GRAS Notice (GRN) No. 1171 with amendments
https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory

# GRAS Determination for the Use of Non-Animal Type 21 Collagen Polypeptide in Selected General Foods and Beverages

**Prepared for:**

Geltor, Inc.
5400 Hollis Street
Emeryville, CA 94608

**Prepared by:**

Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

October 25, 2023

## TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

## LIST OF ABBREVIATIONS

AAHRPP: Association for the Accreditation of Human Research Participation Protection Programs

ACS: American Chemical Society

AE: Adverse Event

AOAC: Association of Official Analytical Collaboration International

ATCC: American Type Culture Collection

CC XXIa: Chicken Collagen Type XXIa

CDC: United States Centers for Disease Control

CFR: United States Code of Food Regulations

DNA: Deoxyribonucleic Acid

EDI: Estimated Daily Intake

FACIT: Fibril-Associated Collagen with Interrupted Triple Helices

FCC: Food Chemicals Codex

FD&C Act: Federal Food, Drug, and Cosmetic Act

FDA: United States Food and Drug Administration

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

FITS: Feeding Infants and Toddlers Study

FNDDS: Food and Nutrition Database for Dietary Studies

FOIA: Freedom of Information Act

GCP: Good Clinical Practices

GMP: Good Manufacturing Practice

GRAS: Generally Recognized As Safe

GRN: GRAS Notification Number

IOM: Institute of Medicine

IPTG: Isopropyl β-D-1-thiogalactopyranoside

LOD: Limit of Detection

MEC: Mobile Examination Center

mITT: Modified Intent-To-Treat

NA: Not Applicable

NCHS: National Center for Health Statistics

ND: Not Detected

NF: National Formulary

NHANES: National Health and Nutrition Examination Surveys

NM: Not Measured

OECD: Organization for Economic Cooperation and Development

OMA: Official Method of Analysis

PDCAAS: Protein Digestibility-Corrected Amino Acid Score

PSU: Primary Sampling Unit

RDA: Recommended Dietary Allowance

RNA: Ribonucleic Acid

RP-HPLC: Reverse Phase High Performance Liquid Chromatograph

RRT: Relative Retention Time

SWATH LC-MS: Sequential Window Acquisition of all Theoretical Mass Spectra

USP: United States Pharmacopiea

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

Geltor, Inc. is hereby submitting a GRAS notice in accordance with subpart E of part 170.

## B. NAME AND ADDRESS OF THE SPONSOR

Geltor, Inc.
5400 Hollis Street
Emeryville, CA USA

## C. CHEMICAL NAME

Non-Animal Type 21 Collagen Polypeptide produced by *Escherichia coli* K-12 fermentation

## D. COMMON OR USUAL NAME

Non-Animal Collagen

Non-Animal Collagen (Type 21)

Fermentation-Derived Collagen

Fermentation-Derived Collagen (Type 21)

Microflora-Derived Collagen

Microflora-Derived Collagen (Type 21)

## E. TRADE SECRET OR CONFIDENTIAL INFORMATION

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

## F. INTENDED USE

Geltor intends to use Non-Animal Type 21 Collagen Polypeptide as an ingredient in selected general foods and beverages (Table 1).

**Table 1. Non-Animal Type 21 Collagen Polypeptide Intended Uses and Use Levels**

| Category | Application | Protein % (w/w)* |
|---|---|---|
| Baked goods and bakery mixes | Cookies | 7 |
| Beverages and beverage bases | Flavored beverages and beverage bases**, excluding soft drinks and energy drinks | 4.5 |
| Coffee and Tea | Packaged coffee & tea beverages | 6 |
| | Instant coffee & tea | |
| | Coffee and tea pods | 2 |
| Fruit, vegetable, and grain drinks | Fruit drinks (drinks, drink bases**, juices, and smoothies) | 4.5 |
| | Vegetable drinks (drinks, drink bases**, and smoothies) | |
| | Grain drinks (drinks, drink bases**, and smoothies) | |
| Dairy products and substitutes | Probiotic and yogurt beverages | 2.5 |
| | Milk substitutes | |
| Coffee creamer | Coffee creamer (liquid and powder** forms) | 20 |
| Cereal products | Cereal bars | 12.5 |
| | Hot breakfast cereals | |
| Nutritional foods | Nutritional beverages and beverage bases** | 4.5 |
| | Nutritional bars | 25 |
| | Protein powders | 95 |
| Soups and soup mixes | Broths | 2.5 |
| Confections | Gummies | 10 |
| | Chocolate confections | |
| Meat, poultry, and fish substitutes | Meat, poultry, and fish substitutes | 12 |

*Non-Animal Type 21 Collagen Polypeptide will be used to provide the amount of protein listed on a weight/weight percent basis. The amount of Non-Animal Type 21 Collagen Polypeptide used to deliver the maximum protein % will vary according to the protein concentration in each lot of Non-Animal Type 21 Collagen Polypeptide. Non-Animal Type 21 Collagen Polypeptide contains at least 80% protein based on a nitrogen conversion factor of 5.55.
**Usage level reflective of reconstituted, ready-to-consume product.

## G. BASIS FOR GRAS DETERMINATION

This GRAS determination for Non-Animal Type 21 Collagen Polypeptide in the intended uses and uses levels specified above is based on scientific procedures as required under the Federal Food, Drug, and Cosmetic Act (FFDCA) and described in 21 CFR §170.30(b). The GRAS status of Non-Animal Type 21 Collagen Polypeptide is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food and is based on generally available and accepted information.

The use of Non-Animal Type 21 Collagen Polypeptide as an ingredient for the intended use in selected general foods and beverages has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. Non-Animal Type 21 Collagen Polypeptide is a fragment of chicken (*Gallus gallus domesticus*) collagen type XXIa (CC XXIa) manufactured by fermentation using a genetically engineered strain of *Escherichia coli* K-12.

2. Non-Animal Type 21 Collagen Polypeptide is intended to provide a source of non-animal collagen in selected general foods and beverages.

3. Non-Animal Type 21 Collagen Polypeptide lacks the post-translational modifications that impart the characteristic triple helical structure of native collagen and resistance to the digestive enzymes of the gastrointestinal tract.

4. Non-Animal Type 21 Collagen Polypeptide is manufactured according to Good Manufacturing Practices (GMPs) in FDA-registered facilities.

    a. *E. coli* K-12 and its derivatives are non-toxigenic, non-pathogenic, known as laboratory "safety-strains" because they possess smaller genomes, fewer genes, and lack the gene families that would allow them to colonize the human gastrointestinal tract and/or produce toxins. They have also been used to manufacture a variety of other food ingredients that are GRAS.

    b. All raw materials and food contact substances used in the manufacturing process are of suitable purity and quality for use in a food product, common ingredients used in the food/enzyme industry, and are either listed in 21 CFR, GRAS, or are Food Chemicals Codex (FCC), United States Pharmacopeia (USP), National Formulary (NF), or American Chemical Society (ACS) grade.

    c. None of the raw materials used during manufacture is a major allergen.

    d. The quality of each lot of Non-Animal Type 21 Collagen Polypeptide is evaluated against a set of product specifications that confirm the identity, protein, ash, moisture, heavy metal, and microbial content of the finished ingredient using compendial or validated methods that are fit-for-use to ensure that the production process is adequately controlled.

    e. Batch data for additional quality attributes, such as macronutrient content, micronutrient content, elemental content, endotoxin content, and host cell protein content further demonstrate control of the production process and quality of the finished ingredient.

5. The addition of Non-Animal Type 21 Collagen Polypeptide to selected general foods and beverages results in mean intakes of 10.31 g/day and 90$^{th}$ % intakes of 22.95 g/day for children and adults ages 2 years and up.

6. Although collagen peptides do not contain tryptophan and are low in cysteine and methionine, the average US diet contains a surplus of these amino acids that allows for the substitution of the total protein intake with collagen peptides. Iterative

PDCAAS calculations showed that a level as high as 36% of collagen peptides can be used as a protein substitute in the daily diet without lowering the overall PDCAAS score below 1.0 while ensuring indispensable amino acid requirements are met and maintenance of dietary protein quality. A comparison of the EDI for Non-Animal Type 21 Collagen Polypeptide with a calculation of the amount of collagen that would be consumed at 36% of substitution for protein demonstrates that the proposed intake in children and adults will not compromise the protein quality of the diet.

7. The safe use of Non-Animal Type 21 Collagen Polypeptide is also supported by a clinical study demonstrating its digestibility and metabolic fate as a protein, and lack of mutagenicity in an Ames assay.

8. Because Non-Animal Type 21 Collagen Polypeptide is derived using the protein sequence from CC XXIa, a component of chicken meat, and similar to bovine and fish collagen or gelatin, the likelihood of sensitizing and/or provoking an allergic response in food allergic individuals may be similar to that following the ingestion of chicken meat, bovine or fish collagen.

Therefore, Non-Animal Type 21 Collagen Polypeptide is safe and GRAS at the proposed level of addition to selected general foods and beverages. Non-Animal Type 21 Collagen Polypeptide is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

## H. PREMARKET APPROVAL

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of intended use.

## I. 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 Dietrich Conze, PhD, Managing Partner, Spherix Consulting Group Inc., at 751 Rockville Pike, Unit 30B, Rockville, MD 20852; Telephone: 240-367-6089; Email: dconze@spherixgroup.com; or be sent to FDA upon request.

## J. 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.

## K. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

To the best of our knowledge, the information contained in this GRAS notification is complete, representative and balanced. It contains both favorable and unfavorable information, known to Geltor and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

Signature of Authorized Representative of Geltor, Inc.

11/3/2023

Date

Printed Name: Nikolay Ouzounov

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

## A. CHEMICAL NAME

Non-Animal Type 21 Collagen Polypeptide produced by *Escherichia coli* K-12 fermentation

## B. OTHER NAMES

Non-Animal Collagen

Non-Animal Collagen (Type 21)

Fermentation-Derived Collagen

Fermentation-Derived Collagen (Type 21)

Microflora-Derived Collagen

Microflora-Derived Collagen (Type 21)

## C. MOLECULAR MASS

17.915 kDa

## D. DESCRIPTION OF NON-ANIMAL TYPE 21 COLLAGEN POLYPEPTIDE

Non-Animal Type 21 Collagen Polypeptide is a chicken (*Gallus gallus domesticus*) collagen type XXIa (CC XXIa) polypeptide manufactured by fermentation using a genetically engineered strain of *Escherichia coli* K-12. Native CC XXIa is a 957 amino acid long fibril-associated collagen with interrupted triple helices (FACIT) that contains Von Willebrand factor A, thrombospondin, and two triple helical domains (Fitzgerald and Bateman, 2001). It is expressed in many tissues including heart, stomach, kidney, skeletal muscle, and placenta. Although the function of CC XXIa is not known, the Von Willebrand factor A, thrombospondin and triple helical domains domain are believed to mediate protein-protein interactions, bind thrombospondin, and mediate collagen oligomerization, respectively (reviewed in Von der Mark, 2006). Non-Animal Type 21 Collagen Polypeptide contains 188 amino acids from the collagen domains found in CC XXIa (Figure 1). Additionally, because it is manufactured through microbial fermentation, Non-Animal Type 21 Collagen Polypeptide lacks the hydroxylated prolines and lysines that impart the triple helical structure that is characteristic of native collagen (Macek et al., 2019).

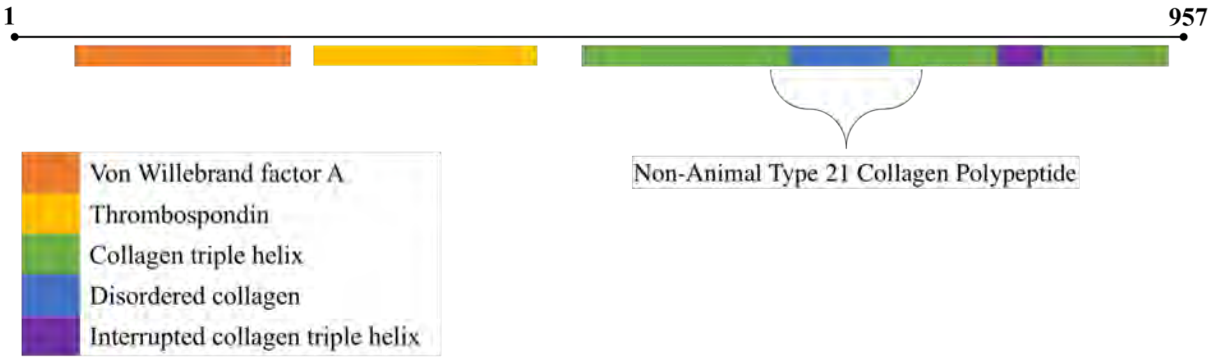



**Figure 1. Chicken Collagen XXIa with Non-Animal Type 21 Collagen Polypeptide Indicated**

To confirm the identity of the subject of this GRAS determination, Non-Animal Type 21 Collagen Polypeptide was subjected to tryptic and Asp-N peptide mapping using reverse phase ultra-performance liquid chromatography. Although several post-translational modifications (methionine oxidation, aspartic acid isomerization, and asparagine succinimide) and non-specific clips were detected, which may be methodological artifacts, the tryptic and Asp-N peptide mapping confirmed that the amino acid sequence of the Non-Animal Type 21 Collagen Polypeptide in the finished product was 100 % identical to the expected amino acid sequence.

