weight (bw)/day, respectively. Relative to intakes in the first six months of life, intake of infant formula in the second six months of life was lower both on a kcal/day and kcal/kg bw/day basis.

Using the estimates of energy intake from infant formula present in Table 9 and the assumption that the infant formula contains 3.8 g WBM solids/100 kcal, the per user intake of WBM from the intended use in infant formula was calculated for the infant subpopulations: 0-2 months, 3-5 months, 6-8 months, and 9-11 months (Table 10). Per user mean intake of WBM solids ranged from 16.5 g to 20.5 g/day and from 1.8 g to 3.0 g/kg bw/day. The highest estimated daily intake was among infants in the subgroup 3-5 months old with a mean of 20.5 g WBM solids/day and a 90th percentile of 31.7 g WBM solids/day. When considering the body weights of the users, the highest estimated daily intake was in infants 0-2 months old with a mean of 3.6 g WBM solids/kg bw/day and a 90th percentile of 5.5 g/kg bw/day. These levels are similar to the per user intake levels of dry whole milk presented in GRN 001041.

Table 9. Per User Estimated Daily Intake of Energy from Infant Formula, WWEIA/NHANES 2011-2018[a]

Age (Months)	Total Sample	Users		Body Weight (kg)	Kcal/day		Kcal/kg bw/day	
		Number	Percent		Mean	90th Percentile	Mean	90th Percentile
0-2	250	148	54.3	5.1	484	766	95	146
3-5	346	229	60.7	7.0	539	833	78	118
6-8	295	212	71.7	8.3	479	735	58	95
9-11	303	210	70.7	9.5	435	694	47	75

Abbreviations: g – gram; kg – kilogram; bw – body weight; WWEIA/NHANES - What We Eat in America / National Health and Nutrition Examination Survey.
[a]Adopted from Table 8 of GRN 001041; total sample represents number of infants with 2 days of recall data in the sample; Users number represents unweighted number of infants reporting use of infant formula on at least one day of dietary recall. Infants not consuming infant formula presumably consumed human milk and/or table foods, or an infant formula excluded from this assessment.

Table 10. Per User Estimated Daily Intake of WBM Solids from the Intended Use in Infant Formula, WWEIA/NHANES 2011-2018*

Age (Months)	g/day		g/kg bw/day	
	Mean	90th Percentile	Mean	90th Percentile
0-2	18.8	29.7	3.7	5.7
3-5	20.9	32.3	3.0	4.6
6-8	18.6	28.5	2.3	3.7
9-11	16.9	26.9	1.8	2.9

Abbreviations: g – gram; kg – kilogram; bw – body weight; WWEIA/NHANES - What We Eat in America / National Health and Nutrition Examination Survey.
*Calculated assuming 2.6 g solid WBM in 100mL reconstituted formula and 3.8 g WBM/100 kcal infant (see Chapter III, Section A. Proposed Use and Use Level), based on a maximum inclusion rated of 153 g WBM/100 g powdered formula and an average of 13g solids/100 g WBM.

C. MAXIMUM ALLOWABLE LEVELS OF NUTRIENTS IN INFANT FORMULA

Per 21 CFR §107.100 infant formula must contain a variety of micro and macronutrients. 21 CFR §107.100 also specifies maximum levels of protein, fat, vitamin A, vitamin D, iron, iodine, selenium, sodium, potassium, and chloride per 100 kcal infant formula (Table 11). Considering the maximum inclusion rate of 153 g WBM solids/100 g infant formula, the reconstitution rate of 12.9 g infant formula powder/100 ml, and the energy density of reconstituted formula of 67 kcal/100 ml, both the average and maximum concentrations of protein, fat, vitamin A, vitamin D, iron, iodine, selenium, sodium, potassium, and chloride in WBM will not exceed the maximum levels in infant formula as specified in 21 CFR §107.100 (Table 11).

Table 11. Nutrients In WBM and Potential Concentration in Infant Formula Vs Maximum Permitted Concentration in Infant Formula

Nutrient (Units)	Average Nutrient Concentration in WBM		Maximum Permitted Level/100 kcal (21 CFR §107.100)
	/100 g WBM[a]	/100 kcal Infant Formula[b]	
Protein (g)	3.36	1.1	4.5
Fat (g)	3.78	1.1	6.0
Vitamin A (IU)	127.9	37.7	750
Vitamin D3 (IU)	<4[c]	<1.2[d]	100
Iron (mg)	<0.1[c]	<0.03[d]	3.0
Iodine (µg)	14	4.1	75
Selenium (µg)	2.3	0.7	7
Sodium (mg)	34	10.0	60
Potassium (mg)	160	47.1	200
Chloride (mg)	100	29.5	150

Abbreviations: n/a - not available; kcal – kilocalorie; CFR – Code of Federal Regulations; IU – International Units; g – gram; kg – kilogram; µg - microgram

[a]Values reflect average of 3 samples from non-consecutive batches (Table 4).

[b]Calculated values in infant formula assuming maximum inclusion rate of 153 g WBM/100 g infant formula, the reconstitution rate of 12.9 g infant formula powder/100 ml, and the energy density of reconstituted formula of 67 kcal/100 ml.

[c]Values reflect max concentration (Table 4).

[d]Represents the amount at the limit of quantitation.

IV. SELF-LIMITING LEVELS OF USE

The amount of WBM added to cow's milk-based infant formula is limited by the nutrient requirements specified in Title 21 CFR §107.100. The maximum intended use of WBM is 153 g/100 g infant formula powder, which is equivalent 3.8 g/100 kcal reconstituted infant formula.

V. COMMON USE IN FOOD BEFORE 1958

The conclusion of GRAS status for the use of WBM as an ingredient in non-exempt infant formula is based upon scientific procedures. Examples of common use in food before 1958 are provided in Part 6 as supplemental information.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

A. HISTORICAL USE OF MILK IN INFANT FORMULA

As summarized in GRN 001041 (pg. 30) recent infant formula feeding practices in the U.S. have not relied on the use of whole milk or milk fat, though historically substitutes for human milk have included cow's milk, which could also be in the forms of evaporated milk and sweetened milk (Innis, 2011; IOM, 2004; Jensen and Jensen, 1992). In the early 1900s, cow's milk was recognized as the most likely foundation for development of infant formula (IOM, 2004). Fomon (2001) cited survey data indicating that, in the 1960s, "60% of infants were fed whole milk by 4 months of age." In 1971, ">30% of infants from 3 to 4 months of age, >40% of infants from 4 to 5 months of age and >60% of infants from 5 to 6 months of age were fed cow's milk." Interest in breast feeding in the last thirty years of the twentieth century led to a deferment of the age of introduction of cow's milk, but "it was generally recommended (American Academy of Pediatrics Committee on Nutrition, 1976) that for non-breastfed infants >6 months old, formula feeding was desirable, but cow's milk plus regular feeding of iron-fortified cereals was a satisfactory alternative" (cited in GRN 000980 pg. 21).

As summarized in GRN 001041 (pg. 30), the modern commercial milk-based infant formulas originated with the development of a formulation called "synthetic milk adapted", which contained non-fat cow's milk, lactose, and fat from vegetable oils. Further modifications to the cow's milk base continued over time, including but not necessarily limited to modifications such as changes in the fatty acid profile, dilution of protein and altering the whey:casein ratio to mimic the ratio in human milk, and adjusting levels of micronutrients. Cow's milk-based formulas produced from non-fat milk and milk-derived ingredients remain the primary source of nutrition for formula fed infants (Corkins and Shurley, 2016; LSRO, 1998; Martin et al., 2016). Data collected in 2003-2010 indicate that cow's milk formula was used by 69% of infants fed formula or milk (Rossen et al., 2016). Dry skim milk is typically the predominant ingredient in milk-based formulas, though these formulas typically contain several other milk-derived ingredients, such as whey and lactose, which are the predominant sources of protein and carbohydrate, respectively. Although commercial infant formulas moved away from the use of whole milk and related products in the 1970s, non-fat milk has been routinely used in infant formula as a source of protein and carbohydrate (in the form of lactose) for decades (Corkins and Shurley, 2016; LSRO, 1998).

B. REGULATED USES OF MILK IN INFANT FORMULA

Recently, dry whole bovine milk, and anhydrous bovine milk fat and dry whole goat milk have been determined to be GRAS in the United States for use as ingredients in infant formula

for term infants (Table 12). Additionally, several infant formulas made with whole milk are now currently available in the United States, such as Kendamil (Kendal Nutricare Ltd., United Kingdom), A2 Infant Formula (A2 Milk Company, Australia), Bubs (Bubs Australia Ltd., Australia), and Bellamy's Organic Infant Formula (Bellamy's Organic, Australia) via letters of enforcement discretion (https://www.fda.gov/food/infant-formula-guidance-documents-regulatory-information/enforcement-discretion-manufacturers-increase-infant-formula-supplies; accessed on February 27, 2024).

Table 12. GRAS Notifications for Use of Milk and Milk Fat in Infant Formula

GRN	Substance	Notifier	Intended Use	Date of Closure
001136	Dry Whole Goat Milk	Jovie USA LLC	Intended for use as a source of protein in ready-to-feed or powdered, non-exempt infant formula for term infants at a typical level of 5.5 g/100 mL of infant formula as consumed.	10/31/23
001041	Dry Whole Milk	Nara Organics, Inc.	Intended for use as an ingredient in cow's milk-based, non-exempt infant formula for term infants at a maximum level of 22% (w/w) powdered infant formula.	5/10/2022
000980	Dry whole milk	ByHeart, Inc.	Intended for use as an ingredient in cow's milk-based, non-exempt infant formula for term infants at a maximum level of 16% (w/w) of powdered infant formula.	7/13/2021
000898	Anhydrous milk fat	Hogan Lovells US LLP	Intended for use as a source of fat in cow's milk-based, calorically dense, ready-to-feed and exempt infant formula for term infants at a maximum level of 7% of the fat blend.	10/28/2020

C. ASSESSMENT OF SAFETY OF THE INTENDED USE OF WBM

1. Basis for Safety for Dry Whole Milk in GRN 000980 and GRN 001041

Because the dry whole milks that are the subjects of GRNs 000980 and 001041 are produced from pasteurized liquid whole bovine milk, the basis for safety of the use of WBM in infant formula relies on the same lines of evidence as presented and accepted in GRNs 000980 and 001041.

The basis for safety for the use of dry whole milk in infant formula presented in GRN 000980 (pg. 28-29) and summarized in GRN 001041 (pg. 32-33) included animal studies; a discussion of milk digestion via well-established metabolic pathways without adverse effects; current safe consumption of whole milk and dry whole milk by infants, toddlers, and children; and controlled clinical trials showing no adverse effects associated with consumption of whole milk or dry whole milk by infants or toddlers other than allergic reactions in susceptible individuals. Additionally, the concerns associated with the use of whole milk as a sole source of

nutrition have no relevance because the intended use of WBM, as well as the subjects of GRNs 000980 and 1041, is as an ingredient in infant formula, which is a complex mixture of proteins, fats, carbohydrates, vitamins, and minerals. WBM, as well as the subjects of GRNs 000980 and 1041, provides only a portion of total infant formula nutrients. Thus, the intended levels of use of WBM, as well as the subjects of GRNs 000980 and 1041, in infant formula do not present safety concerns. In addition, ByHeart reviewed the contributions of milk fat and lipid components of whole milk in infant formula compared with breast milk, and indicated that the intended addition of dry whole milk does not cause the allowable levels of nutrients with maximum allowable levels in infant formula to be exceeded (GRN 000980 pg. 14-16). It is also noteworthy that the use of dry whole milk is not different from the current use of non-fat dry milk and whey powders in infant formula, suggesting that the use of dry whole milk or WBM would be substitutional for other milk-based ingredients currently used in infant formula.

The basis for safety summarized in GRN 001041 (pg. 44-46) included the arguments set forth in GRN 000980, as well as documentation that Nara Organics' dry whole milk is manufactured using standard processes in the dairy industry that have been extensively reviewed for their effect on milk proteins and are consistent with the processes detailed in GRN 000980 for the production of dry whole milk. Physico-chemical similarities and differences between unmodified milk, dry whole milk, and non-fat dry milk arising from processing were discussed, as well as the potential physiological consequences; any effects on processing had no effect on the safety profile of the various forms of milk. Updated literature searches were performed and new studies were incorporated into discussions relevant to the safety assessment. Nara Organics concluded that although processing may affect milk proteins and have physiological effects, the effects of processing, including similarities and differences between dry whole milk, dry non-fat milk and whole milk (unmodified), have no effect on the safety profile. The potential concerns raised with the use of whole milk as a sole source of nutrition (e.g., potential nutrient deficiency, potential renal solute load, fat absorption) were not relevant to the intended use of dry whole milk as an ingredient in a complex infant formula that provides only a portion of nutrients in the total formula. Additionally, infant formulas manufactured with milk-derived ingredients, such as skimmed milk and vegetable oils, are estimated to contain up to 4% residual milk fat and thus, provide infants some exposure to components naturally present in dairy fat that are not found in vegetable oils. Components unique to milk fat include short- and medium-chain fatty acids, branched- and odd-chain fatty acids, trans fatty acids, conjugated linolenic acid (CLA), as well as phospholipids, cholesterol and sphingolipids, which are largely found in the milk fat globule membrane (GRN 001041, pg 38-41). While the specific composition of cow's milk differs from the composition of human milk, these constituents in cow's milk fat are present at some concentration in human milk; thus, breastfeeding infants are routinely exposed to these constituents. A review of the concentration of each constituent in non-exempt infant formula

from the intended use of dry whole milk relative in infant formula provided information that the relative exposure to these components from use of the dry whole milk was consistent with that from exposure to human milk. Lastly, in GRN 001041, Nara Organics summarized clinical studies in which infants and young children consumed bovine whole milk that were identified in GRN 000980; literature searches did not identify any additional clinical studies.

Following our review of the information presented in GRNs 000980 and 001041 to support the intended use of WBM in infant formula, we concur with the conclusions of safety as summarized in GRN 000980 and 001041.

2. Basis for Safety of the Intended Use of WBM

The evidence to support the safety of the intended use of WBM as an ingredient in the infant formula is presented below and, as stated above, relies on the same basis as described in GRN 000980 and GRN 001041. Additionally, a series of literature searches using the search strings enumerated in GRN 001041 (Appendix C. of GRN 001041, pg. 64) was conducted in PubMed to identify more recent information pertinent to the safety review (search conducted for studies published from January 2021 to December 2023). The more recent literature identified in these searches is incorporated in the discussion below.

D. DIGESTION OF MILK IN INFANT FORMULA

The intended use of WBM in infant formula would replace a portion of the non-fat milk commonly used in milk-based formulations. Like non-fat milk, WBM will provide milk protein and lactose in infant formula. WBM will also contribute fat to the total fat profile of the infant formula.

1. Protein

Casein and whey proteins in human milk or infant formula are the main source of proteins for infants. Casein in milk exists mainly in the form of casein micelles, which consist of highly phosphorylated caseins (αs- and β-caseins) interacting and aggregating with calcium phosphate and covered by a κ-casein layer. Casein micelles contain thousands of individual protein molecules that are susceptible to coagulation due to pepsin hydrolysis and low pH in the stomach close to the isoelectric point of caseins. Unlike caseins, whey proteins in their native state hardly coagulate and remain soluble, resulting in more rapid emptying from the stomach to the small intestine than caseins (Jiang et al., 2022). One study analyzed the effect of the ratio of casein to whey protein (40:60, 60:40, 80:20) on the digestibility of cow's milk-based infant formula and showed that as the ratio of casein to whey increased, coagulation was more widespread and protein digestibility was lower. When the ratio is 60:40 (whey:casein), which

closely resembles the ratio in human milk, casein micelles coalesced the least extensively and protein digestibility was the highest (Phosanam et al., 2021).

At the proposed level of addition, WBM will contribute 55% to the total protein content and will, alongside non-fat milk powder, be the main source of casein protein in the infant formula. Other sources of whey proteins will be added to the infant formula to target a resulting whey:casein ratio of 60:40. WBM will also contribute 21% of the total fat and 12.7% of the total carbohydrate. Other ingredients will also be added (vegetable oils to reach the desired fat content, and lactose to reach the desired carbohydrate content, and vitamins and minerals) to ensure the infant formula is nutritionally complete for infants and compliant with 21 CFR §107.100.

In contrast to dry whole milk (the subjects of GRN 000898 and 001041), WBM does not undergo the additional steps of evaporation and drying prior to the addition to infant formula. As summarized by van Lieshout et al. (2020) (GRN 001041 pg 34), pasteurization causes the most protein denaturation. However, unlike liquid whole milk, both the evaporation and drying processes can further decrease protein digestibility and amino acid availability, and thus decrease protein quality.

1.1. Protein Quality

As summarized in GRN 001041 (pg. 33-35), milk proteins in general are recognized as highly digestible and high-quality proteins for human nutrition. Typical processing may modify dairy proteins and in turn protein digestibility or kinetics (van Lieshout et al., 2020). The digestible indispensable amino acid score (DIAAS) and protein digestibility amino acid score (PDCAAS) are measures that have been used to evaluate the relative nutritional quality of different protein sources. PDCAAS and DIAAS data indicate that skim milk powder, whole milk powder, and fluid milk all have scores of not less than 1, indicating that they are complete sources of protein (Burd et al., 2019; FAO/WHO 2013).

Consistent with quality factor requirements for infant formula (21 CFR §106.960), a Protein Efficiency Ratio (PER) bioassay was completed on an infant formula containing WBM at the intended level of use in accordance with the AOAC official method number 960.48 (Unpublished Report, 2023). The standard protocol of 28 days allocation to the test diet in comparison to control animals allocated to a casein standardized protein diet was employed. The daily measurement of weight gain and food intake was utilized to determine the protein quality of the test formulation with a standard formula of:

PER = gain in weight (g) / protein intake (g)

Juvenile rats were maintained on each diet, control and test article, and were evaluated daily over a 28-day period for their rate of growth and consumption of their allocated diets. All treatment groups exhibited the typical rapid rate of growth of young rats. The test diet did not change the increase in rat weight gain as compared to the casein control diet group. A measure of total weight gain over the 28-day period demonstrated no difference between diet groups (102.38±1.34 versus 98.63±1.36 grams, control versus test diet respectively). This was also evident by the average daily weight gain of animals, with no significant difference between the control and test diet animal groups. This study demonstrated that the PER was greater than that of the casein control, thus demonstrating appropriate biological quality of the protein required for an infant formula.

Animal food consumption was recorded for the same 28 day period, with daily food intake measured for each. Analysis of daily food consumption demonstrated a significant decrease in food consumption by the test diet group across the period of evaluation. The known values for protein Nx6.25 for control diet (9.83 g/100g) and test diet (9.75 g/100g) were employed to calculate the average protein intake for each formulated diet over the period of the 28-day study. Analysis demonstrated a significant decrease in average protein intake by animal allocated to the test diet.

Calculation of protein efficiency ratio (PER) for each diet indicated there to be a higher PER value in the test diet formulation of 3.32±0.08 versus 3.15±0.09 for the casein control. This did not reach significance. A calculation of ratio x100 of the test diet formulation to ANRC reference casein PER was determined at 106.16% as compared to the control diet.

a. Effect of Processing Conditions

As summarized in GRN 001041 (pg 34), milk proteins are subjected to a variety of heat processes as part of standard dairy practices. All liquid dairy undergoes a pasteurization step. During this processing step the naturally occurring casein (80% of the protein) and whey (20% of the protein) are subjected to a heating process. Because of a lack of tertiary structure, the caseins are remarkably stable; however, whey proteins are highly sensitive to pasteurization temperatures and tend to easily denature onto the casein micelles. It is important to note that all dairy products subjected to liquid pasteurization undergo the same changes, and WBM is subjected to similar processing and pasteurization conditions as dry whole milk.. Therefore, both WBM and dry whole milk would be expected to have comparable changes in their protein profiles as a result of pasteurization.

Research specifically on milk proteins in infant or enteral formulas indicates that heat treatments can induce protein denaturation, which may enhance digestibility, although heat-induced protein-protein and protein-lipid interactions may counteract this effect (Rudloff and

Lonnerdal, 1992; Wada and Lonnerdal, 2014). Glycation of lysine and amino terminal residues, resulting from the heat induced Maillard reaction, reduces the allergenicity of ß-lactoglobulin, the major milk allergen, by hindering the binding of IgE to the protein epitope. This glycation also reduces protein bioavailability (Sarwar et al, 1989; Perusko et al., 2018). This protein bioavailability reduction is accentuated in liquid formulas that are exposed to a higher heat process compared to powdered formulas (Sarwar et al., 1989, van Lieshout et al., 2020) (cited in GRN 001041 pg 35-36).

As cited in GRN 001041 (pg 35), van Lieshout et al. (2020) reported in a review of 102 peer-reviewed articles that processing affects milk proteins to varying degrees, which may in turn impact protein digestibility, amino acid bioavailability and quality and other physiological consequences of the proteins. Because of a lack of tertiary structure, caseins don't show the typical denaturation and aggregation upon heating. Caseins are, however, sensitive to processing-induced chemical modifications and aggregation. The whey proteins, on the other hand, both denature and aggregate upon heating and can be chemically modified. The ratio in which different reactions (denaturation, aggregation, chemical modification) occur depends to a large extent on the exact processes to which the milk is subjected. This causes liquid and dry dairy products to differ in their degree of protein modifications. Pasteurization can be further subdivided in low and high pasteurization. Low pasteurization refers to a very mild heat treatment of 72ºC for approximately15 seconds or 63ºC for 30 minutes, which both have been shown to have a negligible effect on milk proteins. Some enzymes are denatured and thereby inactivated, but the major milk proteins remain in their native state. High pasteurization is less well defined, but is usually both at higher temperatures (>80ºC) and for longer durations (up to several minutes) compared to low pasteurization, and can lead, depending on the precise heat load, to partial or full denaturation of the whey proteins. Caseins are not directly affected by pasteurization conditions, besides the binding of whey proteins to the casein micelle. Protein denaturation can facilitate gastric hydrolysis, especially of beta-lactoglobulin. Conditions of extreme or high intensity processing of milk protein may have the largest impact on physiological consequences.

Liu et al. (2016) demonstrated, in an in vitro model, that differences in phosphorylation levels between human and bovine caseins can affect the aggregation state of casein during digestion. A de-phosphorylated milk protein concentrate has reduced gastric coagulation and increased gastrointestinal digestibility. Therefore, micellar structure and composition of casein affects the curd formation during gastric digestion of milk, thus improving protein digestion of human milk compared to infant formulas.

A recent review of the effect of milk processing published by Broersen (2020) reported that pasteurization not only ameliorates consumer safety concerns mediated by pathogenic bacteria, but also has an impact on one of the main nutritional whey constituents of milk, the

protein β-lactoglobulin. As a function of heating, as well as homogenization, β-lactoglobulin was reported in this review to become increasingly prone to denaturation, aggregation, and lactose conjugation. Chauvet et al. (2023) also corroborated the effect of processing on protein denaturation levels and composition using four powdered infant formulas based on commercial whey protein ingredients with pasteurization at 72ºC for 2 minutes prior to spray drying. The study demonstrated that the quality (structure and composition) of dairy commercial protein ingredients had a significant impact on the microstructure of infant formulas and that these differences modulated the proteolysis kinetics as well as the deconstruction of the emulsion in the early gastric phase. Whey protein ingredients with a high denaturation level generated star-shaped microstructures favoring protein hydrolysis. The structure and composition of infant formulas also impacted the accessibility of the peptide bonds to enzymes, and the infant formula with the lower denaturation extent presented the highest abundance of peptides.

b. Allergenicity

Milk is among the foods identified as a major allergen in the U.S. Allergy to milk protein is estimated to occur in approximately 2.6% of the population of young children in North America, though an estimated 5-15% of infants may experience cow's milk protein intolerance (Abrams and Sicherer, 2021; Corkins and Shurley, 2016). Thus, this infant formula is not intended for milk allergic individuals.

c. Summary

Overall, processing affects milk proteins to varying degrees, which may in turn impact protein digestibility and quality and other physiological consequences of the proteins. WBM is subjected to the same processing conditions as dry whole milk, with the exception of evaporation and drying; pasteurization conditions for both dry whole milk and WBM comply with those specified in the Pasteurized Milk Ordinance; WBM also complies with Commission Delegated Regulation (EU) No 2020/692 and Regulation (EC) No 853/2004. Thus, the WBM that is the subject of this GRAS Determination is subjected to similar processing and pasteurization conditions as dry whole milk and therefore is expected similar protein profiles as the subjects of GRNs 000980 and 001041. In addition, calculation of PER in the WBM formulation was 3.32 ± 0.08 compared to the casein control of 3.15 ± 0.09, indicating acceptable protein quality.

2. Fat

As summarized in GRN 01041 (pg. 35-36), studies describe the digestion of powder milk-based infant formulas with added milk fat compared to standard milk-based formula with vegetable fat and human milk. Using a static two-phase in vitro digestion model to mimic digestion in the gastric and duodenal phases of digestion, Hageman and colleagues (2019a; 2019b) showed that human milk and infant formula containing different fat blends result in a

similar release of total fatty acids at the end of digestion as a percentage of initial composition. One of the formulas contained only vegetable fat (palm, palm kernel, rapeseed, and sunflower oil) while the other formula contained a blend of 67% bovine milk fat and 33% vegetable fat (rapeseed, sunflower, and coconut oil). In comparisons of the percentage release of individual fatty acids from the two formulas, differences in the release of some short and medium chain fatty acids were noted in the gastric and duodenal phases. However, following total digestion, the only difference between formulas was a lower percentage of C14:0 released from the formula containing milk fat compared to the formula containing only vegetable fat.

Liu and colleagues (2021) (summarized in GRN 001041, pg. 36) compared in vitro digestion of human milk and infant formulas. Two infant formulas were prepared with whole bovine milk and whole goat milk, and two were prepared with skim milk. One of the skim milk-based formulas only had vegetable oils, while the other was formulated with milk fat globule membrane (MFGM) and vegetable oils. The lipolysis rate of human milk was highest at 86.8%, followed by the formula containing MFGM (81.2%), then the formulas containing whole milk (78.0% for the whole goat milk formula and 77.6% for the whole bovine milk formula), and lastly the skim milk, vegetable oil-based formula (70.5%). The presence of MFGM components on the fat surface assisted with lipid hydrolysis. At the end of the simulated intestinal digestion, the concentration of palmitic acid was lower for human milk (158 μmol/g) relative to all of the infant formulas, though the concentration from the formula containing a blend of whole bovine milk and vegetable fat and the formula containing only vegetable fat were comparable at 235 and 251 μmol/g, respectively. The difference noted in palmitic acid concentration is attributed to the differences in triacylglycerol structure between formulas and human milk. Lipids in human milk exist in the form of dispersed droplets called milk fat globules, the core of which is mainly triglycerides, representing more than 95% of lipids in human milk. The external trilayer membrane consisted of polar lipids, proteins, neutral lipids and other minor components (Jiang et al. 2022). The structure of the milk lipids has an important influence on lipid digestion and absorption. Human milk contains more than 200 fatty acids. In mature human milk, triglycerides consist mainly of oleic acid (21–42%), palmitic acid (15–29%) and linoleic acid (7–25%). Up to 70% of palmitic acid in human milk is in the sn-2 position of triglycerides, resulting in a uniquely high level of stereospecific structures oleic-palmitic-oleic (OPO) and oleic-palmitic-linoleic (OPL). This sn-2 position leads to the release of palmitic acid as sn-2 monoglyceride, which is easily absorbed and prevents the formation of calcium-palmitic acid soaps and hardening of stools, and subsequently reduces the incidence of constipation and excretion of calcium and palmitic acid in infants (Jiang et al. 2022). While up to 70% of palmitic acid in human milk occupies the sn-2 position of triglycerides, there is approximately less than 40% of sn-2 palmitic acid in formula with OPO or cow or goat milk fat, and less than 13% of sn-2 palmitic acid in infant formulas that use vegetable oils only as a fat source. The unesterified palmitic acid resulting from the digestion of vegetable oils is able to form indigestible complexes

with calcium in the lumen, called soap formation. Since the fat digestion process mainly hydrolyzes fatty acids at sn-1 and sn-3 positions, addition of milk fat mixed with vegetable oils in cow's milk-based formula may improve fat digestion to be more similar to human milk (Jiang et al. 2022).

Recent work published by George et al. (2023) provided a lipidomics analysis of human milk, infant formula and animal milk demonstrating that there are differences in composition between human milk, infant formula and animal milk. Human milk samples from two birth cohorts, the Barwon Infant Study (n = 312) and University of Western Australia birth cohort (n = 342), were analyzed using four liquid chromatography-mass spectrometry (LC–MS) methods (lipidome, triacylglycerol, total fatty acid, alkylglycerol). Bovine, goat, and soy based infant formula, and bovine and goat milk were analyzed for comparison. Composition was explored as concentrations, relative abundance, and infant lipid intake. Key findings from this study were: (1) the human milk lipidome differs from that of both infant formula and animal milk, and is rich in ether lipids; (2) human milk lipids exhibit longitudinal trends; and (3) the human milk lipidome impacts infant circulating lipids. Ether lipids were significantly higher, in concentration and relative abundance, in human milk compared with infant formula and animal milk. Infant formula and animal milk (including bovine) are similar in that they both have little if any ether lipid concentrations.

A study in piglets evaluated the impact on immunological outcomes relative to milk fat compared to fat derived from vegetable oils in normal birth weight (NBW) compared with intrauterine growth restricted (IUGR) piglets (Baek et al., 2021). Two-day old piglets were selected (NBW, n = 18, IUGR, n = 18) and each group of animals were fed formula based on either vegetable oil or bovine milk fat. Animals were reared until day 23/24 and systemic immune parameters were evaluated. Milk-fat feeding decreased blood neutrophil counts and improved neutrophil function while transiently reducing leucocytes' expression of genes related to adaptive and innate immunity as well as energy metabolism, following in vitro stimulation by live Staphylococcus epidermidis (whole blood, 2 h). However, there were only a few interactions between milk-fat type and birthweight status. Thus, piglets fed milk-fat-based formula had improved neutrophil maturation and suppressed pro-inflammatory responses, compared to those fed vegetable-oil-based formula. In conclusion, the impact of milk-fat-feeding on the developing immune system was moderate. There were no adverse outcomes associated with feeding of formula based on bovine milk fat. The authors speculated that it is possible that diets based on milk fats could help improve the adaptation to extra-uterine life. However, the data indicated that growth restricted neonates do not appear to have specific needs for dietary fat relative to neonates with normal bodyweight.

a. Summary

Recent literature corroborates the findings summarized in GRNs 000980 and 001041 for dry whole milk that the lipolysis rate of human milk is highest followed by infant formula containing MFGM, then formulas containing whole milk, and lastly skim milk, vegetable oil-based formula (70.5%). The presence of MFGM components on the fat surface assists with lipid hydrolysis. Additionally, the differences noted in the palmitic acid concentrations are attributed to the differences in triacylglyceride structure between formulas and human milk. Up to 70% of palmitic acid in human milk is in the sn-2 position of triglycerides, leading to the release of palmitic acid as sn-2 monoglyceride, which is easily absorbed and prevents the formation of calcium-palmitic acid. Collectively, these data indicate that the addition of bovine milk fat to infant formula may result in fat digestion being more similar to that of human milk.