## E. PRODUCTION PROCESS

Non-Animal Type 21 Collagen Polypeptide is produced by fermentation using a genetically engineered strain of *E. coli* K-12 that was transformed with a plasmid encoding Non-Animal Type 21 Collagen Polypeptide to create recombinant *E. coli* K-12 S9188. Following fermentation, the protein is then purified from the fermentation medium, concentrated, and spray-dried into a powder.

### 1. Description of the Production Strain

The host strain used to produce Non-Animal Type 21 Collagen Polypeptide, *E. coli* K-12 S9188, is derived from *E. coli* K-12 NCM3722, which is a prototrophic *E. coli* K-12 strain. *E. coli* K-12 NCM3722 was derived from *E. coli* K-12 NCM 196 (*ntrA7*) via mutagenesis with a frameshift mutagen and transduction to glutamine prototrophy with a P1*vir* phage grown on donor strain *E. coli* CGSC 6300 (Soupene et al., 2003). *E. coli* NCM3722 exhibits a unique phenotype that includes galactose metabolism, response to spectinomycin, and poor survivability in humans. The full genomic sequence of *E. coli* K-12 NCM3722 has been deposited in

GenBank with the accession number CP011495 and consists of a 4,678,045 base-pair chromosome and a 67,545 base pair F-like plasmid (Brown and Jun, 2015).

To develop *E. coli* K-12 S9188, five genetic modifications were made to strain *E. coli* K-12 NCM3722:

1. Deletion of a DNA recombination gene from the *E. coli* K-12 NCM3722 genome via homologous recombination to improve the genetic stability of the strain.

2. Deletion of two membrane-associated protease genes from the genome of the strain resulting from (1) via homologous recombination to improve the heterologous protein stability.

3. Insertion of the viral T7 RNA polymerase expression cassette into the genome of the strain resulting from (2) via homologous recombination to permit expression of heterologous genes under the T7 promoter through use of a chemical inducer.

4. Duplication of the native *lacI* transcriptional repressor gene on the genome of the strain resulting from (3) via homologous recombination to exert greater transcriptional repression of the viral T7 RNA polymerase gene. Following this stage, all modifications to the chromosome and removal of all plasmids was confirmed by polymerase chain reaction and DNA sequencing.

5. Transformation of the strain resulting from (4) with the multi-copy plasmid p9058, which contains DNA encoding a signal peptide sequence from *Rosenbergiella nectarea* translationally fused to the Non-Animal Type 21 Collagen Polypeptide gene (Figure 2). The signal peptide sequence is cleaved from the nascent heterologous polypeptide upon induced expression and secretion of the protein, resulting in mature Non-Animal Type 21 Collagen Polypeptide. Plasmid p9058 also contains the kanamycin resistance gene *aphIII*, which encodes aminoglycoside 3'-phosphotransferase III, and the *E. coli* K-12 NCM3722 *lacI* transcriptional repressor gene (Figure 2).

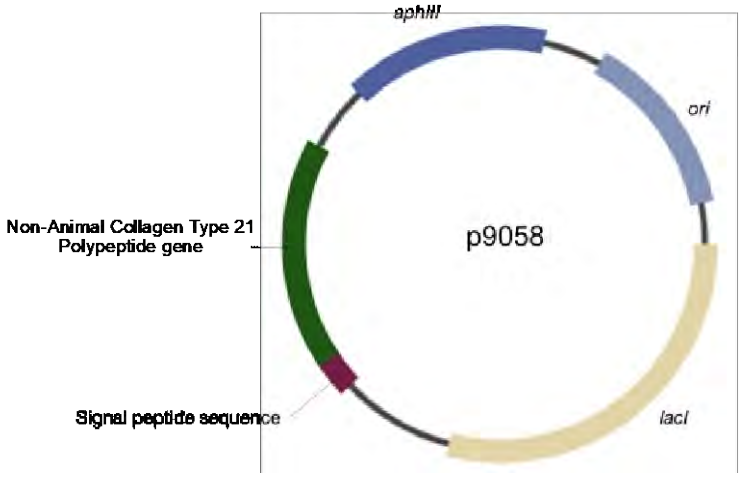



**Figure 2. Map of p9058**

## 2. Manufacturing

### *a. Quality*

The subject of this GRAS Determination is produced by fermentation using *E. coli* K-12 S9188 according to Good Manufacturing Practices (GMPs) in FDA-registered facilities. The production strain has been deposited in the American Type Culture Collection (ATCC). All potable water undergoes reverse osmosis filtration to further limit contaminants prior to being used in the manufacturing process and its quality is routinely tested. Raw materials and food contact substances used in the manufacturing process are of suitable purity and quality for use in a food product and are common ingredients used in the food/enzyme industry. Raw materials and food contact substances are listed in 21 CFR, GRAS, or are Food Chemicals Codex (FCC), United States Pharmacopeia (USP), National Formulary (NF), or American Chemical Society (ACS) grade. None of the raw materials used during manufacture are major allergens.

### *b. Generation and Maintenance of the Production Strain*

To generate *E. coli* S9188, the genetically modified *E. coli* NCM3722 was transformed with the plasmid p9058. The transformants were then selected on kanamycin-containing agar plates and selected for the presence of p9058 by kanamycin resistance. *E. coli* S9188 was subsequently expanded in culture medium to generate working stocks that frozen at -80°C for long-term storage. Kanamycin is not used during the manufacturing process for Non-Animal Type 21 Collagen Polypeptide.

### *c. Production of Non-Animal Type 21 Collagen Polypeptide*

To manufacture Non-Animal Type 21 Collagen Polypeptide, a working stock of *E. coli* K-12 S9188 is sequentially expanded in culture medium to produce the biomass required for Non-Animal Type 21 Collagen Polypeptide production (Figure 3); pH and temperature are adjusted as necessary to facilitate culture growth. During the final fermentation step, Non-Animal Type 21 Collagen Polypeptide expression is induced with the addition of isopropyl β-d-1-thiogalactopyranoside (IPTG) derived from plant-based D-galactose.

The culture is determined to be free of contaminants by microscopy and plate streaking. The rate of collagen production is monitored to ensure there is not an excessive accumulation of the production strain with inactivated expression systems. The fermentation run is ended before this population can adversely affect protein production. The biomass is then removed from the Non-Animal Type 21 Collagen Polypeptide-containing medium using centrifugation and discarded. To purify Non-Animal Type 21 Collagen Polypeptide, the supernatant undergoes microfiltration, ultrafiltration, and diafiltration. The product is then spray-dried into its final form.


**Figure 3. Production Process for Non-Animal Type 21 Collagen Polypeptide**

## F. FINISHED PRODUCT SPECIFICATIONS

To ensure that the production process is adequately controlled, the quality of each lot of Non-Animal Type 21 Collagen Polypeptide is evaluated using aggregated composite samples obtained during manufacturing against a set of product specifications that confirm the identity, protein, ash, moisture, heavy metal, and microbial content of the finished ingredient using compendial or validated methods that are fit-for-use. For protein content, a nitrogen conversion factor of 5.55 is used as it is the standard conversion factor for gelatin/collagen-containing products (Mariotti et al., 2008). Data from composite samples from three non-consecutive lots collected off the production line show that the manufacturing process reproducibly produces a finished product that complies with the product specifications (Table 2).

**Table 2. Final Product Specifications and Lot Data for Non-Animal Type 21 Collagen Polypeptide**

| Parameter | Analytical method | Specification | Lot Number[3] | | |
|---|---|---|---|---|---|
| | | | GLT402321 | GLT402921 | GLT403321 |
| ***Physical Parameters*** | | | | | |
| Appearance | Visual | White to pale yellow powder | Complies | Complies | Complies |
| ***Chemical Parameters*** | | | | | |
| Identity | RP-HPLC[1] | RRT of 0.97-1.03 | Complies | Complies | Complies |
| Protein | AOAC 2001.11; or AOAC OMA 992.15[a] | 80 % | 89.74 | 91.08 | 90.52 |
| Moisture | AOAC OMA 984.25; or AOAC OMA 925.09 | ≤ 9% | 4.6 | 5.4 | 4.5 |
| Ash | AOAC 942.05; or USP <281> | ≤ 6% | 3.73 | 3.14 | 2.96 |
| ***Heavy Metals[2]*** | | | | | |
| Arsenic | AOAC OMA 2015.01; or AAS | ≤ 0.25 ppm | 0.09 | 0.06 | 0.09 |
| Cadmium | AOAC OMA 2015.01; or AAS | ≤ 0.25 ppm | ND | ND | ND |
| Lead | AOAC OMA 2015.01; or AAS | ≤ 0.50 ppm | 0.04 | 0.04 | 0.03 |
| Mercury | AOAC OMA 2015.01; or AAS | ≤ 0.10 ppm | ND | ND | ND |
| ***Microbes*** | | | | | |
| Aerobic Plate Count | AOAC OMA 990.12; or FDA BAM | ≤ 1000 CFU/g | < 10 | < 10 | < 10 |
| Yeast and Mold | AOAC OMA 2014.05; or FDA BAM | ≤ 100 CFU/g | < 10 | < 10 | < 10 |
| *Escherichia coli* | USP <62> | Absent in 10 g | Absent | Absent | Absent |
| *Salmonella spp.* | AOAC OMA 2011.3; or USP<62> | Absent in 25 g; or absent in 10 g | Absent | Absent | Absent |

Abbreviations: RP-HPLC – reverse phase high performance liquid chromatograph; RRT – relative retention time; AOAC – Association of Official Analytical Collaboration International; OMA – Official Method of Analysis; ND – not detected; USP – United States Pharmacopeial Convention
[1]Relative to standard at 0.25 mg/mL; internally validated by Geltor, Inc.
[a]N x 5.55.
[2]Arsenic limit of detection (LOD) = 0.01 ppm; Cadmium LOD = 0.01 ppm; Mercury LOD = 0.01 ppm; Lead LOD = 0.01 ppm.
[3]All lots manufactured with *E. coli* S9188.

## G. OTHER QUALITY PARAMETERS

To document the composition of Non-Animal Type 21 Collagen Polypeptide, additional compositional analyses were conducted by Geltor (macronutrient content, micronutrient content, elemental content, endotoxin content, and host cell protein content). Some of these analyses were conducted using Non-Animal Type 21 Collagen Polypeptide that was manufactured using the production strain *E. coli* S7555, which preceded *E. coli* S9188. Except for lacking the protease ompT, which is expected to increase the level of Non-Animal Type 21 Collagen Polypeptide in the finished product, production stain *E. coli* S9188 contains the same genetic modifications and plasmid as *E. coli* S7555. Because Non-Animal Type 21 Collagen Polypeptide is manufactured using the same process despite the different strains, it is reasonable to conclude that the compositional analyses conducted with Non-Animal Type 21 Collagen Polypeptide manufactured using *E. coli* S7555 are representative to Non-Animal Type 21 Collagen Polypeptide manufactured with *E. coli* S9188.

### 1. Proximate Analysis

To confirm that the product contains predominantly protein, a proximate analysis was conducted with discrete samples obtained from the ingredient packaged in the proposed packaging system. Each lot was analyzed in triplicate to provide accurate estimates of each lot's composition and to permit an assessment of analytical method variability.

Protein accounted for greater than 90% in all lots (Table 3). The next most prevalent components were moisture and ash. Fat accounted for approximately 0.5%. Considering that coefficients of variance for the different methods, these data indicate that Non-Animal Type 21 Collagen Polypeptide contains predominantly protein, which is consistent with the product specifications.

**Table 3. Nutrient Content of Non-Animal Type 21 Collagen Polypeptide**

| Element | Method | Coefficient of Variance (%) | Lot Number[1] | | |
|---|---|---|---|---|---|
| | | | GLT402321 | GLT402921 | GLT403321 |
| Protein (%) | AOAC 2001.11 (N x 5.55) | 0.82 | 90.5 ± 0.09 | 91.2 ± 0.94 | 91.8 ± 1.21 |
| Crude Fat (%) | AOAC OMA 922.06 | 10.82 | 0.50 ± 0.05 | 0.59 ± 0.07 | 0.52 ± 0.06 |
| Moisture (%) | AOAC 984.25 | 4.81 | 3.33 ± 0.25 | 2.40 ± 0.10 | 2.13 ± 0.06 |
| Ash (%) | AOAC 942.05 | 0.72 | 3.66 ± 0.04 | 2.99 ± 0.03 | 3.66 ± 0.04 |

Abbreviations: NA – not applicable
[1]All lots manufactured with *E. coli* S9188; samples were tested in triplicate.

### 2. Residual Culture Minerals

Small amounts of residual culture minerals are expected to be present in Non-Animal Type 21 Collagen Polypeptide. Geltor quantitated the amount of the culture minerals in the finished ingredient manufactured with production strain S9188 using compendial analytical methods. Culture minerals in Non-Animal Type 21 Collagen Polypeptide were either below the limit of detection or present in very low concentrations (<60 ppm).