3. Carbohydrate

As summarized in GRN 001041 (pg 36), like non-fat milk, WBM as an ingredient in infant formula will provide carbohydrate in the form of lactose. Lactose is the primary form of carbohydrate in human milk and the common carbohydrate used in standard non-exempt infant formula (Kien, 1996; Corkins and Shurley, 2016). Lactose is also recognized as safe and appropriate for use in infant formula for healthy term infants (LSRO, 1998).

E. UNMODIFIED WHOLE MILK VS MILK AS AN INGREDIENT

While infant feeding practices in the first half of the twentieth century commonly relied on use of evaporated milk or fresh cow's milk, evidence emerged that unmodified whole milk was not suitable as the sole source of nutrition for infants (Fomon, 2001; Ziegler, 2011). However, WBM is intended to be used as an ingredient in infant formula ingredient, not as a sole source of nutrition (summarized in GRN 001041 pg. 36-37), and the infant formula to which WBM will be added will meet all nutrient specifications specified for infant formula per 21 CFR §107.100.

1. Nutrient Imbalances including Iron Deficiency

As summarized in GRN 001041 (pg 36-37) consumption of fresh milk by infants is associated with iron deficiency, potentially due to the low concentration of iron in milk, as well as iron inhibitors including calcium and casein and intestinal blood loss (Ziegler, 2011). Clinical studies demonstrate that consumption of fresh milk results in early iron deficiency compared to consumption of a milk-based formula despite comparable intake of iron (Woodruff et al., 1972). Consumption of unmodified whole milk also caused dose-dependent increases in intestinal blood loss among some infants, which could contribute to iron deficiency (Fomon, 1981; Wilson et al., 1974; Ziegler et al., 1990). In contrast to unmodified cow's milk, the study by Fomon and colleagues (1981) demonstrated that milk treated under time and temperature conditions

consistent with those used in the manufacture of standard infant formula was not observed to cause fecal blood loss, thus suggesting that a heat-labile protein was at least in part a factor for the whole milk-induced bleeding. Other investigators also have observed a similar effect with heat-treated milk (Wilson et al., 1974). Studies on fecal iron loss show that the youngest infants exhibit the greatest loss, with concerns resolved by age 12 months (Jiang et al., 2000; Ziegler et al., 1999). Regarding concerns of low iron, milk-based formulas fortified with iron at a concentration of 6 to 12 mg/L meet infants' iron needs (Ziegler, 2011). Per 21 CFR §107.100, all non-exempt infant formulas in the United States are required to contain 0.15 to 3.0 mg iron per 100 kcal (equivalent to 0.1005 mg to 2.01 mg/100 kcal assuming that infant formula contains 67 kcal/100 ml). If formula contains less than 1 mg iron/100 kcal, a statement on the label is required, "Additional Iron May Be Necessary", in order to ensure appropriate attention to infant iron needs. Non-exempt infant formulas are also required to contain certain micronutrients to ensure infant health. The low levels of other nutrients in milk that are important for infant health (e.g., vitamin C, zinc, vitamin E, essential fatty acids) are therefore not a concern. The Kendal Nutricare infant formula will meet all nutrient specifications for infant formula listed in 21 CFR §107.100.

2. Potential Renal Solute Load

Additional concerns for infants consuming unmodified cow's milk include the high potential renal solute load which may contribute to the risk of dehydration. This risk has been considered a concern principally during illness (LSRO, 1998). The potential renal solute load of conventional infant formula (20-26 mOsm/100 kcal) was concluded to be an acceptable range (IOM, 2004; Ziegler and Fomon, 1989). The potential renal solute load of the infant formula containing WBM is estimated to be 21.9 mOsmol/100kcal with an intended acceptable range of 20 -26 mOsmol/100kcal.

F. AVOIDING ANIMAL FAT

As cited in GRN 001041 (pg 36-37), an additional concern regarding the use of whole milk as "safe and palatable for human infants" was identified as a need to remove animal fat and substitute butterfat (i.e., milk fat) with vegetable oils. Several reasons were cited to support the use of vegetable oils in infant formulas rather than milk fat; namely vegetable fats 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., 2019c).

In a review of infant nutrition, Fomon (1993) (summarized in GRN 004041, pg. 38) noted that the newborn infant's absorption of 100% milk fat is poor. However, when provided in formula as a blend of 50% milk fat and 50% vegetable oil (equal parts corn and coconut oil), the

fat blend was well absorbed and excretion of fat was within the range of excretion from human milk and infant formula.

KNC whole milk contributes to approximately 21% of the total fat in infant formula. The intended use of WBM in infant formula will provide approximately 10% of total energy from milk fat given that 49% of total energy in the formula is provided by fat and approximately 21% of fat in the formula is provided by WBM. Thus, the amount of milk fat provided by the intended use of WBM is within the level of milk fat identified as well absorbed and therefore does not present a safety concern.

G. COMPONENTS IN WBM NOT FOUND PRESENTLY IN TYPICAL FORMULA

1. Fatty Acids and Cholesterol

Milk fat is a source of several fatty acids that are not common to vegetable oils typically used in the manufacture of infant formula, namely butyric acid, trans-fatty acids, conjugated linoleic acid, odd-chain fatty acids, and branched-chain fatty acids (Gallier et al., 2020). The concentration of selected fatty acids including butyric acid, trans fatty acids, CLA, odd chain fatty acids and cholesterol in WBM intended for use in infant formula are summarized in Table 13.

Table 13. Concentration Of Fatty Acids and Cholesterol in Pasteurized Liquid Whole Bovine Milk (WBM)

Component (unit)[a]	Lot Number			Average ± SD
	KTM08/006	KTM08/007	KTM09/008	
4:0 Butyric (g/100g)	0.136	0.121	0.141	0.133 ± 0.01
Trans Fatty Acids (acid form) (g/100g)	0.093	0.087	0.078	0.086 ± 0.01
18:2 Conjugated Linoleic Acid (g/100g)	0.030	0.027	0.023	0.027 ± 0.004
15:0 Pentadecanoic (g/100g)	0.0427	0.0362	0.0428	0.041 ± 0.004
17:0 Heptadecanoic (g/100g)	0.019	0.018	0.021	0.019 ± 0.001
Cholesterol (mg/100g)	13.50	2.40	12.60	9.50 ± 6.17

Abbreviations and notes:
[a]Fatty Acids analyzed using GC-FID (BS EN ISO 12966). Cholesterol analyzed with a method based on JAOAC International 76, 1993.
SD: Standard Deviation.
GC-FID: Gas Chromatography-Flame ionization Detection.
BS EN: British Standards implementations of English language version of European Standards.
N.B. 'KT codes' are unique laboratory sample codes. Samples were taken from non-consecutive batches.

Based on the maximum intended use of WBM in infant formula, the concentrations of these fatty acids and cholesterol provided from WBM in infant formula were estimated and the percent of total fatty acids in the resulting infant formula was calculated (Table 14). For comparison, the concentrations of these components in human milk and infant formula were summarized from the literature (as a percent of total fatty acids) as presented in GRN 001041 and are therefore incorporated by reference (GRN 001041, pg. 40) (Table 14).

Taken together, these data demonstrate that these components of WBM are also present in varying concentrations in human milk and infant formulas prepared from a variety of fat sources. Additionally, the concentrations of these components in human milk vary with the mother's diet. For example, CLA content is dependent on the intake of dairy products (Martysiak-Zurowska, 2018), Zhang et al. (2022) demonstrated that the proportion of vegetable intake versus odd-chain fatty acid containing food intake, such as ruminant fats, could affect the level of odd-chain fatty acids in secreted milk, odd-chain fatty acid level is impacted by the stage of milk production, and Zhang et al. (2022) found that a comparatively higher level of odd-chain fatty acids is observed in the colostrum milk stage, with a reduction in level during the transition/ mature milk stage.

In summary, the use of WBM in infant formula will result in concentrations of butyric acid, trans-fatty acids, conjugated linoleic acid, odd-chain fatty acids, and branched-chain fatty acids that will not exceed those reported in human milk and infant formula.

Table 14. Estimated Concentration of Selected Fatty Acids and Cholesterol in Infant Formula Containing Pasteurized Liquid Whole Bovine Milk (WBM) vs Concentrations in Human Milk

Component	Average Fatty Acid Level in WBM (mg/100 g)[a]	Level of Fatty Acid in Infant Formula Containing WBM (mg/100 mL)[b]	Percentage of Fatty Acid in Infant Formula Containing WBM[c]	Range of Means in Human Milk (% of Fatty Acids)[d]	Range of Means in Infant Formula (% of Fatty Acids)[e]
4:0 Butyric	133	26.20	0.73	0.0009 - 0.76	ND - 3.1
Trans Fatty Acids (Acid Form)	86	16.94	0.47	1.9 - 2.7	ND - 1.56
18:2 Conjugated Linoleic Acid	27	5.32	0.15	0.07 - 0.49	ND - 0.33
15:0 Pentadecanoic	41	8.08	0.22	0.08 - 0.50	ND - 0.6
17:0 Heptadecanoic	19	3.74	0.10	0.19 - 0.41	ND - 0.4
Cholesterol	9.5	1.87	0.05	9 - 20 mg per 100mL	1.46 - 5.1 mg per 100 mL

Abbreviations and notes:
n/a: not applicable.
[a]Mean of analytical values from 3 non-consecutive batches.
[b]Shown as liquid WBM contribution to KNC infant formula. Calculated values in infant formula assume 19.7 liquid WBM in 100mL reconstituted formula.
[c]Shown as solid WBM contribution to KNC infant formula Fatty Acids profile. Calculated values in infant formula assume 28g total fat per 100g infant formula and 153g of liquid WBM in 100g of KNC infant formula.
[d]Concentrations in human milk reported by Chardigny et al., (1995), Glew et al. (2011), Hageman et al. (2019c), IOM (2005), Koletzko, (2016), Martysiak-Zurowska et al. (2018), Mosley et al. (2005), Mueller et al. (2010), Prentice et al. (2019), Ratnayake et al. (2014), Santillo et al. (2018), Sun et al. (2016), Wan et al. (2010), and Yuhas et al. (2006) [adapted from GRN 000898]).
[e]ND = not detected; Reported by Claumarchirant et al. (2015), Gallier et al. (2020), Hageman et al. (2019c), Rodríguez-Alcalá et al. (2019), Martysiak-Zurowska et al. (2018), McGuire et al. (1997), and Sun et al. (2016).

2. Phospholipids

Human milk consists of 3−5% fat, of which phospholipids only occupy a small portion,. Phospholipids refer to phosphoric acid mono- or di-esters (IUPAC recommendations, 1976), including glycerophospholipids and sphingolipids. Among the latter, sphingomyelins (SMs) are the most abundant in mammalian cells. Phospholipids play a crucial physiological function in maintaining the normal growth and development of the infant (Yang et al., 2022). Glycerol-3-phosphate is a structural moiety common to all glycerophospholipid molecules, and the glycerol molecule containing two hydroxyl groups is esterified with fatty acid. Meanwhile, the phosphate class can be linked with different head groups to form various glycerophospholipids, which primarily include phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidic acid (PA). However, sphingomylins (SM) comprises phosphoric acid, fatty acid, choline, sphingosine, or dihydrosphingosine. It does not contain glycerol, and its fatty acid is connected to the amino group of sphingosine by an amide bond. A study reported by Wang et al. (2023) identified a total of 5048 lipid species and 45 lipid classes in human MFGM and cow MFGM using the UHPLC-Q-Exactive MS-based lipidomics analysis. The lipid profiles of cow MFGM significantly differed from the human MFGM, but the analysis of phospholipid classes revealed that the lipid composition of human MFGM and cow MFGM was more similar than the other dietary-derived lipid such as soybean, krill and yolk. These results provide a rationale for addition of cow-milk derived lipids to infant formula to more closely approximate human MFGM lipid profiles.

The concentration of phospholipids in WBM intended for use in infant formula was examined and summarized in Table 15. The predominant phospholipids are phosphatidyl-choline, phosphatidylethanolamine, and sphingomyelin. Based on the maximum intended use of WBM in infant formula and compositional data on the WBM (Table 15), the concentration of WBM-derived phospholipids in infant formula was calculated and compared to those in dry whole milk (as summarized in Table 14 of GRN 001041, pg. 41) and human milk (Table 16). The levels of the phospholipids in formula prepared using WBM approximate those in dry whole milk, and are lower than those in human milk. Thus, the levels of phospholipids contributed by WBM are consistent with what would be expected and consistent with the exposures from the use of dry whole milk in infant formula.

Table 15. Concentration of Phospholipids in Pasteurized Liquid Whole Bovine Milk (WBM)

Component (unit)	WBM Lot Number KTM08/006	KTM08/007	KTM09/008	Average ± SD	Average Level in Dry WBM[a]	Reference Value in Dry Whole Milk[b]
Phosphatidylcholine (mg/100g)	8.0	9.0	8.0	8.3 ± 0.001	63.85	69.1
Phosphatidylethanolamine (mg/100g)	9.0	9.0	8.0	8.7 ± 0.001	66.92	64.9
Phosphatidylinositol (mg/100g)	1.0	2.0	2.0	1.7 ± 0.001	13.08	38.1
Phosphatidylserine (mg/100g)	3.0	3.0	2.0	2.7 ± 0.001	20.77	34.0
Sphingomyelin (mg/100g)	8.0	9.0	6.0	8.0 ± 0.002	61.54	58.8
Sum of Phospholipids (mg/100g)	29.0	32.0	27.0	29.0 ± 0.003	223.08	294.8

Abbreviations and notes:
[a]Calculated based on the average value of 13g solid in 100g liquid WBM from three batches.
[b]Values reported by Soga et al., 2015 and corrected for moisture content of 3%.
SD: Standard Deviation.
N.B. 'KT codes' are unique laboratory sample codes. Samples were taken from non-consecutive batches.

Table 16. Estimated Concentrations of Phospholipids in Infant Formula Containing Pasteurized Liquid Whole Bovine Milk (WBM) vs Concentrations In Human Milk

Component	Phospholipid Level in WBM (mg/100g)[a]	Phospholipid Level in Infant Formula Containing WBM (mg/100mL)[b]	Reference Values in Human Milk (mg/100mL)[c]
Phosphatidylcholine	8.3	1.6	3.3
Phosphatidylethanolamine	8.7	1.7	7.0
Phosphatidylinositol	1.7	0.3	0.8
Phosphatidylserine	2.7	0.5	3.8
Sphingomyelin	8.0	1.6	6.2
Sum of Phospholipids	29.0	5.7	21.6

Abbreviations and notes:
[a]Mean of analytical values from 3 non-consecutive batches.
[b]Shown as liquid WBM contribution to infant formula. Calculated values in infant formula assume 19.7g liquid WBM per 100mL infant formula.
[c]Average concentration of phospholipids reported by Ma et al. (2017) in human milk samples collected from transitional milk and mature milk at 2, 6, and 12 months of lactation.