### 3. Endotoxin

Although the production strain is derived from *E. coli* K-12, which is non-toxigenic, Geltor quantified endotoxin concentration in three lots of the finished ingredient manufactured using production strain S9188. Endotoxin was not detected in any of the three batches of Non-Animal Type 21 Collagen Polypeptide (Table 4).

**Table 4. Endotoxin Content of Non-Animal Type 21 Collagen Polypeptide**

| Parameter[1] | Method | Lot Number[2] | | |
|---|---|---|---|---|
| | | GLT402321 | GLT402921 | GLT403121 |
| Endotoxin (EU/mg) | USP <85> | <0.500 | <0.500 | <0.500 |

Abbreviations: EU – Endotoxin Unit.
[1]USP <85> Official as of 01-May-2018; limit of quantitation (LOQ) = 0.500 EU/mg.
[2]All lots manufactured with *E. coli* S9188.

### 4. Host Cell Protein Content

To confirm that Non-Animal Type 21 Collagen Polypeptide contains only negligible amounts of proteins from the production strain, Geltor conducted sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH LC-MS/MS) on three lots of the finished ingredient manufactured using the production strain S7555.

Ninety-eight host cell proteins were identified by two or more peptides in the three lots (Table 5). Five to 34 host cell proteins were quantified above the lower limit of quantitation of 25 ng/mg, resulting in 2798 to 5239 ng protein/mg of the total protein in the sample. After normalizing for Non-Animal Type 21 Collagen Polypeptide content, the amount of host cell protein in the finished ingredient ranged from 3082 to 8195 ng/mg Non-Animal Type 21 Collagen Polypeptide. Collectively, these data indicate that the proteins derived from S7555 are approximately six orders of magnitude lower than the amount of Non-Animal Type 21 Collagen Polypeptide. Therefore, the contribution of proteins derived from the production strain to the overall protein content of Non-Animal Type 21 Collagen Polypeptide is negligible.

**Table 5. Host Cell Protein Content of Non-Animal Type 21 Collagen Polypeptide**

| Element | Method | Lot Number[1] | | |
|---|---|---|---|---|
| | | PP7-GL21-20-028-201 | PP7-GL21-20-036-201 | PP7-GL21-20-175-P0001 |
| ng Host Cell Protein/mg total sample protein | SWATH LC-MS/MS[a] | 5239 | 3355 | 2798 |
| ng HCP/mg Non-Animal Collagen Type 21 Polypeptide | | 8195 | 4855 | 3082 |
| Number of HCPs | | 85 | 70 | 35 |
| Number of HCPs (>LLOQ, 25 ng HCP/mg total sample protein) | | 29 | 19 | 5 |
| Number of HCPs (>LLOQ, 25 ng HCP/mg Non-Animal Collagen Type 21 Polypeptide) | | 34 | 27 | 6 |

Abbreviations: SWATH LC-MS - Sequential window acquisition of all theoretical fragment ion spectra mass spectrometry.
[1]All lots manufactured with *E. coli* S7555.
[a]Limit of quantitation is 25 ng/mg.

## H. STABILITY

### 1. Genetic Stability of the Production Strain

Stability of the plasmid within the genome of the production strain was confirmed without antibiotic selection for 37 generations through observation of the ongoing production of the Non-Animal Type 21 Collagen Polypeptide. As the plasmid contains a kanamycin resistance gene, plasmid stability was additionally confirmed using kanamycin selective media.

### 2. Stability of the Finished Ingredient

To support the shelf-life of the Non-Animal Type 21 Collagen Polypeptide, three lots of the finished product were stored in packaging comparable to that used for bulk storage under accelerated conditions (40°C ± 2°C, 40-65% relative humidity). Protein, moisture, and microbial content were evaluated over the course of 6 months. At all time points, all parameters conformed with the product specifications (Table 6). Based on these results, Non-Animal Type 21 Collagen Polypeptide is expected to be stable under the recommended storage conditions of room temperature and ambient humidity for 2 years from the date of manufacture. Importantly, stability studies conducted at room temperature and ambient humidity are currently underway to further support the shelf-life of Non-Animal Type 21 Collagen Polypeptide.

**Table 6. Stability of Non-Animal Type 21 Collagen Polypeptide Stored under Accelerated Conditions (40°C ± 2°C, 40-65% relative humidity)**

| Parameter | Lot Number | Specification | Time (months) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Baseline | 1 | 2 | 3 | 4 | 5 | 6 |
| Protein (%)a | 028 | >80% | 83 | 81 | 82 | 79 | 81 | 81 | 81 |
| | 036 | | 86 | 85 | 84 | 85 | 85 | 84 | 85 |
| | 175 | | 89 | 88 | 89 | 89 | 89 | 89 | 89 |
| Moisture (%) | 028 | ≤ 9% | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | 036 | | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| | 175 | | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Aerobic Plate Count (CFU/g)b | 028 | ≤ 1000 | <10 | NT | NT | <10 | NT | NT | <10 |
| | 036 | | <10 | NT | NT | <10 | NT | NT | <10 |
| | 175 | | <10 | NT | NT | 57 | NT | NT | 187 |
| Yeasts and Mold (CFU/g)b | 028 | ≤ 100 | <10 | NT | NT | <10 | NT | NT | <10 |
| | 036 | | <10 | NT | NT | <10 | NT | NT | <10 |
| | 175 | | <10 | NT | NT | <10 | NT | NT | <10 |
| Escherichia coli (CFU/g)b | 028 | Negative/g | <10 | NT | NT | <10 | NT | NT | <10 |
| | 036 | | <10 | NT | NT | <10 | NT | NT | <10 |
| | 175 | | <10 | NT | NT | <10 | NT | NT | <10 |
| Salmonella (CFU/25 g)c | 175 | ND | ND | NT | NT | ND | NT | NT | ND |
| | 028 | | ND | NT | NT | ND | NT | NT | ND |
| | 036 | | ND | NT | NT | ND | NT | NT | ND |

Abbreviations: ND – not detected; NT – not tested; CFU – colony forming units; g - gram.
[a]Calculated using AOAC 2001.11 using a nitrogen conversion factor of 5.55.
[b]Limit of Detection = 10 CFU/g.
[c]Limit of Detection = ND/25 g.

# III. DIETARY EXPOSURE

## A. INTENDED EFFECT

The intended effect is to provide a source of Non-Animal Type 21 Collagen Polypeptide in selected general foods and beverages.

## B. HISTORY OF EXPOSURE

Although native CC XXIa is expressed in many tissues of the chicken body, the subject of this GRAS Determination is a polypeptide fragment of CC XXIa and therefore has no specific recorded history of exposure.

## C. INTENDED USES

Non-Animal Type 21 Collagen Polypeptide is intended to be added to select general foods and beverages to deliver the amount of protein indicated in Table 7. The amount of Non-Animal Type 21 Collagen Polypeptide product consumed will vary depending upon the amount of protein in the lot used. Because the specifications ensure a minimum protein content of 80%, the maximum amount of Non-Animal Type 21 Collagen Polypeptide consumed will be up to 25% greater than the estimated daily intake of protein in Table 8.

**Table 7. Non-Animal Type 21 Collagen Polypeptide Intended Uses and Use Levels**

| Category | Application | Protein % (w/w)* |
|---|---|---|
| Baked goods and bakery mixes | Cookies | 7 |
| Beverages and beverage bases | Flavored beverages and beverage bases**, excluding soft drinks and energy drinks | 4.5 |
| Coffee and Tea | Packaged coffee & tea beverages | 6 |
| | Instant coffee & tea | |
| | Coffee and tea pods | 2 |
| Fruit, vegetable, and grain drinks | Fruit drinks (drinks, drink bases**, juices, and smoothies) | 4.5 |
| | Vegetable drinks (drinks, drink bases**, and smoothies) | |
| | Grain drinks (drinks, drink bases**, and smoothies) | |
| Dairy products and substitutes | Probiotic and yogurt beverages | 2.5 |
| | Milk substitutes | |
| Coffee creamer | Coffee creamer (liquid and powder* forms) | 20 |
| Cereal products | Cereal bars | 12.5 |
| | Hot breakfast cereals | |
| Nutritional foods | Nutritional beverages and beverage bases** | 4.5 |
| | Nutritional bars | 25 |
| | Protein powders | 95 |
| Soups and soup mixes | Broths | 2.5 |
| Confections | Gummies | 10 |
| | Chocolate confections | |
| Meat, poultry, and fish substitutes | Meat, poultry, and fish substitutes | 12 |

*Non-Animal Type 21 Collagen Polypeptide will be used to provide the amount of protein listed on a weight/weight percent basis. The amount of Non-Animal Type 21 Collagen Polypeptide used to deliver the maximum protein % will vary according to the protein concentration in each lot of Non-Animal Type 21 Collagen Polypeptide. Non-Animal Type 21 Collagen Polypeptide contains at least 80% protein based on a nitrogen conversion factor of 5.55.
**Usage level reflective of reconstituted, ready-to-consume product.

## D. ESTIMATED DAILY INTAKE

### 1. Estimated Daily Protein Intake of Non-Animal Type 21 Collagen Polypeptide

Estimates for the protein intakes of Non-Animal Type 21 Collagen Polypeptide were based on the food uses and maximum protein in Table 6 in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2017-2018 National Health and Nutrition Examination Surveys (NHANES) (Centers for Disease Control, 2018; United States Department of Agriculture and Agricultural Research Service, 2020a). A total of 570 food codes representative of each proposed uses were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS) for the corresponding biennial NHANES survey (United States Department of Agriculture, Department of Agriculture, Ag Data Commons (2020b). Calculations from NHANES for the mean and 90th percentile intakes were performed for representative food uses of Non-Animal Type 21 Collagen Polypeptide as a source of protein.

## 2. Food Consumption Survey Data

### *a. Survey Description*

The most recent National Health and Nutrition Examination Surveys (NHANES) for the years 2017-2018 are available for public use (United States Department of Agriculture, and Agricultural Research Service, 2020a. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. In each cycle, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Hayes and Kruger, 2014). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2017-2017 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provides the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected via stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES survey data were collected from individuals and households via 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented, and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For example, in the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9,754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2. The release data do not necessarily include all the questions asked in a section. Data items may have been removed due to confidentiality, quality, or other considerations. For this reason, it is possible that a dataset does not completely match all the questions asked in a questionnaire section. Each data file has been edited to include only those sample persons eligible for that particular section or component, so the numbers vary.

In addition to collecting information on the types and quantities of foods being consumed, the NHANES surveys collect socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population.

Sample weights are incorporated with NHANES surveys to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (Centers for Disease Control, 2006; United States Department of Agriculture, Department of Agriculture, Ag Data Commons, 2020).

*b. Statistical Methods*

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated in Octave and used to generate estimates for the intakes of protein from Non-Animal Type 21 Collagen Polypeptide by the U.S. population. Estimates for the daily intakes of protein from Non-Animal Type 21 Collagen Polypeptide represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES data; these average amounts comprised the distribution from which the mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating sample weights in order to provide representative intakes for the entire U.S. population. “All-user” intake refers to the estimated intake of Non-Animal Type 21 Collagen protein by those individuals consuming food products containing Non-Animal Type 21 Collagen Polypeptide. Individuals were considered users if they consumed one or more food products containing Non-Animal Type 21 Collagen Polypeptide on either Day 1 or Day 2 of the survey.

**3. Food Usage**

In summary, 62.07% of the total U.S. population 2+ years of age were identified as consumers of Non-Animal Type 21 Collagen Polypeptide as a source of protein from the intended food uses in the 2017-2018 NHANES (Table 8). The amount of protein consumed from Non-Animal Type 21 Collagen Polypeptide exposure was calculated because the intended use of Non-Animal Type 21 Collagen Polypeptide is to deliver protein. The mean intakes of protein from Non-Animal Type 21 Collagen Polypeptide from consumers ages 2+ from the selected food uses were estimated to be 10.31 g protein/person/day or 0.15 g protein/kg body weight/day. The heavy consumer ($90^{th}$ percentile) intakes were estimated to be 22.95 g protein/person/day or 0.33 g/kg body weight/day.

**Table 8. Estimated "All-user" Daily Intake (EDI) of Protein from Non-Animal Type 21 Collagen Polypeptide in Targeted Foods by Population Group (2017-2018 NHANES Data)**

| Age Cohort | N users | N population | % Users | Mean mass (kg) | Mean EDI (g/day) | 90th % EDI (g/day) | Mean EDI (g/kg/day) | 90th % EDI (g/kg/day) |
|---|---|---|---|---|---|---|---|---|
| ages 1-2 | 320 | 642 | 49.84 | 13.24 | 4.89 | 11.16 | 0.37 | 0.84 |
| ages 3-5 | 271 | 529 | 51.23 | 21.08 | 6.04 | 11.16 | 0.29 | 0.53 |
| ages 6-12 | 702 | 1258 | 55.80 | 41.89 | 8.17 | 18.82 | 0.20 | 0.45 |
| ages 13-19 | 508 | 886 | 57.34 | 70.32 | 13.11 | 29.73 | 0.19 | 0.42 |
| ages 20 and up | 3750 | 5493 | 68.27 | 81.70 | 10.63 | 23.16 | 0.13 | 0.28 |
| ages 2 and up | 5231 | 8427 | 62.07 | 69.41 | 10.31 | 22.95 | 0.15 | 0.33 |

# IV. SELF-LIMITING LEVELS OF USE

This part does not apply.