It is important to note that the distribution and concentration of the phospholipid classes in human milk changes at various lactation stages, and may be impacted by dietary habits, individual conditions, stage of lactation, genetics, season and with storage. There are also differences among infant formulas in the distribution and concentration of phospholipids. Wei et al. (2019) measured the composition of phospholipids in eight different infant formulas and reported the following ranges of phospholipids (Table 17).

Table 17. Phospholipid Composition of Infant Formula

Phospholipid	Range of Phospholipids (µmol/100 mL)
PC	10.03 – 33.17
PI	0.84 – 12.11
PS	0.30 – 2.26
SM	5.45 -15.98
PE	7.35 – 20.14
Total PL	24.60 – 82.30

Adapted from Wei et al. (2019).
PS: phosphatidylserine
PC: phosphatidylcholine
PI: phosphatidylinositol
PE: phosphatidylethanolamine
SM: sphingomylins
PL: phospholipid

Similarly, phospholipid composition of human milk is expected to vary; levels of phospholipids measured in mature milk are summarized in Table 18. A recent study of human milk reported by Ding et al. (2023) demonstrated the variability of phospholipids over time by analyzing samples obtained from colostrum, transitional, and mature milk for phospholipid content with ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS). Human milk was obtained from the Obstetrics Unit of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China. Phosphatidylcholine (PC), sphingomyelins (SM), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) were significant phospholipid class molecules in human milk. The total percentage of these phospholipid content was nearly 90% at different lactation stages. PC is always the most prevalent phospholipid group in human milk, whose content was 450.2 mg/L in colostrum, then decreased to 400.6 mg/L in transitional milk, and finally decreased to 288.9 mg/L in mature milk. SM was the second most abundant phospholipid class in human milk, which increased from 155.0 mg/L in colostrum to 234.3 mg/L in mature milk. The average PE content attained its highest value of 136.1 mg/L in transitional milk, while the average PS content declined along the lactation stage. Ingvordsen Lindahl et al. (2019) used liquid chromatography

tandem-mass spectrometry to quantify phospholipid species in colostrum (<5 days postpartum), transitional (≥5 days and ≤2 weeks) and mature milk (>2 weeks and ≤15 weeks) samples from mothers who had delivered preterm and term infants, respectively. The analysis revealed that both gestational age and age postpartum affected the phospholipid composition of human milk (Table 19). Differences related to gestation decreased as the milk matured.

Wei et al. (2021) demonstrated that the nutritional profile of human milk varies significantly during storage, with an impact of storage conditions on phospholipid composition. Phospholipids continuously decreased during storage. After 120 days of storage, the total phospholipids decreased from 19.1 μmol/100 mL milk to 4.8 μmol/100 mL, 8.2 μmol/100 mL and 10.3 μmol/100 mL at 4°C, -20°C and - 80°C, respectively. Glycerophospholipid showed a higher lipolysis rate than sphingomyelin. This may account for some of the variability seen in human milk phospholipid content when reported in studies.

Table 18. Published Values for Phospholipid Content in Human Milk

PL	Wei et al. (2022; full term milk)[a]	Wei et al. (2019; 90-day term)[a]	Ma et al. (2017; average from 2,6 and 12 months term)	Giuffrida et al. (2013; 4 weeks term)	Ingvordsen Lindahl et al. (2019; mature milk)	Ding et al. (2023; mature milk)[c]	Duan et al. (2021; mature milk)	Liu et al. (2023; mature milk)[d]	Song et al. (2021; mature milk)	Yang et al. (2022; mature milk)
PC (mg/100 ml)	5.68 ± 1.47	4.63 ± 0.43	3.3	6.0 ± 1.3	4.5 ± 1.97	29	2.26 ± 0.55	3.9	12.9	4.3 ± 0.08
PI (mg/100 ml)	0.70 ± 0.28	0.65 ± 0.12	7.0	1.1± 0.3	NR	3	NR	0.3	NR	1.9 ± 0.03
PS (mg/100 ml)	1.06 ± 0.35	0.83 ± 0.07	0.8	1.4 ± 0.3	NR	4	NR	NR	11.3	2.2 ± 0.04
SM (mg/100 ml)	6.94 ± 1.69	6.63 ± 0.45	3.8	8.5 ± 1.7	3.29 ± 1.73	23	5.11 ± 1.42	4.6	16.1	6.0 ± 0.09
PE (mg/100 ml)	2.10 ± 0.53	2.13 ± 0.10	6.2	6.8 ± 1.9	29.15 ± 13.04	11	2.00 ± 0.84	3.0	14.7	6.6 ± 0.12
Total PL	Approx. 24.4[b]	Approx. 22.6[b]	21.6	23.8 ± 3.4	36.94 ± 16.41	NR	10.39 ± 3.11	NR	NR	21.1 ± 0.3

PL: phospholipid
PC: Phosphatidylcholine
PE: Phosphatidylethanolamine
PI: Phosphatidylinositol
PS: Phosphatidylserine
SM: Sphingomyelin
NR: not reported
[a]Reported in µmol/100 ml; converted to mg/100 ml assuming the following molecular weights: PC = 814.2 g/mol (https://pubchem.ncbi.nlm.nih.gov/compound/Phosphatidylcholine_22_1_16_1); PI = 863.1 g/mol (https://pubchem.ncbi.nlm.nih.gov/compound/Phosphatidylinositol_18_1_18_1); PS = 972.1 g/mol (https://pubchem.ncbi.nlm.nih.gov/compound/Phosphatidylserine; SM = 813.2 (https://pubchem.ncbi.nlm.nih.gov/compound/C24_1-Sphingomyelin); PE = 299.1 (https://pubchem.ncbi.nlm.nih.gov/compound/Phosphatidylethanolamine)
[b]Estimated because primary data is not available.
[c]Estimated from Figure 1C.
[d]Estimated from Figure 2.

Table 19. Concentration of Phospholipids in Colostrum, Transitional and Mature Milk from Mothers with Preterm and Term Gestations[a]

Phospholipid Class	Preterm						Term					
	Colostrum		Transitional		Mature		Colostrum		Transitional		Mature	
PE (mg/100 ml)	87.85	(27.30) ^{A,a}	75.25	(24.85) ^{A,a}	46.64	(19.81) ^{B,a}	49.40	(13.68) ^{A,a}	37.86	(14.00) ^{A,a}	29.15	(13.04) ^{B,a}
PC (mg/100 ml)	19.31	(11.46) ^{A,a}	13.09	(3.94) ^{B,a}	6.92	(3.23) ^{C,a}	11.44	(2.64) ^{A,b}	6.56	(3.26) ^{B,b}	4.50	(1.97) ^{C,b}
SM (mg/100 ml)	10.13	(6.24) ^{A,a}	7.21	(1.95) ^{B,a}	3.93	(1.63) ^{C,a}	6.90	(1.26) ^{A,b}	4.23	(1.88) ^{B,b}	3.29	(1.73) ^{C,b}
Total PL (mg/100 ml)	117.30	(42.08) ^{A,a}	95.49	(29.43) ^{B,a}	57.49	(23.77) ^{C,a}	67.74	(14.47) ^{A,b}	48.65	(18.11) ^{B,b}	36.94	(16.41) ^{C,b}

[a]Adopted from Ingvordsen Lindahl et al. (2019).

Two-way ANOVA with Tukey's test of concentration means and standard deviations (in brackets). PE: Phosphatidylethanolamine, PC: Phosphatidylcholine, SM: Sphingomyelin, PL: phospholipid. Values with different superscript letters are significantly different ($p < 0.05$) within a row (upper case letters A, B, C indicate significant differences related to lactational stage; lower case letters a, b indicate significant differences related to gestational age).

H. CONSUMPTION OF WHOLE MILK BY INFANTS

Clinical studies in which infants and young children consumed bovine whole milk were identified and summarized in GRN 000980 and reviewed in GRN 001041 (Table 1, Appendix D). No more recent interventions were identified in the literature search conducted for this GRAS Determination. Among the 23 studies cited in GRN 000980, eight studies represented prospective interventions in infants, including seven repeat intake studies with intake from 6 days to one year and one study examining acute effects of milk consumption. Key details from the eight prospective randomized trials, including infants (<12 months of age) in the study population, are presented in Table 1, Appendix D in GRN 001041 and the table is reproduced below in Table 20. In none of these studies were any adverse events attributable to feeding of whole milk reported other than iron deficiency among children not receiving iron fortification or supplementation. We agree with the conclusion reached in GRN 001041 that no adverse health consequences are attributed to whole milk ingestion in children.

GRN 000980 (Table 9, pg. 22-27) also summarized nine studies in which toddlers or young children were given whole milk (Houghton et al., 2011; Svahn et al., 2000; van der Gaag and Forbes 2014; van der Gaag et al., 2017; van der Gaag et al., 2020; Vanderhout et al. 2016a; Vanderhout et al. 2016b; Wong et al., 2019) and no adverse effects attributable to whole milk were noted. We agree with this conclusion.

Table 20. Prospective Randomized Trials of Infants Consuming Whole Bovine Milk

Reference	Population	Intervention	Key Results
Alarcon et al. (1991)	Population: 85 Peruvian infants and children, hospitalized for acute diarrhea Age range: 5-24 months, stratified into ages 5-6 months and 7-24 months Mean age: 11.9±4.2 months	Duration: 6 days Test material: 1) Mixture of dried whole milk, potato flour, carrot flour, sucrose & vegetable oil 2) Mixture of wheat flour, pea flour, carrot flour, sucrose, & vegetable oil 3) Soy-protein isolate, lactose-free formula Intake of test material: 110 kcal/kg bw/day Intake of whole milk powder (1st diet): 6.46 g No additional foods allowed	Key results: Children in all groups gained weight; no differences were observed in anthropometric status, energy intakes, energy absorption, nitrogen retention, or fecal output and no differences in treatment failure. Authors' conclusion: The " locally available, low-cost staple food mixtures [i.e., interventions 1 and 2] offer a safe and nutritionally adequate alternative to a commercially produced lactose-free formula for the dietary management of young children with acute diarrhea in this setting."
Brown et al. (1991)	Population: 116 Peruvian male infants and toddlers with acute diarrhea Age range: 3-24 months Mean age: 12.5±6.1 months	Duration: 6 days Test material: 1) Modified whole milk & wheat noodles with vegetable oil 2) Lactose-hydrolyzed whole milk & wheat noodles with vegetable oil 3) Modified whole milk with corn syrup solids 4) Lactose-hydrolyzed milk formula with corn syrup solids Intake of test material: 55 (first two days of treatment) and 110 kcal/kg bw/day for the following 4 days. -Intake of full-fat dried milk (modified) when fed alone: 17.4 g -Intake of full-fat dried milk (modified) when fed with wheat noodles: 8.7 g -No additional foods allowed.	Key results: The combination of milk and noodles resulted in reduced stool outputs, shorter durations of diarrhea, and lower rates of treatment failure than did milk alone. Authors' conclusion: "the noodle-milk diets employed during this study were safer than the milk diets for the dietary management of children with acute diarrhea."

Table 20. Prospective Randomized Trials of Infants Consuming Whole Bovine Milk

Reference	Population	Intervention	Key Results
Fomon et al. (1981)	Population: 81 normal healthy infants Age: 112-196 days	Duration: 12 weeks Test material: Pasteurized whole milk (n = 39) or heat treated milk (n=22) or Enfamil (n = 20) Intake of test material: 126-130 mL/kg bw/day at 112-139 days (~79% energy), 110-118 mL/kg bw/day at 140-167 days (~75% energy), 96-102 mL/kg bw/day at 168-195 days (~73% energy) -Weaning foods allowed, including milk	Key results: Incidence of blood in stool was greater among infants fed whole milk vs heat treated milk or formula from age 112 to 140 days; no difference thereafter. No significant differences observed in mean hemoglobin, hematocrit, serum iron, total iron- binding capacity, or transferrin saturation. *(Note: no iron supplementation was provided with whole milk)* Authors' conclusion: Pasteurized cow's milk should not be administered prior to 140 days of age.
Hjelt et al. (1989)	Population: 52 infants and children hospitalized with acute gastroenteritis after oral rehydration Age range: 6-46 months Mean age: 19 months	Duration: 7 days Test material: Rapid refeeding (lactose-limited whole milk as only fluid intake; n = 27) or gradual refeeding (stepwise intake with each step lasting 1+ days, first three steps excluding whole milk, 2nd step including "small amounts of cultured milk products," 3rd step including presumably typical amounts of cultured milk products, and 4th and last step including whole milk in "increasing amounts"; n = 25) In rapid refeeding (lactose-hydrolyzed) provided 47-59% of daily energy intake. No whole milk quantity provided for gradual refeeding. No limitations on additional foods & liquids in rapid refeeding	Key results: Both regimens produced similar results with regard to duration and severity of diarrhea and vomiting. The rapid-refeeding group derived more energy from fat and protein and less from carbohydrate compared to the gradual-refeeding group. Milk provided 47-59% of the daily energy intake of the rapid-refeeding group. Authors' conclusion: Whole milk was well accepted and no signs of cow's milk protein intolerance were observed. Additionally, the milk-based rapid- refeeding regimen can be employed "without the fear of negative effects on the outcome."

Table 20. Prospective Randomized Trials of Infants Consuming Whole Bovine Milk

Reference	Population	Intervention	Key Results
Isoulauri et al. (1986)	Population: 65 infants and toddlers hospitalized for acute gastroenteritis Age range: 6-34 months Mean age: 14.7months	Duration: acute Test material: Whole milk and milk products (gruel, sour milk, yogurt, ice cream) (n = 38) or no milk (n = 27) Milk-based products made up 30-90% total caloric intake; mean 50%, or approximately 400 kcal in first 18 h. All children received mixed diet:	Key results: No difference observed between the groups in the clinical recovery from diarrhea; no prolonged diarrhea reported in any child; no new cases of clinical atopy were observed at 1-month follow-up; and no significant increases in the total or milk-specific IgE levels were reported. In addition, serum IgG and IgA antibodies to β-lactoglobulin and α-casein were initially present in the majority of the children, but no appreciable changes in the antibodies were reported after gastroenteritis regardless of the type of diet. Authors' conclusion: Cow's milk and milk products can be safely administered in acute gastroenteritis as parts of the mixed diet for children > 6 months of age
Larnkjær et al. (2009)	Population: 83 healthy infants Mean age: 9.1±0.3 months, followed to age 12.1±0.3 months	Duration: 3 months Test material: Whole milk or infant formula, with or without fish oil. No recommendations on the amount of milk intake. Intake of test material: 300 ml/day, 30% of daily protein intake. Weaning foods allowed, including milk	Key results: Intake of whole milk significantly increased protein energy percentage and serum urea nitrogen; no effect on anthropometric measures of growth was observed; whole-milk intervention increased IGF-I in boys but not in girls; intake of fish oil had no effect on the outcomes. Authors' conclusion: "Randomization to whole milk had no overall effect on growth. However, the positive effect of whole milk on IGF-I in boys and the positive association between protein energy percentage and IGF-I at 9 and 12 months is consistent with the hypothesis that a high milk intake stimulates growth."
Stekel et al. (1988)	Population: 554 infants with birthweight >2500 g Age: 3-15 months (Measured at 3, 9, 15 months)	Duration: 12 months Test material: Whole milk with sucrose and corn flour supplemented with ferrous sulfate & ascorbic acid (n=276) or control milk without additives (n=278) Intake of test material: not reported Weaning foods allowed, including milk. Those breastfeeding were allowed to continue to do so.	Key results: 2.5% of infants in the group receiving whole milk + supplements had iron deficiency anemia compared with 25.7% of the control group. Authors' conclusion: "the acceptability of this milk was excellent."