# V. COMMON USE IN FOOD BEFORE 1958

While it is obvious that CC XXIa has been present in human food for centuries, the comparable Non-Animal Type 21 Collagen Polypeptide produced from *E. coli* K-12 was not present in the food supply prior to 1958, and a GRAS determination based upon common use in food before 1958 does not apply.

# VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

The basis for GRAS includes documentation of the safety of the production strain, control of the production process, nutrient composition, digestibility, metabolic fate, lack of mutagenicity and a comparable likelihood of sensitization and/or provoking an allergic response in food allergic individuals to that posed by ingestion of chicken meat, bovine or fish collagen. Protein ingredients from a wide variety of sources including plant, dairy, animal, fungal, and yeast sources have also been the subject of GRAS notifications to FDA for use in select foods. Many of these protein ingredients are provided as an isolate or concentrate. Canola protein (GRN 000683; GRN 000386; GRN 000327), pea protein (GRN 000788; GRN 000608; GRN 000581), hemp seed protein (GRN 000771), mung bean protein (GRN 000684), oat protein (GRN 000575), potato protein (GRN 000086; GRN 000447), soy protein (GRN 000134), and wheat protein (GRN 000026) all received "no questions" responses from FDA.

Because Non-Animal Type 21 Collagen Polypeptide is derived from a collagen source endogenous to chicken to which humans are already exposed, and a clinical study shows that it is absorbed and metabolized by humans similar to other proteins, there is no need for toxicology testing. Additionally, the intended ingestion level has been demonstrated to be consistent with the intakes of other alternative protein sources and will not compromise dietary intake of high quality protein in children or adults. Therefore, based on the totality of publicly available evidence, Geltor concludes that Non-Animal Type 21 Collagen Polypeptide is GRAS per the intended uses.

## A. SAFETY OF THE PRODUCTION STRAIN

The production strain used to manufacture Non-Animal Type 21 Collagen Polypeptide, *E. coli* S9188, is a genetically engineered prototrophic *E. coli* K-12 strain. It is derived from *E. coli* NCM3722, which was derived from *E. coli* NCM 196 (*ntrA7*) via mutagenesis with a frameshift mutagen and transduction to glutamine prototrophy with a P1*vir* phage grown on donor strain *E. coli* CGSC 6300 (Soupene et al., 2003). *E. coli* K-12 and its derivatives are non-toxigenic, non-pathogenic, and known as laboratory "safety-strains" because they possess smaller genomes, fewer genes, and lack the gene families that would allow them to colonize the human gastrointestinal tract and/or produce toxins (Direct Food Substances Affirmed as Generally Recognized as Safe: Chymosin Enzyme Preparation Derived from *Escherichia Coli* K-12, 55 Federal Register (FR) 10932 (March 23, 1990); Environmental Protection Agency, 1997; Manning et al., 1977; Smith, 1978; Lukjancenko et al., 2010; Lyons et al., 2011; Brown and Jun, 2015). They are also widely used in industry in the manufacture of food ingredients, including food ingredients that have been determined GRAS (GRN 000484; GRN 000650; GRN

000659; GRN 000815; GRN 000880; GRN 000881). Additionally, except for the presence of p9058, all genomic modifications in *E. coli* S9188 were accomplished by homologous recombination and do not encode secreted proteins that are toxic. Non-Animal Type 21 Collagen Polypeptide also contains undetectable levels of DNA residues derived from the production strain. Therefore, it is reasonable to conclude that the production strain is safe for its intended use in the manufacture of Non-Animal Type 21 Collagen Polypeptide.

## B. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF NON-ANIMAL TYPE 21 COLLAGEN POLYPEPTIDE

Proteins in food exist almost exclusively as polypeptides and require extensive hydrolysis to di- and tri-peptides and amino acids prior to absorption (reviewed in Stevens, 2006). Following ingestion, the digestion of proteins begins in the stomach where hydrochloric acid and pepsins partially denature and hydrolyze the proteins into smaller polypeptides and amino acids. The polypeptides and amino acids then enter the small intestine where the polypeptides are further digested to small peptides two to eight amino acids in length and free amino acids. The free amino acids, dipeptides, and tripeptides are transported via specific transporters across the apical membranes of the epithelial cells, whereas the larger peptide fragments are further hydrolyzed by epithelial brush-boarder membrane-bound enzymes. For the majority of the di- and tripeptides transported into the enterocyte, additional cytosolic aminopeptidases complete hydrolysis generating free amino acids that then exit the basolateral membrane into the portal blood. It is noteworthy that certain dipeptides and a small fraction of (~1%) of undigested proteins can be absorbed intact and/or initiate antioxidant or immune responses within the intestinal wall or systemically or serve to modulate neural and endocrine functions.

To evaluate the digestibility of Non-Animal Type 21 Collagen Polypeptide, Geltor evaluated the in vitro protein digestibility of two batches of the finished product, two samples of commercially available gelatin, and the reference protein casein. All samples and the reference protein were digested with trypsin and chymotrypsin in a neutral buffer to simulate the physiological conditions of gastric and intestinal digestion. The undigested proteins were then removed by trichloroacetic acid precipitation, and the concentration of the remaining free amines were compared to the concentration of the remaining free amines from casein. The limiting amino acid in Non-Animal Type 21 Collagen Polypeptide and gelatin was tryptophan, which is characteristic of collagen (Paul et al., 2019); the PDCAAS scores for Non-Animal Type 21 Collagen Polypeptide and gelatin were 0.01; and the in vitro digestibility scores for Non-Animal Type 21 Collagen Polypeptide, gelatin and casein were 1.00. This indicates that although Non-Animal Type 21 Collagen Polypeptide is similar to gelatin regarding protein quality, the protein

is digested to the same degree as casein. Therefore, it is reasonable to conclude that Non-Animal Type 21 Collagen Polypeptide will be digested, absorbed, metabolized, and excreted in a manner similar to other proteins present in the diet.

## C. CLINICAL PHARMACOKINETIC STUDY OF NON-ANIMAL TYPE 21 COLLAGEN POLYPEPTIDE

To confirm that Non-Animal Type 21 Collagen Polypeptide is metabolized similarly to hydrolyzed collagen, Geltor compared the digestion and absorption of Non-Animal Type 21 Collagen Polypeptide and bovine collagen peptides in a clinical study.

### 1. Overview

Generally healthy adults (n = 48) aged 21 to 79 years, BMI 18.5 – 32.0 kg/m$^2$, underwent screening (Visit 1, Day -7) and two test visits (Visits 2 and 3, Day 0 and 1) and were enrolled in a randomized, double-blind, placebo-controlled study that evaluated the pharmacokinetics of amino acids for 24 hours after a single oral dose of either 2.5 g, 10 g, 20 g of Non-Animal Type 21 Collagen Polypeptide, 24.0 g of maltodextrin (placebo), or 20 g of bovine collagen peptides. At Visit 2, subjects consumed the study product once at t = 0 min, and blood samples (10 mL) were obtained at t = -15 min (± 10 min), 20, 40, 60, 90, 120, 190, 240 and 480 min ± 5 min post-dose for measurements of plasma amino acids. Participants returned the next day (Visit 3) for the final blood draw at 24 h ± 30 min. Subjects completed clinic visit procedures, including a review of inclusion/exclusion criteria, concomitant medication/supplement use, body weight, vital signs assessment, and last menses query (as applicable). Adverse events (AEs) were assessed, and subjects were queried about study instructions compliance.

The clinical study complied with Good Clinical Practices (GCP) as described in the ICH guidelines for GCP 1996, the US Code of Federal Regulations (CFR) (i.e., 21 CFR parts 50 and 56), and the Declaration of Helsinki. The study protocol, recruitment materials, and informed consent form were approved by Sterling IRB, which is fully accredited by the Association for the Accreditation of Human Research Participation Protection Programs (AAHRPP). All participants gave their informed consent before starting the study, which consisted of a screening visit (Visit 1; Day -7), one test visit (Visit 2; Day 0), and one follow-up visit (Visit 3; Day 1), illustrated in detail below in Figure 4.

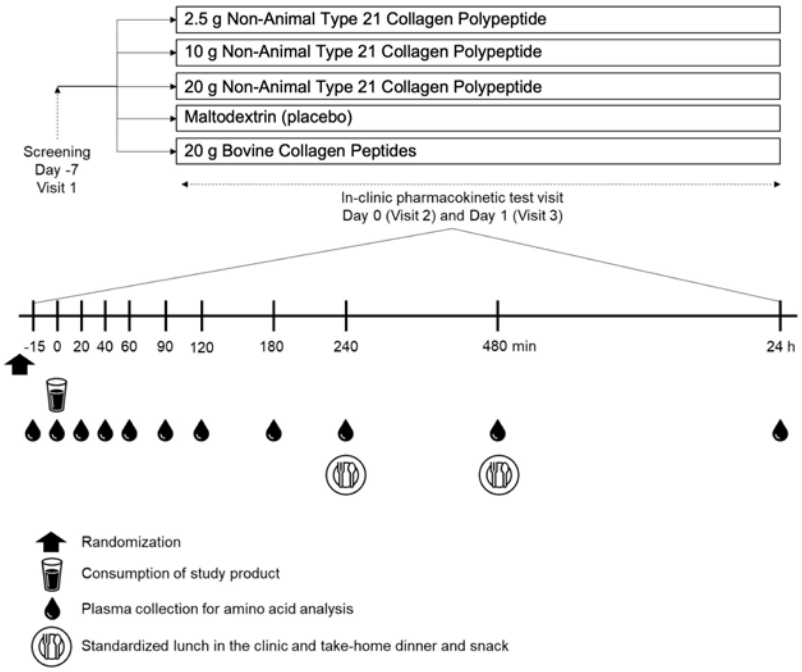



**Figure 4. Overall Study Design, Non-Animal Type 21 Collagen Polypeptide and Servings, and Schedule for Pharmacokinetics (PK) Visits**

### 2. Inclusion and Exclusion Criteria

Each subject was required to meet all of the inclusion criteria and none of the exclusion criteria in order to participate in the study.

Inclusion criteria included:

1. Healthy men and women between 21 – 70 years old, inclusive.
2. BMI between 18.5 to 32 kg/m$^2$, inclusive.
3. A score of 7 to 10 on the Vein Access Scale at Visit 1 (Day -7).
4. Willing to maintain habitual dietary, lifestyle, and physical activity (with exceptions per study instructions) during the trial and willing to adhere to dietary, lifestyle, and physical activity requirements of the study.
5. Non-user or former user of the following products: tobacco, smoking products (including, but not limited to cigarettes, cigars, chewing tobacco, e-cigarettes), and

nicotine products (e.g., nicotine gum and/or nicotine patches) within 90 days of Visit 1 (Day -7) and during the study period.

6. Non-user or former user of any marijuana or hemp products within 90 days of Visit 1 (Day -7) and during the study period. No washout is required for topical marijuana or hemp products, but subjects are required to abstain from these products during the study period.

7. Willing to refrain from exclusionary medications, supplements, and products during the study.

8. No health conditions that would prevent him/her from fulfilling the study requirements as judged by the Clinical Investigator on the basis of medical history and routine laboratory test results.

9. Understands the study procedures and signs forms providing informed consent to participate in the study and authorizes the release of relevant protected health information to the Clinical Investigator.

Exclusion criteria included:

1. Any known food allergy as well as intolerance or sensitivity to study product ingredients and all other foods/beverages provided in this study.

2. Abnormal laboratory test results of clinical significance at Visit 1 (Day -7), at the discretion of the Clinical Investigator. One re-test will be allowed on a separate day prior to Visit 2 (Day 0), for subjects with abnormal laboratory test results.

3. Uncontrolled and/or clinically important pulmonary (including uncontrolled asthma), hepatic, renal (except history of kidney stones in participants who are symptom free for 6 months), cardiac (including, but not limited to, atherosclerotic disease, history of myocardial infarction, peripheral arterial disease, stroke), endocrine (including Type 1 and Type 2 diabetes mellitus), hematologic, immunologic, neurologic (such as Alzheimer's or Parkinson's disease), psychiatric (including depression and/or anxiety disorders) or biliary condition(s). Conditions which are well-controlled or resolved will be assessed by the Clinical Investigator on a case-by-case basis.

4. Clinically important gastrointestinal condition that would potentially interfere with the evaluation of the study product (e.g., inflammatory bowel disease, irritable bowel syndrome, gastric reflux, indigestion, dyspepsia, Crohn's disease, celiac

disease, history of surgery for weight loss, gastroparesis, and clinically significant lactose or gluten intolerance or other food or ingredient allergies).

5. Uncontrolled hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) as defined by the blood pressure measured at Visit 1 (Day -7). One re-test will be allowed on a separate day prior to Visit 2 (Day 0), for subjects whose blood pressure exceeds either of these cut points at Visit 1 (Day -7), in the judgment of the Clinical Investigator. If taken, the repeat blood pressure measurement will be used to determine eligibility. Stable use of hypertension medication is allowed [defined as no change in medication regimen within 90 days of Visit 1 (Day -7)].

6. History of or current diagnosis of collagen-related diseases (including, but not limited to, osteogenesis imperfecta, chondrodysplasias, Ehlers-Danlos syndrome, Alport syndrome, Bethlem myopathy, epidermolysis bullosa, Knobloch syndrome, osteoporosis, arterial aneurysms, osteoarthrosis, and intervertebral disc disease).

7. Weight loss or gain > 4.5 kg within 90 days of Visit 1 (Day -7).

8. Currently or planning to be on a weight loss regimen or musclebuilding/ strengthening program during the study.

9. Signs or symptoms of an active infection of clinical relevance within 5 days of Visit 1 (Day -7). The visit may be rescheduled such that all signs and symptoms have resolved (at the discretion of the Clinical Investigator) at least 5 days prior to Visit 1 (Day -7).

10. Major trauma or any other surgical event within 90 days of Visit 1 (Day -7).

11. History or presence of cancer in the prior 2 years, except for non-melanoma skin cancer.

12. Use of proton pump inhibitors, H2 receptor antagonists, anticoagulants (with the exception of 81 mg aspirin), corticosteroids, antibiotics, antifungals, or antiparasitics within 90 days of Visit 1 (Day -7).

13. Regular (>3 days/wk) use of NSAIDs within 90 days of Visit 1 (Day -7).

14. Use of insulin or diabetes medication within 90 days of Visit 1 (Day -7).

15. Nutritional yeast, whey protein and protein supplements within 7 days prior to Visit 1 (Day -7).

16. Exposure to any non-registered drug product within 30 days prior to Visit 1 (Day -7).

17. Subject is a female who is pregnant, planning to be pregnant during the study period, lactating, or is of childbearing potential and is unwilling to commit to the use of a medically approved form of contraception during the study period. The method of contraception must be recorded.

18. Recent history (within 12 months of screening; Visit 1; Day -7) of alcohol or substance abuse. Alcohol abuse is defined as >14 drinks/week (1 drink = 12 oz beer, 5 oz wine, or 1ó oz distilled spirits).

19. Has a condition the Clinical Investigator believes would interfere with his/her ability to provide informed consent, comply with the study protocol, which might confound the interpretation of the study results, or put the subject at undue risk.

### 3. Study Products and Standard Meals

Subjects received one of 5 study products: 2.5 g,10.0 g, or 20 g of Non-Animal Type 21 Collagen Polypeptide (Geltor Inc.), 24 g of maltodextrin (placebo), or 20 g of bovine collagen peptides. All doses of Non-Animal Type 21 Collagen Polypeptide and bovine collagen peptides were standardized to 24 g with maltodextrin. The crude protein and amino acid composition of Non-Animal Type 21 Collagen Polypeptide and bovine collagen peptides are presented in Table 9. Both products contained a majority of the amino acids and lacked tryptophan. They also had a similar amount of crude protein, as well as a variety of amino acids, including glycine and proline, which were used as the biomarkers of Non-Animal Type 21 Collagen Polypeptide and bovine collagen peptides digestion and absorption.

During Visit 2, the study products were consumed in the clinic under study staff supervision. All subjects arrived fasted for 12 ± 2 h (water only). The powders were mixed with 8 oz of water within 24 hours of consumption and stored under refrigeration. Subjects were instructed to consume the beverages containing the study product in their entirety. Once consumed, an additional 8 oz of water was added to the empty cup. Subjects were then required to swirl the water in the cup to rinse out any residual study product and consume the 8 oz of water in its entirety. Subjects were instructed to drink 8 oz of the study product and the additional 8 oz of water within 5 min.

Subjects were provided a standard lunch immediately after the t = 240 min blood draw, which was consumed within 30 min. Subjects were instructed to eat until comfortably full. Adverse events were assessed after the final blood draw at t = 480 ± 5 min. A take-home dinner

and snack were provided with instructions to consume the dinner and snack such that the subjects could fit in a 12 ± 2 h fast (water only) before the 24 h blood draw. No other foods or beverages (except water) were allowed until the t = 24 h blood draw was completed. Subjects were provided one 16 oz bottle of water and were instructed to consume the water approximately 1 hour before arrival at the clinical for Visit 3.

**Table 9. Protein and Amino Acid Characterization of Geltor's Non-Animal Type 21 Collagen Polypeptide and Bovine Collagen Peptides**

| Parameter | Non-Animal Type 21 Collagen Polypeptide (% weight product)* | Bovine Collagen Peptides (% weight product) |
|---|---|---|
| Crude Protein | 90.52 | 92.52 |
| Alanine | 2.07 | 7.63 |
| Arginine | 5.28 | 6.31 |
| Asparagine/Aspartic acid | 4.51 | 5.12 |
| Cysteine | ND | ND |
| Glutamic acid/Glutamine | 16.70 | 8.86 |
| Glycine | 17.67 | 17.46 |
| Histidine | 0.66 | 0.48 |
| Isoleucine | 3.83 | 1.26 |
| Leucine | 4.79 | 2.55 |
| Lysine | 9.45 | 3.03 |
| Methionine | 1.21 | 0.70 |
| Phenylalanine | 2.14 | 1.57 |
| Proline | 12.22 | 10.60 |
| Sarcosine | ND | ND |
| Serine | 3.80 | 2.67 |
| Threonine | 1.85 | 1.49 |
| Tryptophan | ND | ND |
| Tyrosine | 0.90 | 0.38 |
| Valine | 0.47 | 1.85 |

Abbreviations: ND, not detected.
*Average of two measurements due to exclusion of an outlier.

### 4. Clinical Laboratory Measurements

Fasted (12 ± 2 h) blood samples were collected at Visit 1 (Day -7) to assess the following: albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, calcium, chloride, creatinine, blood urea nitrogen, potassium, sodium, total protein, carbon dioxide, osmolality, glucose, thyroid hormones, white blood cell count, red blood

cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelet count. An in-clinic urine pregnancy test was performed on all women < 60 years old.

### 5. Pharmacokinetics of Amino Acids in Plasma

Subjects arrived at the clinic fasted (12 ± 2 h; water only) on Visit 2 (Day 0). Subjects were randomized and began the test day with an intravenous catheter insertion at least 5 min before the first blood draw. Blood samples were obtained at t = -15 (± 10 min), 20, 40, 60, 90, 120, 180, 240, and 480 min ± 5 min for each measurement of plasma amino acids. Four aliquots at each time point were stored at -80°C.

### 6. Primary Endpoint

The primary endpoint was the net incremental area under the curve ($niAUC_{0\text{-}8h}$) for total plasma amino acids ($AA_T$), which was calculated by the trapezoidal method as follows:

$$niAUC_{0-8} = \frac{1}{2}\sum_{n=1}^{8}(time_n - time_{n-1}) * (AA_{T,n} + AA_{T,n-1})$$

The time before product consumption (t = -15 min) was used as time 0, and the baseline was subtracted from each post-baseline measurement. The unit of time was expressed as hours. The $AA_T$ was defined as the sum of the concentrations (μM) of the following 21 amino acids: L-proline, L-(+)-lysine, DL-5-hydroxy-DL-lysine, trans-4-hydroxy-L-proline, glycine, DL-alanine, L-valine, DL-leucine, L-isoleucine, L-phenylalanine, DL-arginine, L-histidine, L-aspartic acid, L-asparagine, D-glutamic acid, L-glutamine, DL-serine, L-(-)-threonine, DL-tyrosine, L-tryptophan, DL-methionine.

### 7. Secondary Endpoints

Secondary endpoints included the total $AUC_{0\text{-}8h}$, the maximum concentration ($C_{max}$), the time to reach maximum concentration ($T_{max}$), and the relative bioavailability of total amino acids for total plasma acids, as well as the $niAUC_{0\text{-}8h}$, total $AUC_{0\text{-}8h}$, $C_{max}$ and $T_{max}$ for L-proline, glycine and L-proline + glycine, for L-proline, glycine, and L-proline + glycine. Because their levels were similar in Non-Animal Type 21 Collagen Polypeptide and the bovine collagen peptides (< 1.5-fold difference), L-proline and glycine were considered accurate “biomarkers” for comparing the absorption and digestibility of Non-Animal Type 21 Collagen Polypeptide and the bovine collagen peptides.

Total $AUC_{0\text{-}8h}$ was calculated by the trapezoidal method as follows:

$$AUC_{0-8} = \frac{1}{2}\sum_{n=1}^{8}(time_n - time_{n-1}) * (AA_{T,n} + AA_{T,n-1})$$

The relative bioavailability of total amino acids was calculated as follows:

$$F_{rel} = {AUC_{active\ (AAT)}}/{AUC_{comparator\ (AAT)}}$$

## 8. Results

### *a. Analysis Population*

Sixty-nine subjects were screened and of these, 51 subjects were enrolled and randomized to one of the five treatment groups. All enrolled subjects fulfilled the required inclusion and exclusion criteria. The modified intent-to-treat (mITT) population included all randomized subjects that consumed study product, excluding one due to failed catheter placement (n = 50). The per protocol (PP) population consisted of 48 subjects because two subjects were excluded due to adverse events experienced at Visit 2, following consumption of 10 g of Non-Animal Type 21 Collagen Polypeptide (one subject experienced nausea, vomiting, abdominal bloating, and diarrhea which started following consumption of the study product, and one subject experienced near vasovagal syncope (lightheadedness) due to a difficult catheter insertion). Additionally, all subjects in each treatment group consumed the study products in their entirety at Visit 2.

### *b. Baseline Subject Characteristics*

The baseline characteristics of the PP population of each group are provided in Table 10 and there were no differences between the groups.

| Table 10. Baseline Subject Characteristics | | | | | | |
|---|---|---|---|---|---|---|
| **Characteristic** | | **20 g Bovine Collagen Peptides (n = 10)** | **Placebo (n = 10)** | **2.5 g Non-Animal Type 21 Collagen Polypeptide (n = 10)** | **10 g Non-Animal Type 21 Collagen Polypeptide (n = 8)** | **20 g Non-Animal Type 21 Collagen Polypeptide (n = 10)** |
| Sex | Female | 6 (60%) | 7 (70%) | 7 (70%) | 6 (75%) | 7 (70%) |
| | Male | 4 (40%) | 3 (30%) | 3 (30%) | 2 (25%) | 3 (30%) |
| BMI | Mean ± SD | 25.3 ± 3.6 | 26.7 ± 3.4 | 24.4 ± 2.9 | 24.9 ± 4.4 | 27.6 ± 3.2 |
| Age (years) | Mean ± SD | 49.3 ± 13.3 | 40.2 ± 12.9 | 51.8 ± 11.8 | 45.6 ± 13.0 | 47.0 ± 15.9 |
| Race | Asian | 1 | 1 | 0 | 0 | 0 |
| | Black/African American | 0 | 1 | 1 | 1 | 1 |
| | Multiracial | 0 | 1 | 0 | 0 | 0 |
| | White | 9 | 7 | 9 | 7 | 9 |
| Ethnicity | Hispanic/Latino | 1 | 1 | 0 | 0 | 1 |
| | Not Hispanic/Latino | 9 | 10 | 10 | 8 | 9 |
| Systolic BP (mmHg) | Mean ± SD | 118.5 ± 11.8 | 115.6 ± 5.1 | 106.9 ± 8.6 | 111.3 + 11.1 | 118.7 ± 9.4 |
| Diastolic BP (mmHg) | Mean ± SD | 73.6 ± 9.0 | 72.0 ± 7.7 | 67.4 ± 8.8 | 68.8 ± 5.7 | 73.5 ± 7.7 |
| Heart Rate (bpm) | Mean ± SD | 68.1 ± 7.5 | 67.3 ± 8.8 | 68.2 ± 13.1 | 70.8 ± 6.5 | 70.3 ± 12.6 |
| Abbreviations: BMI, body mass index; BP, blood pressure; n, sample size; SD, standard deviation | | | | | | |

*c. Total Plasma Amino Acids*

The ingestion of placebo and 2.5 g Non-Animal Type 21 Collagen Polypeptide resulted in negative niAUC values, which were likely due to the fasting state of the subjects and the inability of 2.5 g Non-Animal Type 21 Collagen Polypeptide to significantly increase the levels of the total plasma amino acids to a degree that would overcome the decreasing levels of plasma amino acids induced by the fasting state. In contrast, the ingestion of 10 g 20 g Non-Animal Type 21 Collagen Polypeptide resulted in positive niAUC values that increased dose-dependently from approximately 1391 to 4418 μmol*h/L, respectively (Table 11). The ingestion of the 20 g bovine collagen peptides also resulted in a positive niAUC value that was comparable to the niAUC value in the 20 g Non-Animal Type 21 Collagen Polypeptide-treated group. Although there were no negative total AUC values in any of the treatment groups, due to the fact that the baseline values were not subtracted from each post baseline measurement, the ingestion of increasing levels of Non-Animal Type 21 Collagen Polypeptide also led to dose-dependent increases in the total AUC and $C_{max}$ values, and $T_{max}$ values ranging between 0.6 and 1 hr. Importantly, the total plasma amino acid exposure as assessed via total AUC was comparable in the 20 g Non-Animal Type 21 Collagen Polypeptide- and 20 g bovine collagen peptides-treated groups, indicating that both products were digested and absorbed to a similar degree.