Table 20. Prospective Randomized Trials of Infants Consuming Whole Bovine Milk

Reference	Population	Intervention	Key Results
Ziegler et al. (1990)	Population: 52 healthy term infants Age: 24 weeks	Duration: 12 weeks Test material: Whole cow's milk or infant formula (n=26/group) Intake of test material: not reported. Weaning foods allowed, including milk	Key results: No differences reported between groups in parental reports of regurgitation, vomiting, constipation, or other feeding-related behavior; stool hemoglobin concentration increased with the introduction of whole cow's milk from 622±527 µg/g dry stool at baseline to 3598± 10,479 µg/g dry stool during the first 28 days of ingestion of whole cow's milk. No increase in stool hemoglobin among formula-fed infants and levels were significantly less than in the whole milk group. Stools with occult blood increased from 3.0% at baseline to 30.3% in the whole-milk group during the first 28 days of the trial, while the proportion of positive stools remained low (5.0%) with formula feeding. The proportion of occult-blood-positive stools among whole-milk-fed infants declined later, but remained significantly elevated for the entire trial. Authors' conclusion: "A large proportion of normal nonanemic infants respond to the feeding of pasteurized cow's milk [i.e., whole milk as the sole source of nutrition and no added iron] with increased fecal loss of blood."

I. Consumption of Milk Fat by Infants from Infant Formula

Four clinical trials in which a specified amount of cow's milk fat (2.8%, 20%, 48%, and 50% of fat) was included in infant formula were identified in the published literature (Breij et al., 2019; de Souza et al., 2018; Leite et al., 2013; Manios et al., 2020 (two trials)); these trials are summarized in Table 21 (adopted from GRN 001041, Table 2 in Appendix D).

The source of milk fat (e.g., dry whole milk, cream, anhydrous milk) used in these formulations is not specified. All forms of milk fat provide a source of the fatty acids found in dry whole milk, though anhydrous milk fat provides little or no components located in the MFGM (Huppertz and Kelly, 2006). Results from 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 provide supporting evidence for the safety of the intended use of up to 153 g WBM/100 g powdered infant formula, which accounts for 21% of total fat as milk fat based on representative product data (Table 21).

Table 21. Clinical Studies of Infants Consuming Formula with Dairy Fat

Reference	Population	Intervention	Key Results
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	Consumed from 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 tolerated and safe for use in infants."
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	Consumed for 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
Manios et al. (2020)	Crossover study 16 healthy, formula-fed infants, age 9-14 weeks	Consumed for 2 weeks: Test: 50% milk fat; blend with vegetable fat Control: vegetable fat	-No difference in formula intake, weight or length measurements -No difference in total free fatty acids, though proportions of some individual fatty acids differed (excluding palmitic acid) -No difference in palmitic acid concentration in stool, but proportion of palmitic acid in stool relative to total free fatty acids was decreased compared to vegetable fat control -Decreased calcium concentration in stool compared to vegetable fat control -Decreased stool consistency and more reports of watery stools compared to vegetable fat control

Table 21. Clinical Studies of Infants Consuming Formula with Dairy Fat

Reference	Population	Intervention	Key Results
Manios et al. (2020)	Crossover study 17 healthy, formula-fed infants, age 9-14 weeks	Consumed for 2 weeks: Test: 20% milk fat; blend with vegetable fat Control: vegetable fat	-No difference in formula intake, weight or length measurements - No difference in total free fatty acids, though proportions of some individual fatty acids differed (excluding palmitic acid) - Decreased calcium concentration in stool compared to vegetable fat control -No difference in stool consistency
Schouten (2013) [unpublished, as cited in GRN 000898]	Single arm trial; open label 50 healthy term infants	Consumed for 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.

Adapted from GRN 000898. Abbreviations: AE - adverse events; ARA - arachidonic acid; DHA - docosahexaenoic acid; RBC - red blood cells; SAE - serious adverse events

J. CLINICAL STUDY: A NON-INFERIORITY ASSESSMENT OF AN INFANT FORMULA CONTAINING WBM VERSUS A USA-LEADING COMMERCIALLY AVAILABLE INFANT FORMULA ON GROWTH VELOCITY IN INFANTS (REPORT R0414)

To evaluate the safety and tolerance of an infant formula containing WBM, a randomized, double-blind, comparator-controlled, multicenter clinical study was conducted in healthy, term infants with a birth weight between 2,600 g and 3,800 g (inclusive). All enrolled infants were not more than 2 weeks (14 days) of age at the time when the legally authorized representative signed the informed consent. The test product was Kendamil® Organic First Infant Milk (TP), which contained WBM, and the comparator product was Similac®Advance® (RP). Study participants were engaged in the study for up to 16 weeks.

A total of 176 infants were screened for eligibility to obtain the required sample size of 120 infants. All enrolled infants were randomized in a 1:1 ratio to Kendamil infant formula (TP) or Similac infant formula (reference product, RP), which served as the sole source of nutrition during the study.

In the per protocol (PP) population, the lower bound of the 95% confidence interval (CI) for the difference between TP and RP in average weight gain over the study period was -1.296 g/day (95% CI: -1.296 to 0.557) and did not exceed the non-inferiority margin of -3.0 g/day. Additionally, there was no statistically significant difference in overall daily body weight gain between the TP- and RP-treated groups in the change from baseline in body weight for age (Figures 2 and 3), body length for age (Figures 4 and 5), head circumference for age (Figures 6 and 7), and weight for length (Figures 8 and 9) throughout the study. There were also no statistically significant differences in average volume of formula intake/day or formula intake/kilogram weight/day, the number of total diaper rash events or any other GI events (i.e., colic, cramps, flatulence or gassiness, and regurgitation/reflux), the number of infants experiencing stool events over the whole study period.

Overall, 13 infants experienced 17 adverse events (AEs) during the study period. There were 7 infants (8.2%) reporting 10 AEs in the TP group and 6 infants (6.6%) reporting 7 AEs in the RP group. All of the reported AEs across the study product groups were mild (76.5%) to moderate (23.5%) in severity. All of the reported AEs across the study groups were assessed as unrelated to the study products, based on the criteria outlined in the study protocol. The majority of the AEs were reported in the gastrointestinal disorders with 6 infants reporting infantile colic (3.4%) and 4 infants reporting infantile diarrhea (2.3%), followed by respiratory, thoracic, and mediastinal disorders with 4 infants reporting rhinorrhea (2.3%). One moderate SAE of upper respiratory tract infection was reported. This SAE was deemed unrelated to study product. No infants had an AE that led to permanent discontinuation of study product.

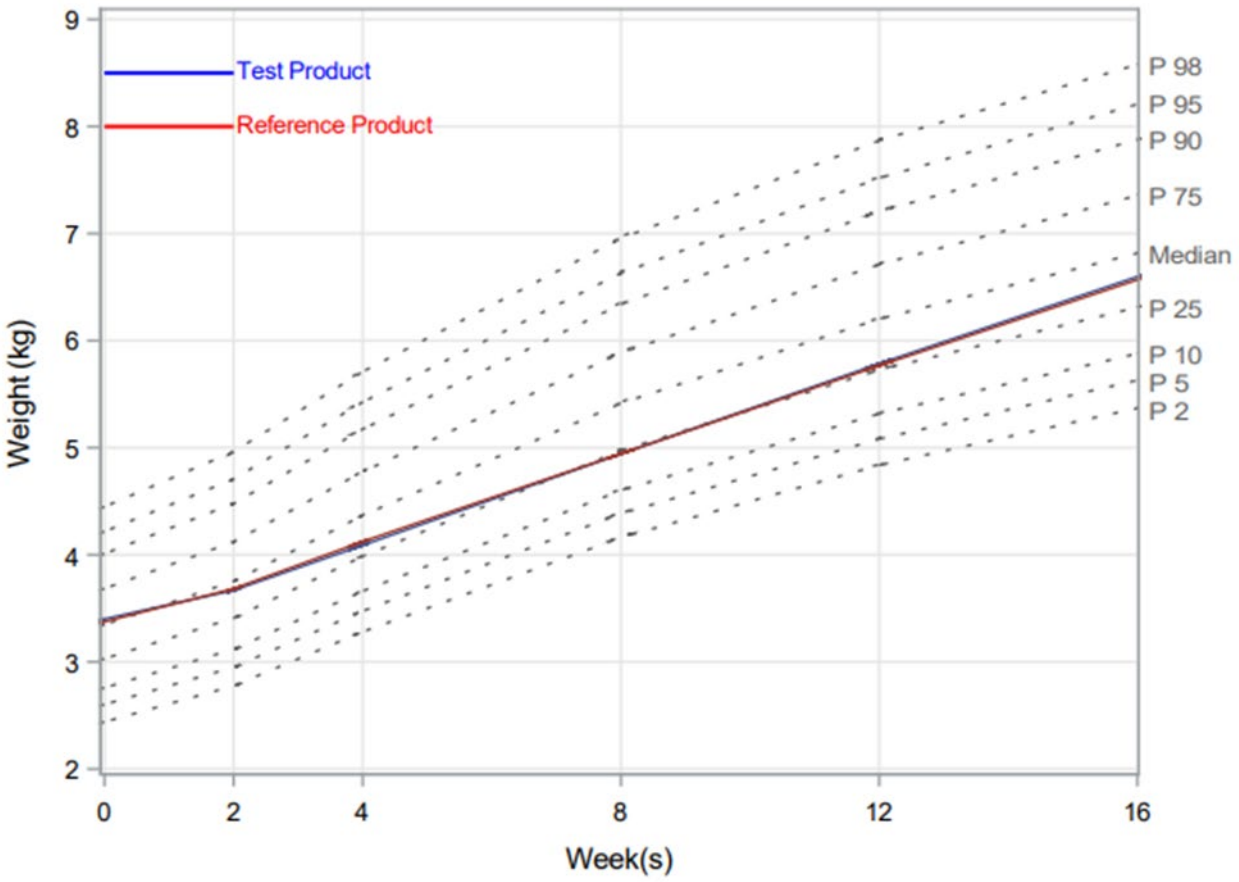

Figure 2. Male Body Weight for Age

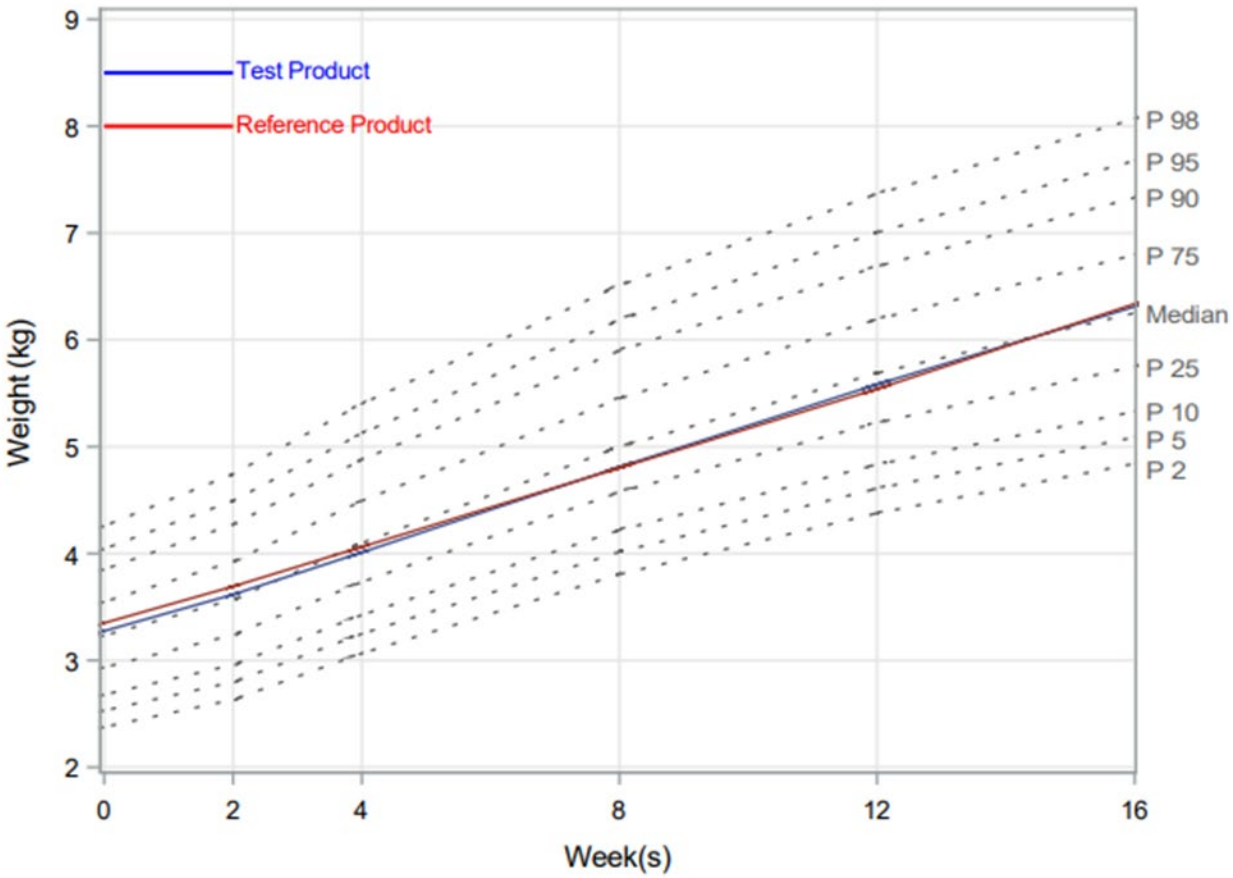

Figure 3. Female Body Weight for Age

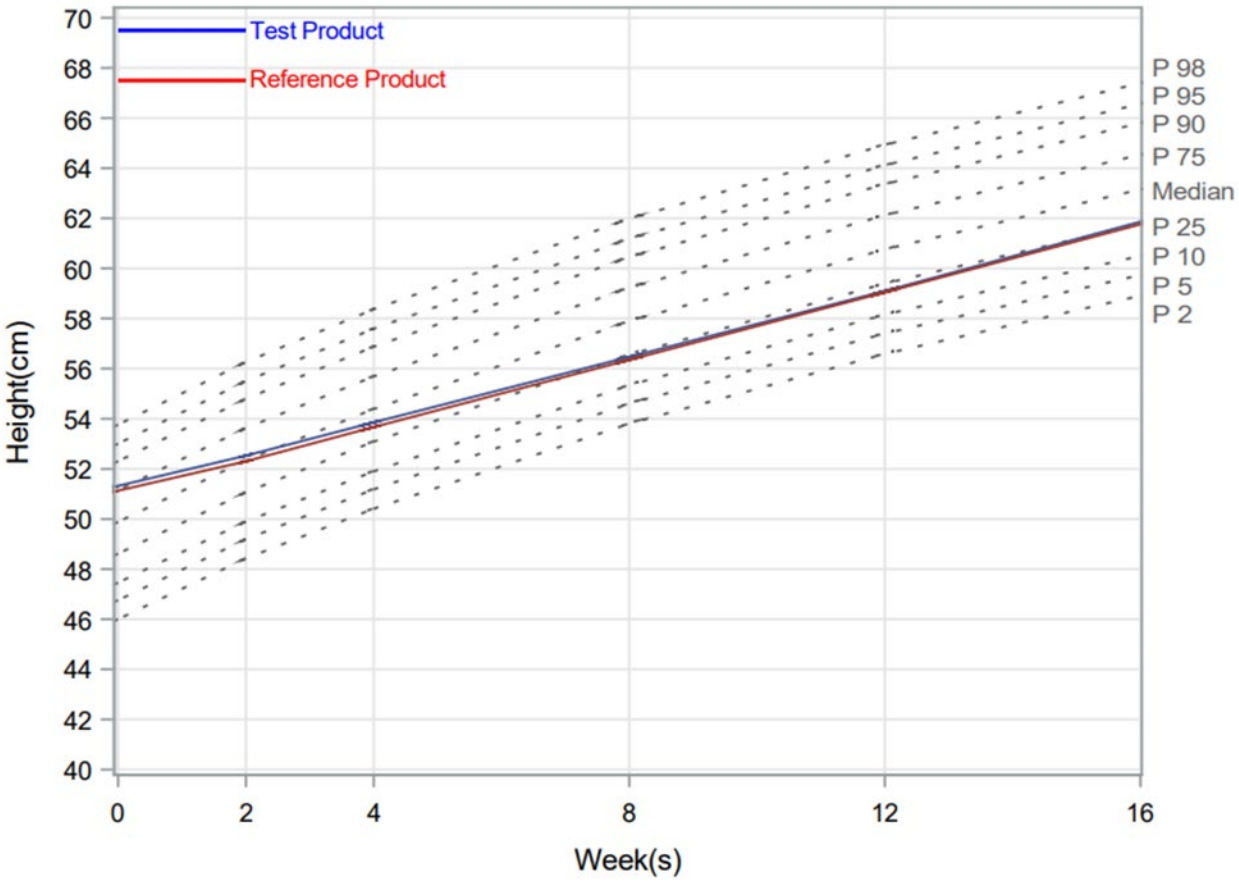

Figure 4. Male Body Length for Age

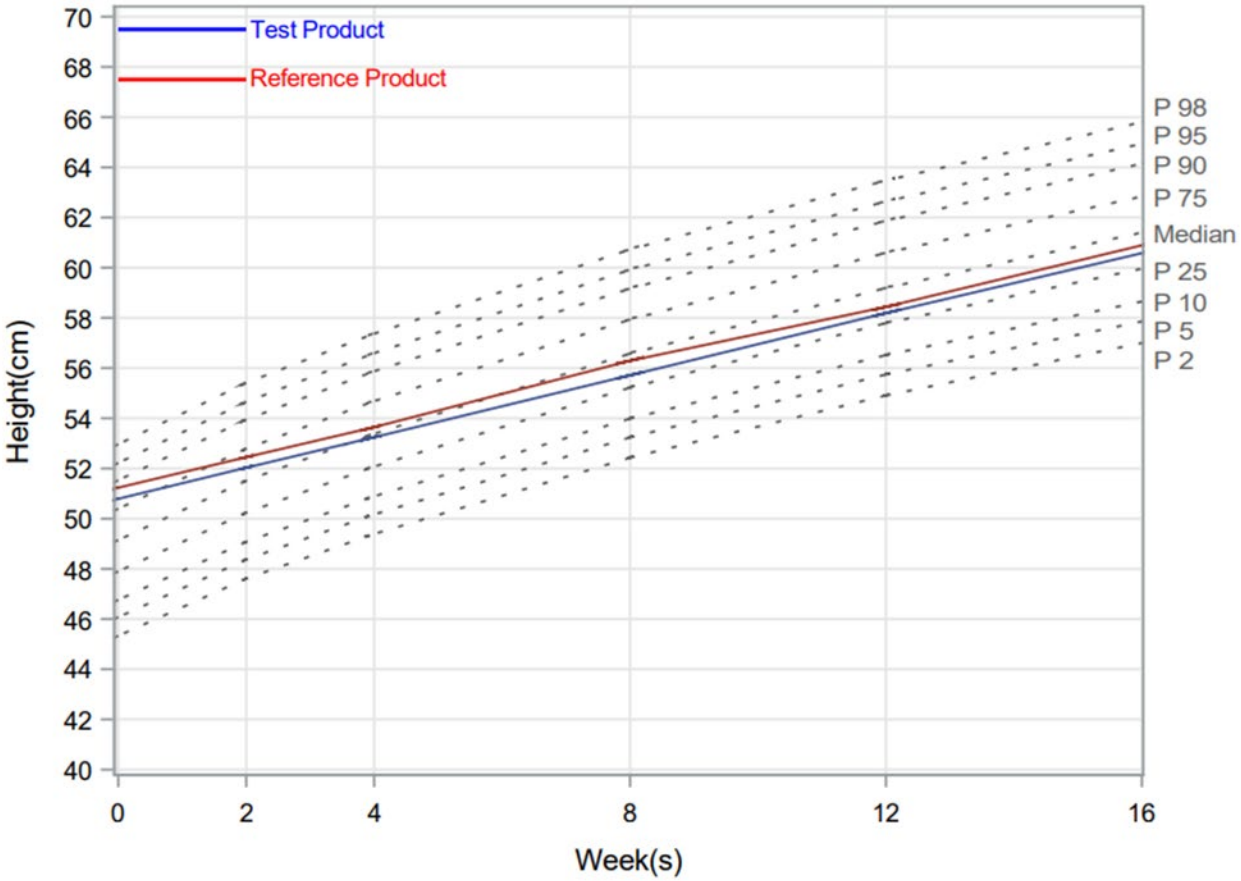

Figure 5. Female Body Length for Age

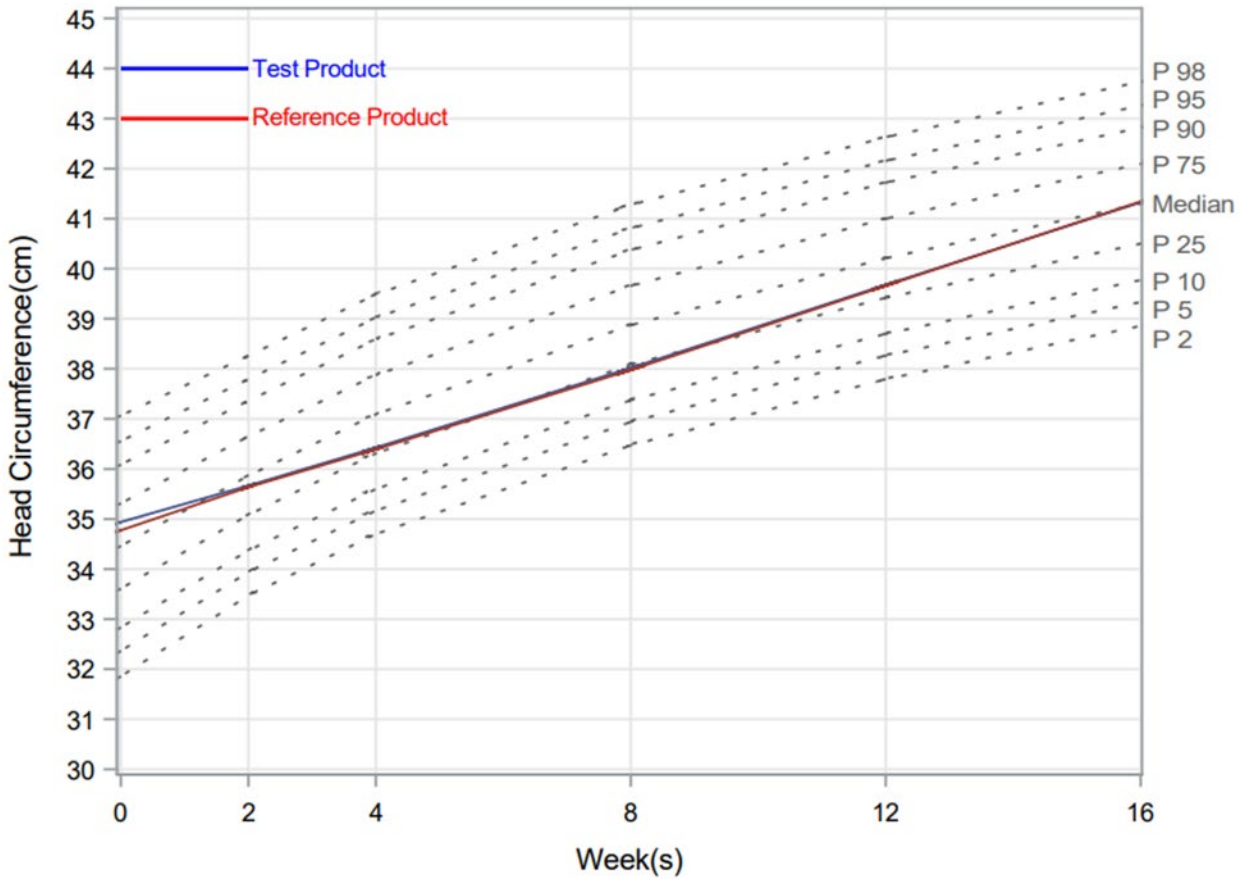

Figure 6. Male Head Circumference for Age

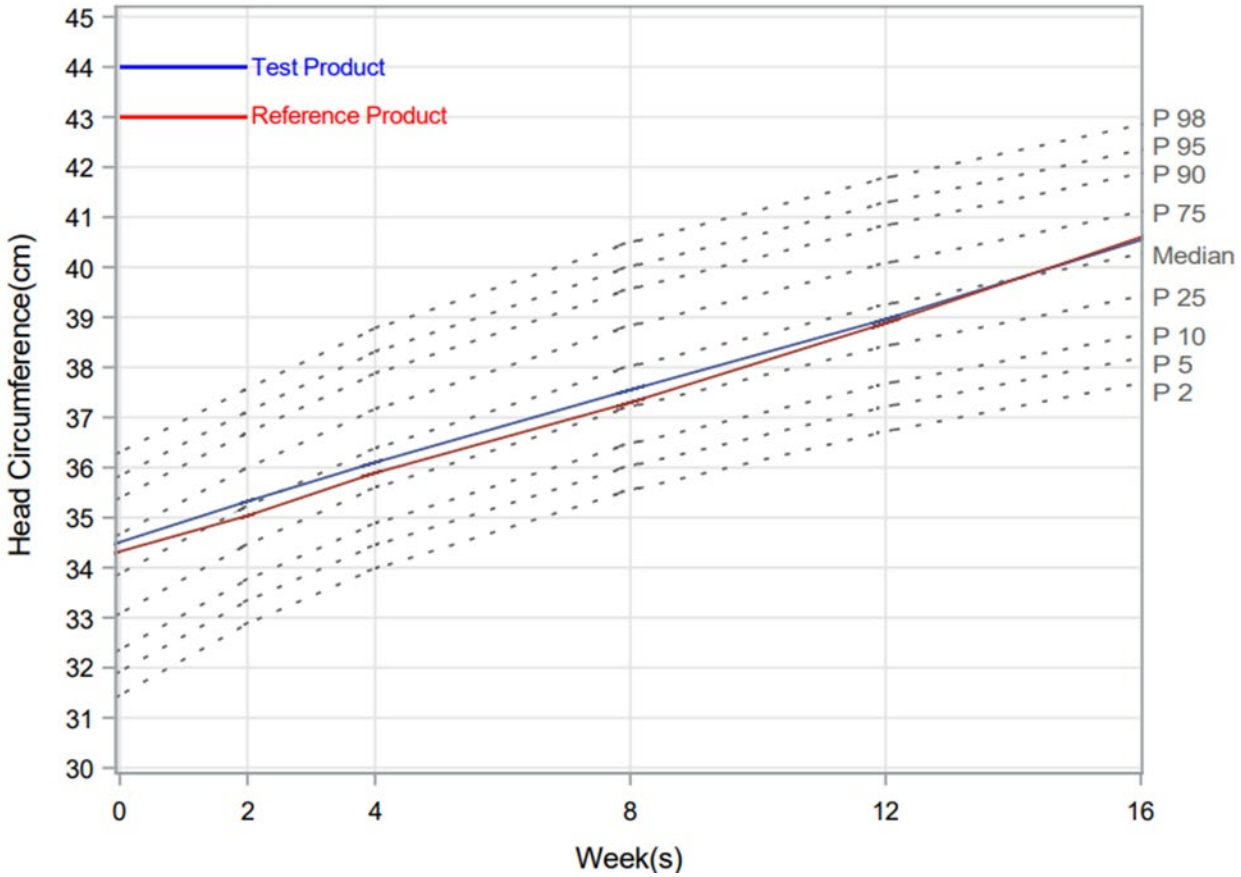

Figure 7. Female Head Circumference for Age

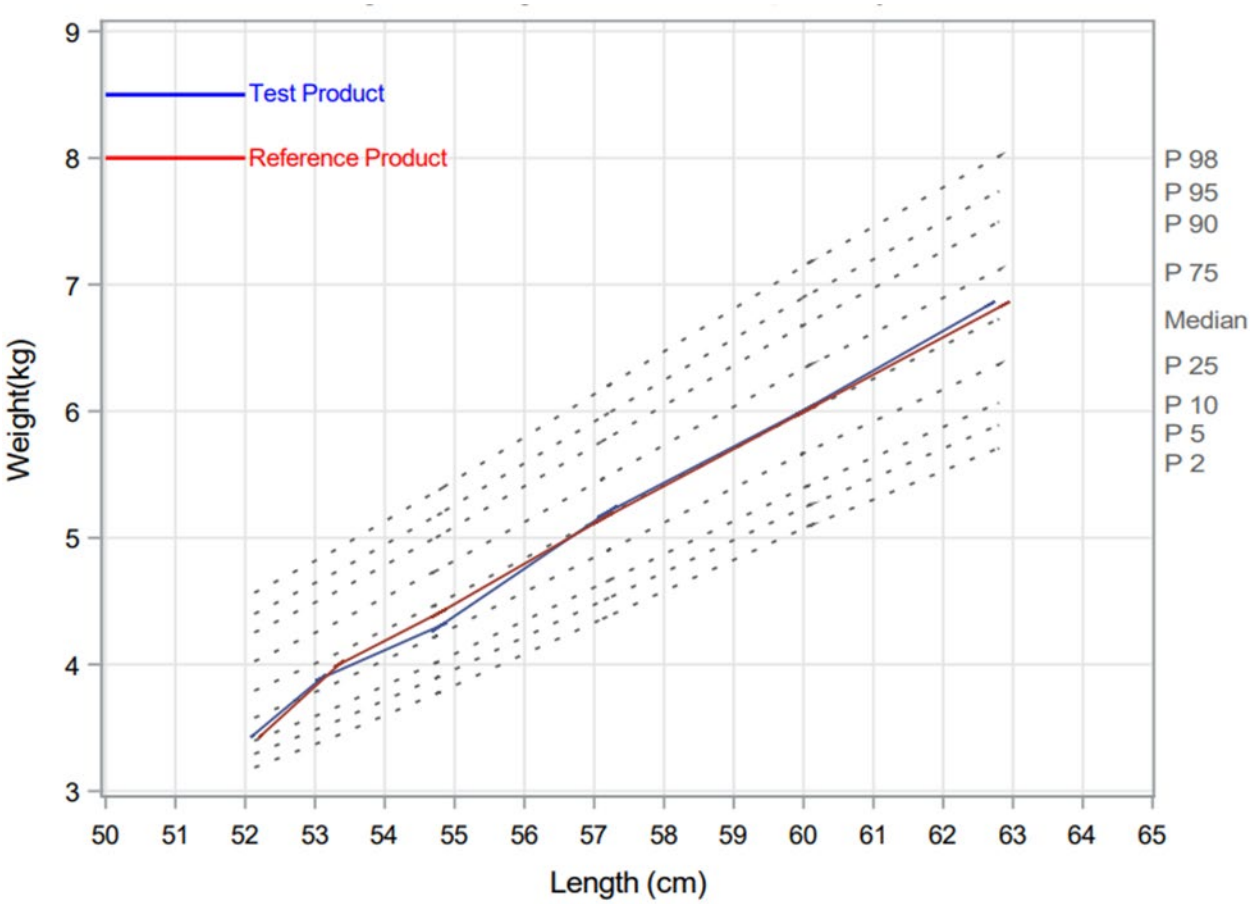

Figure 8. Male Weight for Length

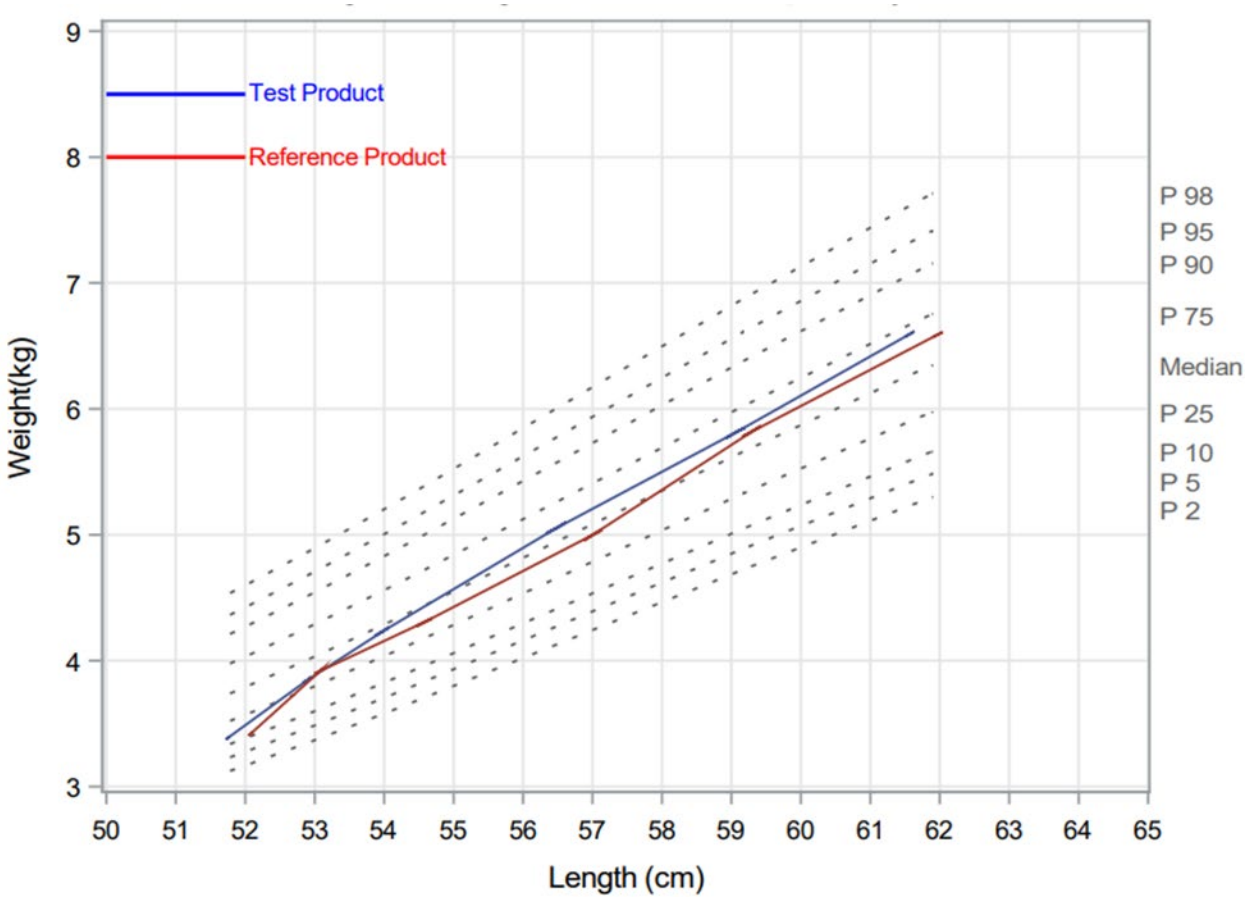

Figure 9. Female Weight for Length

K. 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:

> 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 Notices, 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. The safety standard requires 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.

L. SAFETY ASSESSMENT

The substance that is the subject of this GRAS Determination is WBM, which is intended to be used as an ingredient in milk-based, non-exempt infant formula suitable as the sole source of nutrition from the first day of life for healthy term infants at a maximum use level of 153 g/100 g infant formula,

WBM contributes 21% of the total fat in infant formula. The intended use of WBM in infant formula will provide an estimated 10.3 % of total energy from milk fat given that 49% of

total energy in the formula is provided by fat and 21% of fat in the formula is provided by WBM. Thus, the amount of milk fat provided by the intended use of WBM is within the level of milk fat identified as well absorbed and therefore does not present a safety concern.

M. SAFETY CONCLUSION

The use of dry whole milk was previously concluded to be safe for the intended use as an ingredient at a level of 16 g per 100 g infant formula powder (GRN 000980) and at a maximum level of 22 g per 100 g infant formula powder (GRN 001041). The intended use of WBM that is the subject of this notice is 153 g WBM/100 g infant formula powder. At a moisture value for WBM of approximately 87%, the average solids value is approximately 13 g/100 g WBM. Therefore, the maximum solid whole milk inclusion level in the infant formula powder is 19.9 g/100 g infant formula powder and is comparable to the intended use levels determined GRAS in GRN 000980 and GRN 001041.

The WBM is prepared from Grade "A" raw milk meeting specifications that ensure its safety as a food ingredient in the diet of infants. Based on the typical concentration of macronutrients in WBM, the intended use of WBM in the infant formula, and the total nutrient profile of the infant formula, the WBM ingredient will contribute a portion of the formula's total protein (55%), total fat (21%), and total carbohydrate (12.7%). Milk and milk products have a long history of use in the U.S. food supply, including consumption by infants and toddlers in the transition from a diet of exclusive human milk and/or formula, to foods. Milk products that have been consumed with no adverse effects attributable to the milk other than the well documented occurrence of allergic reactions in susceptible individuals (Abrams and Sicherer, 2021).

Because WBM is subjected to the same processing conditions as dry whole milk, with the exception of evaporation and drying comparable changes in key physico-chemical properties arising from processing are expected; these changes were discussed and have no effect on the safety profile of the various forms of milk. The use and use level of WBM is not fundamentally different from the current use of dry whole milk in infant formula. These ingredients are regarded as safe and GRAS.

Published clinical studies of infants consuming whole milk support the safety of whole milk as a component of the diet (e.g., Alarcon et al., 1991; Brown et al., 1991; Hjelt et al., 1989; Isoulauri et al., 1986; Larnkjær et al., 2009; Stekel et al., 1988). Potential concerns with the consumption of fluid whole milk as a sole source of nutrition (e.g., potential nutrient deficiency, potential renal solute load; fat absorption) have been raised in the literature. However, the intended use of WBM is as an ingredient in infant formula (a complex food matrix), and

therefore provides only a portion of nutrients in the total formula. Thus, the intended use of WBM does not present the same concerns as the direct consumption of fluid milk.

The use of WBM in infant formula will provide a source of constituents typically present in lower concentrations in formula, namely phospholipids and other lipids present in milk fat and not present in vegetable oils. The level of these components provided by the intended use of WBM will result in levels similar to or well below mean concentrations reported in human milk as shown in Table 13 and Table 15, and thus are not a safety concern. Published and unpublished clinical studies in which dairy fat accounts for up to approximately half the fat in infant formula (Breij et al., 2019; De Souza et al., 2018; Leite et al., 2013; Manios et al., 2020; Schouten, 2013, as cited in GRN 000898 and GRN 001041) provide supportive evidence that the level of milk fat provided by the intended use of dry whole milk does not present safety concerns.

N. 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, and that there is a reasonable certainty that a substance is not harmful under the intended conditions of use in foods. The aforementioned regulatory, scientific reviews, and compositional data related to the consumption and safety of WBM have been published in the scientific literature and, therefore, are generally available and generally known among the community of qualified food ingredient safety experts. Thus, there is broad-based and widely disseminated knowledge concerning WBM. The data and publicly available information supporting the safety of the proposed use of dry whole milk, for the intended use in infant formula, are not only widely known and disseminated, but are also commonly accepted among qualified food safety experts. Crossway Foods, Ltd. has determined that the proposed use of WBM at a maximum concentration of 153 g WBM/100 g infant formula powder can be concluded to be safe and GRAS through scientific procedures.

O. DISCUSSION OF INFORMATION INCONSISTENT WITH GRAS DETERMINATION