*d. Plasma Glycine, L-Proline, and Glycine + L-Proline*

The ingestion of the placebo, 2.5, 10, and 20 g Non-Animal Type 21 Collagen Polypeptide, and 20 g bovine collagen peptides had a similar effects on the niAUC, total AUC, $C_{max}$ , and $T_{max}$ values for glycine, L-proline, and glycine + L-proline compared to those observed when considering total plasma amino acids (Tables 12, 13, and 14). However, negative niAUC values for glycine, L-proline, and glycine + L-proline were only observed in the placebo group, and the ingestion of 2.5, 5, and 10 g Non-Animal Type 21 Collagen Polypeptide led in dose-dependent increases in the niAUC, total AUC, and $C_{max}$ values. The $T_{max}$ values also ranged between 0.9 and 1 hr. Additionally, although there were significant differences in the niAUC and $C_{max}$ values for proline and the $C_{max}$ value for proline + glycine, the ingestion of 20 g bovine collagen peptides resulted in comparable niAUC, total AUC, $C_{max}$, and $T_{max}$ values for glycine, total AUC and $T_{max}$ values for proline, and niAUC, total AUC, $T_{max}$ values following the ingestion of 20 g Non-Animal Type 21 Collagen Polypeptide and 20 g bovine collagen peptides.

**Table 11. Total Plasma Amino Acids**

| Characteristic | 20 g Bovine Collagen Peptides (n = 10) | Placebo (n = 10) | 2.5 g Non-Animal Type 21 Collagen Polypeptide (n = 10) | 10 g Non-Animal Type 21 Collagen Polypeptide (n = 8) | 20 g Non-Animal Type 21 Collagen Polypeptide (n = 10) |
|---|---|---|---|---|---|
| niAUC (µmol*h/L) | 4318.6 ± 1932.7 | -1677.7 ± 1612.2 | -685.9 ± 1099.8* | 1391.1 ± 1773.4*## | 4418.0 ± 2366.7## |
| Total AUC (µmol*h/L) | 28148.30 ± 3638.1 | 20217.1 ± 1737.5 | 22190.6 ± 2282.1* | 23469.2 ± 2554.5*## | 27500.8 ± 2729.0## |
| $C_{max}$ (µmol/L) | 5285.9 ± 909.6 | 2831.8 ± 291.3 | 3365.4 ± 343.1*## | 4577.2 ± 658.9*## | 5812.5 ± 532.3*## |
| $T_{max}$ (h) | 1.0 ± 0.4 | 0.2 ± 0.3 | 0.6 ± 0.2*## | 0.9 ± 0.3## | 1.0 ± 0.3## |

Abbreviations: niAUC, net incremental area under the curve; AUC, area under the curve; $C_{max}$, maximum concentration; $T_{max}$, time to reach maximum concentration
Data are presented as mean ± SD; *p ≤ 0.05 vs. comparator; ##p ≤ 0.05 vs. placebo

**Table 12. Plasma Glycine**

| Characteristic | 20 g Bovine Collagen Peptides (n = 10) | Placebo (n = 10) | 2.5 g Non-Animal Type 21 Collagen Polypeptide (n = 10) | 10 g Non-Animal Type 21 Collagen Polypeptide (n = 8) | 20 g Non-Animal Type 21 Collagen Polypeptide (n = 10) |
|---|---|---|---|---|---|
| niAUC (µmol*h/L) | 1576.3 ± 824.0 | -92.8 ± 268.9 | 325.0 ± 421.7* | 1016.8 ± 832.8## | 1877.9 ± 881.4## |
| Total AUC (µmol*h/L) | 3793.4 ± 1276.5 | 2050.1 ± 573.6 | 2872.1 ± 1416.9 | 3423.0 ± 1492.5## | 4195.6 ± 1245.8## |
| $C_{max}$ (µmol/L) | 1042.4 ± 439.8 | 354.9 ± 167.9 | 477.5 ± 198.9* | 951.6 ± 458.2## | 1275.4 ± 235.5## |
| $T_{max}$ (h) | 1.0 ± 0.4 | 2.8 ± 2.2 | 0.8 ± 0.3## | 0.9 ± 0.3## | 1.0 ± 0.3## |

Abbreviations: niAUC, net incremental area under the curve; AUC, area under the curve; $C_{max}$, maximum concentration; $T_{max}$, time to reach maximum concentration
Data are presented as mean ± SD; *p ≤ 0.05 vs. comparator; ##p ≤ 0.05 vs. placebo

**Table 13. Plasma L-Proline**

| Characteristic | 20 g Bovine Collagen Peptides (n = 10) | Placebo (n = 10) | 2.5 g Non-Animal Type 21 Collagen Polypeptide (n = 10) | 10 g Non-Animal Type 21 Collagen Polypeptide (n = 8) | 20 g Non-Animal Type 21 Collagen Polypeptide (n = 10) |
|---|---|---|---|---|---|
| niAUC (µmol*h/L) | 1285.9 ± 527.6 | -190.5 ± 231.5 | 9.4 ± 166.1* | 643.3 ± 385.8*## | 1746.2 ± 672.1*## |
| Total AUC (µmol*h/L) | 2842.3 ± 484.3 | 1368.0 ± 482.7 | 1592.9 ± 424.3* | 2014.8 ± 567.9*## | 3246.4 ± 982.2## |
| $C_{max}$ (µmol/L) | 649.0 ± 199.4 | 216.9 ± 84.6 | 281.3 ± 63.9* | 487.4 ± 144.1*## | 811.6 ± 241.9*## |
| $T_{max}$ (h) | 1.1 ± 0.3 | 1.5 ± 2.6 | 0.6 ± 0.2 | 0.9 ± 0.3 | 1.2 ± 0.3 |

Abbreviations: niAUC, net incremental area under the curve; AUC, area under the curve; $C_{max}$, maximum concentration; $T_{max}$, time to reach maximum concentration
Data are presented as mean ± SD; *p ≤ 0.05 vs. comparator; ##p ≤ 0.05 vs. placebo

**Table 14. Plasma L-Proline + Glycine**

| Characteristic | 20 g Bovine Collagen Peptide (n = 10) | Placebo (n = 10) | 2.5 g Non-Animal Type 21 Collagen Polypeptide (n = 10) | 10 g Non-Animal Type 21 Collagen Polypeptide (n = 8) | 20 g Non-Animal Type 21 Collagen Polypeptide (n = 10) |
|---|---|---|---|---|---|
| niAUC (µmol*h/L) | 2862.3 ± 1319.7 | -283.3 ± 406.9 | 334.4 ± 455.7* | 1660.1 ± 1074.5*## | 3624.1 ± 1438.2## |
| Total AUC (µmol*h/L) | 6635.8 ± 1542.6 | 3418.1 ± 804.3 | 4465.0 ± 1689.7* | 5437.8 ± 1794.0## | 7441.9 ± 2019.6## |
| $C_{max}$ (µmol/L) | 1627.8 ± 547.7 | 535.0 ± 195.9 | 733.2 ± 255.9* | 1439.0 ± 519.0*## | 2056.6 ± 399.5*## |
| $T_{max}$ (h) | 0.9 ± 0.3 | 1.7 ± 1.3 | 0.8 ± 0.3## | 0.9 ± 0.3## | 1.1 ± 0.4 |

Abbreviations: niAUC, net incremental area under the curve; AUC, area under the curve; $C_{max}$, maximum concentration; $T_{max}$, time to reach maximum concentration
Data are presented as mean ± SD; *p ≤ 0.05 vs. comparator; ##p ≤ 0.05 vs. placebo

*e. Relative Bioavailability*

To confirm that 20 g Non-Animal Type 21 Collagen Polypeptide and 20 g bovine collagen peptides are equally bioavailable, the relative bioavailability was calculated as described in Chapter VI, Section C.7. The plasma amino acid response following a single dose of 20 g Non-Animal Type 21 Collagen Polypeptide was similar to a single dose of 20 g bovine collagen peptides with a relative bioavailability of 97.7% (Table 15), demonstrating that Non-Animal Type 21 Collagen Polypeptide is digested and absorbed to a similar extent as bovine collagen peptides.

**Table 15. Relative Bioavailability**

| **20 g Non-Animal Type 21 Collagen Polypeptide** | | **20 g Bovine Collagen Peptides** | | |
|---|---|---|---|---|
| **Dose** | **AUC $_{Active\ (AAT)}$** | **Dose** | **AUC $_{Comparator\ (AAT)}$** | **Relative Bioavailability (%)** |
| 20 g | 27500.8 | 20 g | 28148.3 | 97.7 |

Abbreviations: AUC, area under the curve; $AA_T$,
Relative bioavailability of $AA_T$ was calculated by dividing the $AUC_{Active(ATT)}$ by the $AUC_{Comparator(ATT)}$.

*f. Adverse events*

As described in Chapter VI, Section C.8.a, two subjects reported five adverse events after the ingestion of 10 g of Non-Animal Type 21 Collagen Polypeptide. One subject experienced catheter insertion and placement complications that triggered a near vasovagal syncopal reaction, which was deemed to be not associated with the product. The other subject experienced four adverse events, which included moderate nausea and vomiting, mild abdominal bloating, and two moderate episodes of diarrhea. All of these effects were deemed to be possibly related to the study product. The clinic staff provided the subject with ice chips and saltines; vomiting and bowel movements helped resolve the nausea. All the adverse events in both subjects resolved, and both subjects were excluded from the study and not included in the assessment. It is important to note that nausea, vomiting, diarrhea, and abdominal cramps are possible side effects of Synthroid (https://www.rxabbvie.com/pdf/synthroid.pdf), which the subject had been taking since 1994 for hypothyroidism. Thus, although these adverse effects were deemed to be possibly related to the study product, the lack of similar adverse effects in the 2.5 and 20 g Non-Animal Type 21 Collagen Polypeptide-treated groups and the potential for Synthroid to have similar adverse side effects, suggests that the adverse events experienced by the subject may have been complicated by the subject's medical situation. Non-Animal Type 21 Collagen Polypeptide was otherwise well-tolerated in all other subjects.

### 9. Conclusions

Although negative niAUC values were obtained following the ingestion of placebo and 2.5 g Non-Animal Type 21 Collagen Polypeptide, the weight of evidence obtained from this randomized, double-blind, placebo-controlled study indicate that the ingestion of increasing amounts of Non-Animal Type 21 Collagen Polypeptide leads to dose-dependent increases in the amount total plasma amino acids, including glycine and proline. Additionally, consistent with the results of the PDCAAS and in vitro digestibility assessment by Geltor, the comparable niAUC, total AUC, $C_{max}$, and $T_{max}$ values for Non-Animal Type 21 Collagen Polypeptide and bovine collagen peptides indicate that Non-Animal Type 21 Collagen Polypeptide is digested and absorbed in a manner that is consistent with digestion and absorption of other dietary proteins, such as bovine collagen peptides.

## D. NON-ANIMAL TYPE 21 COLLAGEN POLYPEPTIDE EXPOSURE AND INTAKE OF PROTEIN

Adequate protein intake is critical to health. Protein deficiency has adverse effects on all organs including the brain, immune system, gut mucosal function and permeability and kidney function (Institute of Medicine, 2002). To protect against inadequate protein intake, IOM calculated the Recommended Dietary Allowance (RDA) for protein for various age groups. The RDA is defined as the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group and for protein is based on short-term nitrogen balance studies. In developing an RDA, an emphasis is placed on the reasons underlying the choice of the criterion of nutritional adequacy used to establish the requirement. Nitrogen balance is the difference between nitrogen intake and the amount excreted in urine, feces, skin, and miscellaneous losses. Nitrogen balance remains the only method that has generated sufficient data for the determination of the total protein (nitrogen) requirement. It is assumed that when needs are met or exceeded adults come into nitrogen balance; when intakes are inadequate, negative nitrogen balance results. The established RDAs for children and adults are shown in Table 16.

**Table 16. RDA Established by IOM for Protein**

| Age Groups (yrs) | RDA Protein – Males (g/day) | RDA Protein – Females (g/day) |
|---|---|---|
| 1-3 | 13 | 13 |
| 4-8 | 19 | 19 |
| 9-13 | 34 | 34 |
| 14-18 | 52 | 46 |
| 19 and up | 56 | 46 |

The actual intake of protein in the US by children and adults exceeds the RDAs which are established to protect against inadequate protein intake. NHANES/WWEIA 2017-2018 was used to determine the mean amount of protein consumed (data from the FITS study (Bailey et al. 2018) was used for children 1-2 years old because protein intakes were not available for children in this age group from NHANES). A comparison of the actual intakes of protein shown in Table 17 with the RDAs shown in Table 15 demonstrates that intakes are approximately 2 to 3-fold higher than the RDA for each age group. This finding is consistent with results of dietary surveys elsewhere which show that protein intake by infants during the complementary feeding period in industrialized countries is generally 2 to 3 times the RDA (Wu, 2016).