No information has been identified that would be inconsistent with a finding that the proposed use of dry whole milk in non-exempt infant formula, meeting appropriate specifications specified herein and used according to cGMP, is safe and GRAS based on scientific procedures, under the conditions of intended use in food.

VII. REFERENCES

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(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

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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 Mairead Ni Chuinn	Position or Title Director		
	Organization *(if applicable)* Crossway Foods, Ltd.			
	Mailing Address *(number and street)* Unit 2017 Orchard Avenue Citywest Business Campus			
City Dublin	State or Province Dublin	Zip Code/Postal Code D24 AXR0	Country Ireland	
Telephone Number +353866097015	Fax Number	E-Mail Address nichuinn.mairead@gmail.com		
1b. Agent or Attorney *(if applicable)*	Name of Contact Person Dietrich Conze	Position or Title Managing Partner		
	Organization *(if applicable)* Spherix Consulting Group, Inc.			
	Mailing Address *(number and street)* 751 Rockville Pike Unit 30-B			
City Rockville	State or Province Maryland	Zip Code/Postal Code 20852	Country United States of America	
Telephone Number 240-367-6089	Fax Number	E-Mail Address dconze@spherixgroup.com		

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Pasteurized Liquid Whole Bovine Milk (WBM)

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

_____

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 001041

☐ 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.

Pasteurized Liquid Whole Bovine Milk is intended to be used as an ingredient in non-exempt, cow's milk-based infant formula at the maximum level of 153 g WBM/100 g infant formula powder.

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 Crossway Foods, Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Pasteurized Liquid Whole Bovine Milk (WBM)
(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. Crossway Foods, Ltd. 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)

Unit 2017 Orchard Avenue, Citywest Business Campus, Dublin, Ireland D24 AXR0
(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)
Dietrich B. Conze, PhD Digitally signed by Dietrich B. Conze, PhD Date: 2024.03.27 15:31:14 -04'00'	Dietrich Conze, Managing Partner, Spherix Consulting Grou	03/27/2024

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)
	Form3667.pdf	Administrative
	COSM_3667_18933_CrosswayFoodsLtd.pdf	Administrative
	CrosswayFoodsLiquidWholeMilkGRASNotice3-26-24-Signed.pdf	Administrative
	AAP1976.pdf	Administrative
	AbramsandSicherer2021.pdf	Administrative
	Alarcon1991.pdf	Administrative
	Baeketal.2021.pdf	Administrative
	Breij2019.pdf	Administrative
	Broersen2020.pdf	Administrative

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	Brown1991.pdf	Administrative
	Burd2019.pdf	Administrative
	Chardigny1995.pdf	Administrative
	Chauvet2023.pdf	Administrative
	Claumarchirant2015.pdf	Administrative
	CommissionRegulationEUNo605-2010.pdf	Administrative
	Corkins2016.pdf	Administrative
	deSouza2017.pdf	Administrative
	Ding2023.pdf	Administrative

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Duanetal.2021.pdf	Administrative
	FAO2013.pdf	Administrative
	Fomon1981.pdf	Administrative
	Fomon1993pp104-111.pdf	Administrative
	Fomon2001.pdf	Administrative
	Gallier2020.pdf	Administrative
	George2023.pdf	Administrative
	Giuffridaetal.2013.pdf	Administrative
	Glew2011.pdf	Administrative

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Hageman2019a-Freefattyacid.pdf	Administrative
	Hageman2019b-Correctionfreefattyacid.pdf	Administrative
	Hageman2019c-Comparison.pdf	Administrative
	Hjelt1989.pdf	Administrative
	Houghton2011.pdf	Administrative
	IngvordsenLindahletal.2019.pdf	Administrative
	Innis2019.pdf	Administrative
	IOM2004.pdf	Administrative
	IOM2005.pdf	Administrative

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Ismail2017.pdf	Administrative
	Isolauri1986.pdf	Administrative
	JensenandJensen1992.pdf	Administrative
	Jiang2022-Development.pdf	Administrative
	Jiang2000.pdf	Administrative
	Kien1996.pdf	Administrative
	Koletzko2016.pdf	Administrative
	Larnkjær2009.pdf	Administrative
	Leite2013.pdf	Administrative

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Liu2016.pdf	Administrative
	Liu2021-Simulated.pdf	Administrative
	Liu2023-Comparison.pdf	Administrative
	LSRO1998.pdf	Administrative
	Ma2017.pdf	Administrative
	Manios2020.pdf	Administrative
	Martin2016.pdf	Administrative
	Martysiak-Żurowska2018.pdf	Administrative
	McGuire1997.pdf	Administrative

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Mosley2005.pdf	Administrative
	Mueller2010.pdf	Administrative
	Perusko2018.pdf	Administrative
	Phosanam2021.pdf	Administrative
	Prentice2019.pdf	Administrative
	Ratnayake2014.pdf	Administrative
	Rodríguez-Alcalá2019.pdf	Administrative
	Rossen2016.pdf	Administrative
	Rudloff1992.pdf	Administrative

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	Santillo2018.pdf	Administrative
	Sarwar1989.pdf	Administrative
	Soga2015.pdf	Administrative
	Song2021.pdf	Administrative
	Stekel1988.pdf	Administrative
	Sun2016.pdf	Administrative
	Svahn2000.pdf	Administrative
	vanderGaag2014.pdf	Administrative
	vanderGaag2017.pdf	Administrative

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	vanderGaag2020.pdf	Administrative
	vanLieshout2020.pdf	Administrative
	Vanderhout2016a.pdf	Administrative
	Vanderhout2016b.pdf	Administrative
	Wada2014.pdf	Administrative
	Wan2010.pdf	Administrative
	Wang2023.pdf	Administrative
	Wei2019.pdf	Administrative
	Wei2021.pdf	Administrative

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	Wei2022.pdf	Administrative
	Wilson1974.pdf	Administrative
	Wong2019.pdf	Administrative
	Woodruff1972.pdf	Administrative
	Yang2022.pdf	Administrative
	Yuhas2006.pdf	Administrative
	Zhang2022.pdf	Administrative
	Ziegler1989.pdf	Administrative
	Ziegler1990.pdf	Administrative

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Ziegler1999.pdf	Administrative
	Ziegler2011.pdf	Administrative

CROSSWAY FOODS LIMITED

Unit 2017 Orchard Avenue, Citywest Business Centre,
Dublin D24 AXRO, Ireland

August 9, 2024

Dear Dr. Morissette,

FDA's questions for GRN 001179 are in italicized text and Crossway Foods, Ltd. (Crossway's) responses to each question are below in plain text.