**Table 17. Mean Protein Intakes by Age Group in the US**

| Mean Protein Consumed (NHANES/WWEIA 2017-2018) Age Groups (yrs) | Mean Protein Consumed – Males (g/day) | Mean Protein Consumed – Females (g/day) |
|---|---|---|
| 1-2* | 46 | |
| 2-5 | 56.7 | 49.3 |
| 6-11 | 68.8 | 60.7 |
| 12-19 | 86.5 | 62.0 |
| 20 and up | 95.4 | 69.4 |
| 2 and up | 90.1 | 67.0 |

Based on Bailey et al. (2016)

Non-Animal Type 21 Collagen Polypeptide is intended to substitute for protein at the use levels and in the various food products listed in Chapter III. However, collagen protein lacks one indispensable amino acid (tryptophan) and is therefore categorized as an incomplete protein source. Paul et al. (2019) evaluated the maximum level of dietary collagen peptides that can be incorporated in the Western pattern diet while maintaining its indispensable amino acid balance. PDCAAS-based protein quality scores are used to adjust dietary protein intakes to meet the daily requirements of indispensable amino acids. Ideally, the amino acid scores (AAS) of a protein or protein mixture should not exceed 1.0, i.e., fulfill 100% of the indispensable amino acid requirements while minimizing excess. This is due to the fact that the body's metabolic needs include both indispensable and dispensable amino acids. As a consequence, if one or more of the indispensable amino acids are present in excess of requirements, the diet becomes limited in dispensable amino acids, thus unbalanced, even though the PDCAAS remains equal to 1.0 (Paul et al., 2019). Because collagen protein is never consumed as the sole or primary source of protein, its nutritional contribution must always be evaluated in the context of a mixed protein diet. The study by Paul et al. showed that even though collagen peptides do not contain tryptophan and are low in cysteine and methionine, the average US diet contains a surplus of these amino acids that allows for the substitution of the total protein intake with collagen peptides. Iterative PDCAAS calculations showed that a level as high as 36% of collagen peptides

can be used as a protein substitute in the daily diet without lowering the overall PDCAAS score below 1.0 while ensuring indispensable amino acid requirements are met and maintenance of dietary protein quality (Paul et al., 2019). Table 18 provides a calculation of the amount of collagen that would be consumed at 36% of substitution for protein without compromising the quality of the diet.

**Table 18. Amount of Collagen That Can Be Consumed As a Substitute for Other Proteins Without Compromising Diet Quality**

| Mean Protein Consumed (NHANES/WWEIA 2017-2018) Age Groups (yrs) | Mean Protein Consumed – Males (g/day) | Mean Protein Consumed – Females (g/day) | Collagen Amt (36% of consumed protein) Males | Collagen Amt (36% of consumed protein) Females |
|---|---|---|---|---|
| 1-2[a] | 46 | | 16.6 | |
| 2-5 | 56.7 | 49.3 | 20.4 | 17.7 |
| 6-11 | 68.8 | 60.7 | 24.8 | 21.9 |
| 12-19 | 86.5 | 62.0 | 31.1 | 22.3 |
| 20 and up | 95.4 | 69.4 | 34.3 | 25.0 |
| 2 and up | 90.1 | 67.0 | 32.4 | 24.1 |

[a]Obtained from the Feeding Infants and Toddler Study (FITS), 2016 (Bailey et al., 2018)

When comparing the values in Table 18 with the mean EDI of protein from Non-Animal Type 21 Collagen Polypeptide provided in Chapter III (reproduced here in Table 19), it can be reasonably concluded that intake is at a level that is not expected to compromise protein quality of the diet.

**Table 19. Mean Protein Consumed from Intended Uses and Use Levels of Non-Animal Type 21 Collagen Polypeptide**

| Population Group | N users | N population | % Users | Mean EDI (g/day) |
|---|---|---|---|---|
| ages 1-2 | 320 | 642 | 49.84 | 4.89 |
| ages 3-5 | 271 | 529 | 51.23 | 6.04 |
| ages 6-12 | 702 | 1258 | 55.80 | 8.17 |
| ages 13-19 | 508 | 886 | 57.34 | 13.11 |
| ages 20 + | 3750 | 5493 | 68.27 | 10.63 |
| ages 2 + | 5231 | 8427 | 62.07 | 10.31 |

The intake levels of Non-Animal Type 21 Collagen Polypeptide are also consistent with the uses of other proteins derived from sources other than meat. Previous notifications have provided estimated intakes of these protein containing ingredients that range from 6-210 g/day (Table 20).

| Table 20. GRAS Notifications of Alternative Protein Sources | | |
|---|---|---|
| **GRN #** | **Ingredient** | **Maximum Estimated Daily Intake (g/day)** |
| 000127 | *Arthrospira platensis* (Spirulina dried biomass) | 6 |
| 000394 | *Arthrospira platensis* (Spirulina dried biomass) | 15.5 |
| 000417 | *Arthrospira platensis* (Spirulina dried biomass) | 6 |
| 000447 | Potato protein isolate | 30.0 |
| 000519 | *Chlorella protothecoides* S106 flour | 5.6 |
| 000575 | Oat protein | 56 |
| 000581 | Pea protein | 10.76 |
| 000608 | Pea protein concentrate | 30.0 |
| 000609 | Rice protein concentrate | 20.5 |
| 000684 | Mung bean protein isolate | 22.5 |
| 000683 | Canola/Rapeseed protein isolate | 45 |
| 000771 | Hemp Seed protein | 13.84 |
| 000773 | *Chlamydomonas reinhardtii* (dried biomass) | 38.3 |
| 000742 | Lemnaceae protein powder | 201 |

In summary, the intake of Non-Animal Type 21 Collagen Polypeptide from the proposed uses and use levels has been shown to be at a level that is reasonably expected not to compromise total diet quality in children or adults and is consistent with intakes of other proteins from alternative sources.

## E. GENOTOXICITY

To confirm that Non-Animal Type 21 Collagen Polypeptide is not mutagenic, Geltor, Inc. evaluated the mutagenicity of Non-Animal Type 21 Collagen Polypeptide in a Good Laboratory Practice (GLP)-, OECD 471-compliant bacterial reverse mutation assay. Non-Animal Type 21 Collagen Polypeptide (Lot #GLT403621) was determined to be completely soluble up to 5 mg in sterile deionized water, which was the solvent used in the study. The mutagenicity of 0.05, 0.1, 0.5, 1.0, 5.0 mg of Non-Animal Type 21 Collagen Polypeptide was then evaluated using the tester strains *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 *uvr*A in the presence and absence of Aroclor 1254-induced rat liver S9 homogenate. The negative control was sterile deionized water and the positive controls were sodium azide for strains TA100 and TA1535, 2-nitrofluorene for strain TA98, ICR 191 acridine for TA1537, methyl methanesulfonate for *E. coli* WP2 *uvr*A and 2-aminoanthracene for all strains when in the presence of S9 homogenate. All dose levels of Non-Animal Type 21 Collagen Polypeptide, and the solvent control and positive controls were plated in triplicate.

All tester strains were sensitive to the positive control mutagens and showed the appropriate mutagenic response (i.e., positive control counts were greater than 2.5 times the negative solvent control. Additionally, the spontaneous reversion rate was well within the accepted values of each strain, indicating that under the test conditions, the strains were sensitive to the detection of potentially genotoxic agents. Non-Animal Type 21 Collagen Polypeptide was not cytotoxic up to 5.0 mg, there was no precipitation at any of the concentrations either with or without the S9 homogenate, and there was no mutagenic activity at any of the concentrations either with or without the S9 homogenate (Table 21).

**Table 21. Bacterial Reverse Mutation Assay with Non-Animal Type 21 Collagen Polypeptide**

| | Test Item | Test Concentrations (mg/plate) | Mean Colonies/Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA98 | TA100 | TA1535 | TA1537 | WP2uvrA pKM101 |
| With S9 Mix | Water | NA | 20 ± 2.08 | 107 ± 8.33 | 9 ± 1.53 | 10 ± 2.65 | 109 ± 0.58 |
| | Non-Animal Type 21 Collagen Polypeptide | 5.0 | 20 ± 1.00 | 106 ± 6.66 | 9 ± 30.58 | 10 ± 1.53 | 102 ± 1.53 |
| | | 1.0 | 21 ± 3.51 | 105 ± 4.58 | 10 ± 3.51 | 10 ± 0.58 | 108 ± 6.51 |
| | | 0.5 | 18 ± 0.58 | 107 ± 5.29 | 8 ± 1.73 | 11 ± 1.73 | 106 ± 4.04 |
| | | 0.1 | 20 ± 1.00 | 104 ± 3.79 | 9 ± 2.52 | 9 ± 1.003 | 105 ± 6.43 |
| | | 0.05 | 21 ± 2.65 | 102 ± 1.53 | 9 ± 1.53 | 9 ± 1.00 | 109 ± 3.61 |
| | 2-Aminoanthracene | NA | 1281 ± 11.37 | 1274 ± 10.00 | 545 ± 19.97 | 536 ± 11.79 | 1063 ± 32.23 |
| Without S9 Mix | Water | NA | 20 ± 2.31 | 103 ± 10.82 | 7 ± 1.15 | 10 ± 3.06 | 110 ± 6.11 |
| | Non-Animal Type 21 Collagen Polypeptide | 5.0 | 19 ± 3.61 | 100 ± 10.26 | 9 ± 1.73 | 10 ± 1.00 | 112 ± 11.24 |
| | | 1.0 | 19 ± 4.00 | 103 ± 4.73 | 9 ± 1.73 | 8 ± 1.00 | 108 ± 9.17 |
| | | 0.5 | 21 ± 4.58 | 105 ± 4.51 | 9 ± 1.00 | 10 ± 0.58 | 106 ± 4.73 |
| | | 0.1 | 21 ± 2.52 | 103 ± 7.51 | 9 ± 2.08 | 8 ± 1.53 | 105 ± 2.00 |
| | | 0.05 | 20 ± 2.00 | 100 ± 3.46 | 7 ± 1.15 | 10 ± 2.08 | 108 ± 5.86 |
| | 2-Nitrofluorene | NA | 1252 ± 21.55 | - | - | - | - |
| | ICR 191 Acridine | NA | - | - | - | 536 ± 13.58 | - |
| | Sodium Azide | NA | - | 1237 ± 15.72 | 541 ± 28.50 | - | - |
| | Methyl methanesulfonate | NA | - | - | - | - | 1040 ± 12.66 |

NA- not applicable; "-" denotes not tested
Values are mean +/- standard deviation.

## F. ALLERGENICITY

Allergens, by definition, are antigens that are recognized by IgE antibodies and provoke IgE-mediated hypersensitivity responses (Aalberse, 2000). The Food Allergen Labelling and Consumer Protection Act of 2004 identifies eight foods as major food allergens: milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, soybeans, and sesame. Although Non-Animal Type 21 Collagen Polypeptide is not derived from a major food allergen, rare allergic reactions have been reported to chicken meat in food allergic subjects (0.5-6%) (Sampson and Albergo, 1984; Rancé et al., 1999). The two major allergens are Gal d 5 and Gal d 7, which are chicken serum albumin and myosin light chain 1, respectively (Klug et al., 2020; Quirce et al., 2001). Allergic reactions to bovine and fish collagen and gelatin have also been reported (Mullins et al., 1996; Sakaguchi et al., 2000; Kalic et al., 2020). Because Non-Animal Type 21 Collagen Polypeptide is derived using the protein sequence from CC XXIa, a component of chicken meat, and similar to bovine and fish collagen or gelatin, the likelihood of sensitizing and/or provoking an allergic response in food allergic subjects may be similar to that following the ingestion of chicken meat, bovine or fish collagen.

# VII. SUPPORTING DATA AND INFORMATION

## A. REFERENCES

All information included in the following list of references is generally available.

Aalberse, R.C. (2000). Structural biology of allergens. J Allergy Clin Immunol *106*, 228–238.

Bailey RL, Catellier DJ, Jun S, Dwyer JT, Jacquier EF, Anater AS, Eldridge AL. (2016). Total Usual Nutrient Intakes of US Children (Under 48 Months): Findings from the Feeding Infants and Toddlers Study (FITS) J Nutr. 2018 Sep 1;148(9S):1557S-1566S. doi: 10.1093/jn/nxy042. PMID: 29878255; PMCID: PMC6126633.

Brown, S.D., and Jun, S. (2015). Complete Genome Sequence of Escherichia coli NCM3722. Genome Announc *3*, e00879–15.

Centers for Disease Control (2006). Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES). National Center for Health Statistics, Centers for Disease Control and Prevention; Hyattsville, Maryland. Available from: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf

Centers for Disease Control (2018). National Health and Nutrition Examination Survey (NHANES) 2017-2018. Available from: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015.

Direct Food Substances Affirmed as Generally Recognized as Safe: Chymosin Enzyme Preparation Derived from *Escherichia Coli* K-12, 55 Federal Register (FR) 10932 (March 23, 1990).

Environmental Protection Agency. (1997). Attachment 1 — Final Risk Assessment of Escherichia coli K-12 Derivatives.

Feeding Infants and Toddler Study (FITS): Findings and Thoughts on the Third Data Cycle. (2018). Stallings, VA, J Nutr. Sep 1;148(suppl_3):1513S-1515S. doi: 10.1093/jn/nxy158. PMID: 30247581; PMCID: PMC6126629.