We hope that our responses address your questions.

Sincerely,

Mairead Ni Chuinn
Director, Crossway Foots, Ltd.

Regulatory:

1) *"On p. 23, the notice references 21 CFR 106.960, which does not exist. Please clarify which regulation was intended here."*

"Quality factor requirements for infant formula (21 CFR §106.960)" should read "Quality factor requirements for infant formula (21 CFR §106.96)"

The additional 0 was a typographical error.

2) *"On p. 60 at the end of the last paragraph of section M. Safety Conclusion, the notice states: Published and unpublished clinical studies in which dairy fat accounts for up to approximately half the fat in infant formula (Breij et al., 2019; De Souza et al., 2018; Leite et al., 2013; Manios et al., 2020; Schouten, 2013, as cited in GRN 000898 and GRN 001041) provide supportive evidence that the level of milk fat provided by the intended use of* ***dry whole milk*** *does not present safety concerns. Please clarify if liquid whole cow milk is intended in this sentence instead."*

Liquid Whole Milk is intended for this sentence instead of dry whole milk.

3) *"…In the first paragraph of section N. Conclusion Regarding Safety and General Recognition of Safety, the notice states: The data and publicly available information supporting the safety of the proposed use of dry whole milk, for the intended use in infant formula, are not only widely known and disseminated, but are also commonly accepted among qualified food safety experts. Please clarify if liquid whole cow milk is intended in this sentence instead."*

Liquid Whole Milk is intended for this sentence instead of dry whole milk.

Chemistry:

4) *"Crossway provides the results from three non-consecutive batch analyses of liquid whole cow milk to describe the composition of proximates, fatty acids, amino acids, and various nutrients (Tables 1-4, pp. 4-6), as well as to describe measured levels or absence of heavy metals (Table 5, p. 6), aflatoxin, and microorganisms (Tables 7 and 8, p. 11). In addition to the specifications listed in Table 6 (p. 9) for raw milk, Crossway describes a variety of analyses that are conducted on raw milk from new suppliers and analyses conducted on a yearly basis for all raw milk, which includes tests for composition and contaminants, such as heavy metals (p. 7).*

Please elaborate on the testing parameters indicated for new suppliers and parameters for the yearly testing of raw milk that are used. For example, how many batches are tested, what are the methods used and are they validated for the intended purpose, and what are the acceptable limits for the parameters tested? Crossway confirms that the results from the batch analyses meet the specifications for heavy

metals (on a dry matter basis) described in GRNs 000980 and 001041. For ingredients intended for use in infant formula, we request that manufacturers establish specification limits for potential contaminants in the subject of the notice, such as lead, arsenic, cadmium, mercury, and microorganisms, such as Cronobacter sakazakii, Listeria monocytogenes, and Salmonella spp., to ensure that the ingredient is manufactured consistently and is safe under the conditions of its intended use."

Due to the storage requirements of not more than 72 hr at not more than ≤7.2°C, the heavy metals and microbes noted in your question cannot be tested on each incoming batch of raw milk. The time needed for complete heavy metal and microbiological results are summarized below:

- Heavy metals: 5 working day turnaround. Accounting for necessary preparation, transit and reporting turnaround, this increases to 7 working days.
- Microbiological: For the parameters listed on our testing protocol, microbiological testing is 6 working days turnaround.

Below we have outlined the heavy metal and microbiological controls tested on the ingredient itself (where direct ingredient testing is possible given the above storage restrictions) or controlled for through the production process the ingredient undergoes (where direct ingredient testing is possible given the above storage restrictions), to ensure the ingredient is manufactured consistently and safe under the conditions of its intended use.

<u>Heavy metal controls – raw liquid milk testing plan</u>

To minimize the levels of heavy metal in the finished product, Crossway works closely with suppliers through a supplier approval and material onboarding process to ensure that only the best possible quality raw materials with the lowest achievable levels of contaminants are used in the production of the finished infant formula.

Due to the storage requirements (per the Grade "A" Pasteurized Milk Ordinance (PMO), which outlines the sanitary standards for Grade "A" raw milk for pasteurization, ultra-pasteurization, aseptic processing and packaging, retort processed after packaging or ferment required for the production of pasteurized milk in the United States) and retrospective nature of testing for pasteurized liquid milk, lead, cadmium, arsenic and mercury are tested as routine in the first 3 batches of pasteurized liquid milk, where the raw milk is from a new supplier. Upon completion of the supplier onboarding process, the supplier then moves to a monitoring programme, whereby the milk is tested quarterly. The ingredient specification parameters, acceptance criteria, and methods used in the testing program for new suppliers, as well as the yearly testing program for raw milk (the monitoring plan), which includes lead, cadmium, arsenic, and mercury, are outlined below in Table 1.

Table 1. Crossway Foods raw milk testing plan.

Raw liquid milk monitoring specification					
Test	Method	Reference	Method description	Limits[1]	Testing frequency
Sodium	ICP-OES	ICP/003	The sample is microwave digested in acid and determined by ICP-OES. Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	30 - 57mg/100g	First 5 batches and annually
Iron	ICP-OES	ICP/003	The sample is microwave digested in acid and determined by ICP-OES. Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	≤0.75mg/100g	First 5 batches and annually
Potassium	ICP-OES	ICP/003	The sample is microwave digested in acid and determined by ICP-OES. Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	122 - 182mg/100g	First 5 batches and annually

CROSSWAY FOODS LIMITED

Unit 2017 Orchard Avenue, Citywest Business Centre,
Dublin D24 AXRO, Ireland

Test	Method	Reference	Method description	Limits[1]	Testing frequency
Chloride	ICP-OES	Q/012	Volhard Titration. Organic matter in the sample is destroyed by wet digestion with a mixture of potassium permanganate and nitric acid. This method uses a back titration with potassium thiocyanate to determine the concentration of chloride ions in a solution. In the presence of excess silver nitrate, chloride is precipitated as silver chloride. Urea is added to decompose nitrites and the excess silver nitrate is titrated with potassium thiocyanate, using ferric iron as the indicator. Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	86 - 129mg/100g	First 5 batches and annually
Iodine	ICP-MS	ICPMS/002	Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	18 - 70µg/100g	First 5 batches and all batches received Nov-April
Cholesterol	GC	T-AA08-WO3656	Lipids in sample are saponified at high temperature with ethanolic KOH solution. Unsaponifiable fraction containing cholesterol and other sterols is extracted on SPE cartridge. Sterols are derivatized to trimethylsilyl (TMS) esters and then quantified by GC-FID. Internally validated method COFRAC TESTING (scope on www.cofrac.fr) 1-7085	≤15mg/100g	First 5 batches and annually
Ash	Gravimetric	EC 152/2009		≤2%	Once per year
Vitamin A	HPLC	EN 12823-1 2014		≤100µg/100g	Once per year
Vitamin D3	HPLC	ISO 20636:2018		≤0.5µg/100g	Once per year

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Dublin D24 AXRO, Ireland

Test	Method	Reference	Method description	Limits[1]	Testing frequency
Lead	ICP-MS	ICPMS010	Microwave assisted digestion followed by ICP-MS Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	≤ 6.25µg/kg	First 3 supplier batches and annually
Cadmium	ICP-MS	ICPMS010	Microwave assisted digestion followed by ICP-MS Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	≤ 6.25µg/kg	First 3 supplier batches and annually
Nitrates	Colorimetry BS4401/7	BS 4401-7:1976		≤ 7mg/kg	Once per year
Nitrites	Colorimetry BS4401/8	BS 4401-7:1976		≤ 0.4mg/kg	Once per year
Melamine	LC-MS/MS	LA-LCMS-019-15*	Methanolic extraction / LC/MS/MS with ESI+ and ESI- (acc FDA, modified) Internally validated method DIN EN ISO/IEC 17025:2018 DAkkS D-PL-19579-02-00	<0.3ppm	Once per year
Mycotoxins - Aflatoxin M1	LC-FLD	CHROM/319*	Determination of aflatoxin M1. The sample is cleaned up by immunoaffinity. Analysis is by HPLC post-column derivatization and fluorescence detection. Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	≤ 0.025µg/kg	Once per year
Chromium	ICP-MS	ICPMS010	Microwave assisted digestion followed by ICP-MS Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	<0.3mg/kg	First 3 supplier batches and Investigatory

CROSSWAY FOODS LIMITED

Unit 2017 Orchard Avenue, Citywest Business Centre,
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Test	Method	Reference	Method description	Limits[1]	Testing frequency
Arsenic	ICP-MS	ICPMS010	Microwave assisted digestion followed by ICP-MS Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	<12.5µg/kg	First 3 supplier batches and annually
Mercury	ICP-MS	ICPMS010	Microwave assisted digestion followed by ICP-MS Internally validated method BS EN ISO/IEC 17025:2017 UKAS 0342	≤ 6.25µg/kg	First 3 supplier batches and annually
PCDDs/PCBs	GC-MS/MS	GLS DF 110:2024-07-11	Internally validated method DIN EN ISO/IEC 17025:2018 Dakks D-PL-14629-01-00	Sum of Dioxins: ≤ 2.5pg/g fat Sum of Dioxins and Dioxin like PCBs: ≤ 5.5pg/g fat Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180: ≤ 40ng/g fat	First 3 supplier batches and Investigatory
Pesticides/Biocides	Various: GC-ECD / GC-NCI-MS / GC-FPD / GC-MS / LC-MS/MS	P-14.195-5	Internally validated method DIN EN ISO/IEC 17025:2018 Dakks D-PL-14629-01-00	Not detected	First 3 supplier batches and Investigatory

[1] Results based on a liquid basis, PCDD = Polychlorinated dibenzo-p-dioxins, PCB = Polychlorinated biphenyls, ICP-OES = Inductively Coupled Plasma Optical Emission spectroscopy, ICP-MS = Inductively coupled plasma mass spectrometry, GC = Gas Chromatography, HPLC = High-performance Liquid chromatography, GC = Gas Chromatography with tandem mass spectrometry, GC-ECD = gas chromatography-electron capture detector, GC-NCI-MS = Gas chromatography negative ion chemical ionization mass spectrometry, GC-FPD = Gas chromatography-Flame Photometric Detector, LC-MS/MS = Liquid Chromatography with tandem mass spectrometry, Investigatory = Where any indication that materials may be in danger of contributing to out of specification finished product, Crossway undertakes 'ad hoc' testing aimed at worst case scenario in order to fully evaluate any issues and address with the supplier.

Some of the tests outlined in Table 1 are done in-house. Others are tested at accredited 3rd party laboratories. All tests use validated methods and are suitable for the product matrix, i.e. liquid milk.

In addition to the controls on the raw material, Crossway Foods goes beyond the regulatory requirements outlined within 21 CFR 107.100 by undertaking analytical testing for lead, cadmium, arsenic and mercury on each batch of finished product manufactured for the United States. In doing so, Crossway Foods is serving to proactively test for and control the levels of heavy metals in line with FDA's Closer to Zero initiative. Crossway also routinely produces infant formulas destined for the EU market, where strict heavy metal contaminant limits on infant formula are enforced. These limits in the EU are outlined in Table 2 and are voluntarily undertaken by Crossway on each batch of finished product manufactured for the United States.

Table 2. Contaminant limits for infant formula produced with pasteurized whole liquid milk

Heavy metal specification - Finished product						
Contaminant	Basis	Sample type	Limit[2]	Unit	Test method	Method reference
Lead	As sold	Composite auto sample	0.02	mg/kg	ICP-MS	ICPMS010
Cadmium	As sold	Composite auto sample	0.01	mg/kg	ICP-MS	ICPMS010
Arsenic	As sold	Composite auto sample	0.02	mg/kg	ICP-MS	ICPMS010
Mercury	As sold	Composite auto sample	0.02	mg/kg	ICP-MS	ICPMS010

[2] Results based on a powder basis, ICP-MS = Inductively coupled plasma mass spectrometry

Microbiological controls – to address the risk of Cronobacter sakazakii, Listeria monocytogenes, and Salmonella spp. in leu of batch-by-batch testing

As noted above, the turnaround time for microbiological testing does not facilitate the direct testing of the ingredient itself. Rather, it is through the production process and validated heat treatment (including PMO-compliant heat treatment steps) that we ensure the absence of any such microorganisms of public health concern in the finished product.

The subject of the GRAS Notice is received and pasteurized on-site and stored in accordance with the conditions specified in the PMO. We confirm that this pasteurization process produces a product whose microbial content complies with the levels as detailed within Table 8 of the GRAS Notice.

The liquid milk is then stored in silos at ≤7.2°C for ≤72h after pasteurization and mixed with other ingredients according to established recipes and formulated to meet the nutritional requirements specified in 21 CFR 107.100. After storage and mixing, at least one PMO-compliant heat treatment is applied with the requisite hold time to eliminate

microorganisms of public health concern and ensure the safety of the finished product by eliminating the risks that may arise from their presence in the raw materials. The product is then evaporated and spray dried to produce a powdered intermediate. The heat treatment steps are validated annually at a minimum at each production facility.

To confirm the effectiveness of heat treatment steps, Crossway tests each batch of the powdered intermediate for the presence of Cronobacter spp. and Salmonella spp. After passing the relevant quality checks, which include the microbiological parameters outlined above, the powdered intermediate is then positively released and dry mixed with finishing components where further testing for Listeria monocytogenes, Cronobacter spp., and Salmonella spp within the finished infant formula occurs before its eventual positive release.

The microbiological testing program for the intermediate product is outlined in Table 3. The microbiological testing program for the finished product is outlined in Table 4.

Microbiological testing is conducted either in-house or at accredited 3rd party laboratories. All test methods are validated and suitable for the product matrix, i.e. powdered infant formula.

Therefore, the risks associated with the outlined microorganisms is well controlled through the heat steps throughout the production process and through analytical testing at the intermediate and final product stages.

Table 3. Crossway Foods microbiological testing of Intermediate product.

Microbiology Specification - Intermediate product		
Organism	Limit[3]	Method reference
Cronobacter spp.	Absent in 300g	ISO 22964:2017
Salmonella spp.	Absent in 250g	AFNOR SOL37/01-06/13

[3] Results based on a powder basis, ISO = International standards organisation, AFNOR = Association Française de Normalisation (English: French Standardization Association)

Table 4. Crossway Foods microbiological testing of Finished product.

Microbiology Specification - Finished product			
Organism	Limit[4]	Method reference	Regulation
Cronobacter spp.	Absent in 300g	ISO 22964:2017	21 CFR §106
Salmonella spp.	Absent in 1500 g	AFNOR SOL37/01-06/13	21 CFR §106
Listeria monocytogenes	Absent in 250g	AFNOR SOL37/02-06/13	EC 2073/2005

[4] Results based on a powder basis, ISO = International standards organisation, AFNOR = Association Française de Normalisation (English: French Standardization Association)

Taken together, our positive release process ensures that every finished product is within specification and therefore safe for its intended use prior to sale.