Fitzgerald, J., and Bateman, J.F. (2001). A new FACIT of the collagen family: COL21A1. FEBS letters *505*, 275–280.

GRN 000026. (1999). Isolated wheat protein, Manildra Group. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=26.

GRN 000086. (2002). Coagulated potato protein, hydrolyzed potato protein, or clarified hydrolyzed potato protein, AVEBE b.a. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=86.

GRN 000134. (2004). Soy protein hydrolyzate with enzyme-modified lecithin, Kyowa Hakko U.S.A., Inc. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=134.

GRN 000327. (2010). Cruciferin-rich canola/rapeseed protein isolate and napin-rich canola/rapeseed protein isolate, Archer Daniels Midland Company. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=327.

GRN 000386. (2011). Canola protein isolate and hydrolyzed canola protein isolate, BioExx Specialty Proteins Ltd. Available at:, https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=386.

GRN 000447. (2013). Potato protein isolates, Solanic B.V. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=447.

GRN 000484. (2014). Galacto-oligosaccharides, Clasado Inc. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=484.

GRN 000575. (2015). Oat protein, Tate & Lyle. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=575.

GRN 000581. (2016). Un-hydrolyzed and hydrolyzed pea protein, World Food Proessing. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=581.

GRN 000608. (2016). Pea protein concentrate, Axiom Foods Inc. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=608.

GRN 000650. (2016). 2'-O-fucosyllactose, Glycom A/S. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=650.

GRN 000659. (2016). Lacto-N-neotetraose, Glycom A/S. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=659.

GRN 000683. (2017). Canola protein isolate, DSM Nutritional Products, LLC. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=683.

GRN 000684. (2017). Mung bean protein isolate, Hampton Creek, Inc. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=684.

GRN 000771. (2018). Hemp seed protein, Fresh Hemp Foods Ltd. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=771.

GRN 000788. (2018). Pea protein concentrate, Yantai Oriental Protein Tech Co., Ltd. Available at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=788.

GRN 000815. (2019). 2'-fucosyllactose and difucosyllactose, Glycom A/S. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=815.

GRN 000880. (2020). 3'-sialyllactose sodium salt, Glycom A/S. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=880.

GRN 000881. (2020). 6'-sialyllactose sodium salt, Glycom A/S. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=881.

Hayes, A.W., and Kruger, C.L. (2014). Food Safety and Foodborne Toxicants. In Hayes' principles and methods of toxicology, eds. (Boca Raton: CRC Press), pp. 631.

Institute of Medicine (IOM). (2002). Dietary Reference Intakes For Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Panel on Macronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board. Institute of Medicine of the National Academies, The National Academies Press, Washington, DC. 2002.

Kalic, T., Kamath, S.D., Ruethers, T., Taki, A.C., Nugraha, R., Le, T.T.K., Humeniuk, P., Williamson, N.A., Hira, D., Rolland, J.M., O'Hehir, R.E., Dai, D., Campbell, D.E., Breiteneder, H., and Lopata, A.L. (2020). Collagen-An Important Fish Allergen for Improved Diagnosis. J Allergy Clin Immunol Pract *8*, 3084–3092.e10.

Klug, C., Hemmer, W., Román-Carrasco, P., Focke-Tejkl, M., Quirce, S., Boyano-Martínez, T., Gaubitzer, E., Wank, H., and Swoboda, I. (2020). Gal d 7-a major allergen in primary chicken meat allergy. J Allergy Clin Immunol *146*, 169–179.e5.

Lukjancenko O, Wassenaar TM, Ussery DW. Comparison of 61 sequenced Escherichia coli genomes. Microb Ecol. 2010 Nov;60(4):708-20. doi: 10.1007/s00248-010-9717-3. Epub 2010 Jul 11. PMID: 20623278; PMCID: PMC2974192.

Lyons E, Freeling M, Kustu S, Inwood W. Using genomic sequencing for classical genetics in E. coli K12. PLoS One. 2011 Feb 25;6(2):e16717. doi: 10.1371/journal.pone.0016717. PMID: 21364914; PMCID: PMC3045373.

Macek, B., Forchhammer, K., Hardouin, J., Weber-Ban, E., Grangeasse, C., and Mijakovic, I. (2019). Protein post-translational modifications in bacteria. Nat Rev Microbiol *17*, 651–664.

Manning, P.A., Pugsley, A.P., and Reeves, P. (1977). Defective growth functions in mutants of Escherichia coli K12 lacking a major outer membrane protein. J Mol Biol *116*, 285–300.

Mariotti, F., Tomé, D., and Mirand, P.P. (2008). Converting nitrogen into protein--beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr *48*, 177–184.

Mullins, R.J., Richards, C., and Walker, T. (1996). Allergic reactions to oral, surgical and topical bovine collagen: Anaphylactic risk for surgeons. Australian and New Zealand journal of ophthalmology *24*, 257–260.

Paul, C., Leser, S., and Oesser, S. (2019). Significant Amounts of Functional Collagen Peptides Can Be Incorporated in the Diet While Maintaining Indispensable Amino Acid Balance. Nutrients *11*, E1079.

Quirce, S., Marañón, F., Umpiérrez, A., de las Heras, M., Fernández-Caldas, E., and Sastre, J. (2001). Chicken serum albumin (Gal d 5*) is a partially heat-labile inhalant and food allergen implicated in the bird-egg syndrome. Allergy *56*, 754–762.

Rancé, F., Kanny, G., Dutau, G., and Moneret-Vautrin, D.A. (1999). Food hypersensitivity in children: clinical aspects and distribution of allergens. Pediatr Allergy Immunol *10*, 33–38.

Sakaguchi, M., Toda, M., Ebihara, T., Irie, S., Hori, H., Imai, A., Yanagida, M., Miyazawa, H., Ohsuna, H., Ikezawa, Z., and Inouye, S. (2000). IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. J Allergy Clin Immunol *106*, 579–584.

Sampson, H.A., and Albergo, R. (1984). Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. J Allergy Clin Immunol *74*, 26–33.

Smith, H.W. (1978). Is it safe to use Escherichia coli K12 in recombinant DNA experiments. J Infect Dis *137*, 655–660.

Soupene, E., van Heeswijk, W.C., Plumbridge, J., Stewart, V., Bertenthal, D., Lee, H., Prasad, G., Paliy, O., Charernnoppakul, P., and Kustu, S. (2003). Physiological studies of Escherichia coli strain MG1655: growth defects and apparent cross-regulation of gene expression. J Bacteriol *185*, 5611–5626.

Stevens, B.R. (2006). Digestion and Absorption of Protein. In Biochemical, physiological, and molecular aspects of human nutrition, Stipanuk, M.H., ed. (St. Louis: Elsevier Health Sciences), pp. 200–218.

United States Department of Agriculture, and Agricultural Research Service (2020a). USDA Food and Nutrient Database for Dietary Studies 2017-2018. Food Surveys Research Group Home Page, http://www.ars.usda.gov/nea/bhnrc/fsrg. Available at https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds-download-databases/.

United States Department of Agriculture, Department of Agriculture, Ag Data Commons (2020b). What We Eat In America (WWEIA) Database. https://data.nal.usda.gov/dataset/what-we-eat-america-wweia-database.

Von der Mark, K. (2006). Structure, biosynthesis and gene regulation of collagens in cartilage and bone. Dynamics of Bone and Cartilage Metabolism. Principles and Clinical Applications 3–40.

Wu G. (2016). Dietary protein intake and human health. Food Funct. Mar;7(3):1251-65. doi: 10.1039/c5fo01530h. PMID: 26797090.

## B. EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of Non-Animal Type 21 Collagen Polypeptide in general foods. This GRAS determination for the use of Non-Animal Type 21 Collagen Polypeptide for the intended use and use level specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of Non-Animal Type 21 Collagen Polypeptide has been determined to be GRAS and is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food and is based on generally available and accepted information.

The intended use of Non-Animal Type 21 Collagen Polypeptide in selected conventional foods and beverages is determined to be safe based on the following:

1. Non-Animal Type 21 Collagen Polypeptide is a fragment of chicken (*Gallus gallus domesticus*) collagen type XXIa (CC XXIa) manufactured by fermentation using a genetically engineered strain of *Escherichia coli* K-12.

2. Non-Animal Type 21 Collagen Polypeptide is intended to provide a source of non-animal collagen in selected general foods and beverages.

3. Non-Animal Type 21 Collagen Polypeptide lacks the post-translational modifications that impart the characteristic triple helical structure of native collagen and resistance to the digestive enzymes of the gastrointestinal tract.

4. Non-Animal Type 21 Collagen Polypeptide is manufactured according to Good Manufacturing Practices (GMPs) in FDA-registered facilities.

    a. *E. coli* K-12 and its derivatives are non-toxigenic, non-pathogenic, known as laboratory “safety-strains” because they possess smaller genomes, fewer genes, and lack the gene families that would allow them to colonize the human gastrointestinal tract and/or produce toxins. They have also been used to manufacture a variety of other food ingredients that are GRAS.

    b. All raw materials and food contact substances used in the manufacturing process are of suitable purity and quality for use in a food product, common ingredients used in the food/enzyme industry, and are either listed in 21 CFR, GRAS, or are Food Chemicals Codex (FCC), United States Pharmacopeia (USP), National Formulary (NF), or American Chemical Society (ACS) grade.

c. None of the raw materials used during manufacture is a major allergen.

d. The quality of each lot of Non-Animal Type 21 Collagen Polypeptide is evaluated against a set of product specifications that confirm the identity, protein, ash, moisture, heavy metal, and microbial content of the finished ingredient using compendial or validated methods that are fit-for-use to ensure that the production process is adequately controlled.

e. Batch data for additional quality attributes, such as macronutrient content, micronutrient content, elemental content, endotoxin content, and host cell protein content further demonstrate control of the production process and quality of the finished ingredient.

5. The addition of Non-Animal Type 21 Collagen Polypeptide to selected general foods and beverages results in mean intakes of 10.31 g/day and 90$^{th}$ % intakes of 22.95 g/day for children and adults ages 2 years and up.

6. Although collagen peptides do not contain tryptophan and are low in cysteine and methionine, the average US diet contains a surplus of these amino acids that allows for the substitution of the total protein intake with collagen peptides. Iterative PDCAAS calculations showed that a level as high as 36% of collagen peptides can be used as a protein substitute in the daily diet without lowering the overall PDCAAS score below 1.0 while ensuring indispensable amino acid requirements are met and maintenance of dietary protein quality. A comparison of the EDI for Non-Animal Type 21 Collagen Polypeptide with a calculation of the amount of collagen that would be consumed at 36% of substitution for protein demonstrates that the proposed intake in children and adults will not compromise the protein quality of the diet.

7. The safe use of Non-Animal Type 21 Collagen Polypeptide is also supported by a clinical study demonstrating its digestibility and metabolic fate as a protein, and lack of mutagenicity in an Ames assay.

8. Because Non-Animal Type 21 Collagen Polypeptide is derived using the protein sequence from CC XXIa, a component of chicken meat, and similar to bovine and fish collagen or gelatin, the likelihood of sensitizing and/or provoking an allergic response in food allergic subjects may be similar to that following the ingestion of chicken meat, bovine or fish collagen.

Therefore, Non-Animal Type 21 Collagen Polypeptide is safe and GRAS under the intended conditions of use, is excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

Peter Pressman, MD, MS, FACN
GRAS Expert Panel Member
Medicine Public Health & Nutrition
The Daedalus Foundation

Signature: 

Date: October 25, 2023

Roger Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
School of Pharmacy
University of Southern California

Signature: 

Date: October 25, 2023

Thomas E. Sox, PhD, JD
GRAS Expert Panel Member
Principal, Pondview Consulting LLC

Signature: 

Date: October 25, 2023

Dietrich Conze, PhD
Scientific Advisor to the Panel
Spherix Consulting Group, Inc.

Signature: 

Date: October 25, 2023

**From:** Dietrich Conze
**To:** Downey, Jason
**Cc:** Claire Kruger; Kathy Brailer
**Subject:** Re: [EXTERNAL] GRN 001171 - Geltor"s collagen polypeptide Questions to the Notifier
**Date:** Monday, June 3, 2024 5:52:56 PM
**Attachments:** Geltor Response to FDA on GRN 001171 - 6-3-24.pdf



Hi Jason,
Attached are our responses.
Regards.
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

On May 8, 2024, at 9:07 AM, Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:

Hi Dietz,
No worries. Sure, June 03 or earlier should be fine. Thanks for the heads-up.
Jason

---

**From:** Dietrich Conze <dconze@spherixgroup.com>
**Sent:** Wednesday, May 8, 2024 8:53 AM
**To:** Downey, Jason <Jason.Downey@fda.hhs.gov>
**Cc:** Claire Kruger <ckruger@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
**Subject:** [EXTERNAL] Re: GRN 001171 - Geltor's collagen polypeptide Questions to the Notifier

Hi Jason,

Sorry for the delay in getting back to you; we needed to coordinate with our client. Would it be possible to submit our answers to your questions on June 3, 2024, at the latest?

Regards.
Dietz

> On May 6, 2024, at 11:21 AM, Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:
>
> Hi Dietz,
>
> During our evaluation of GRN 001171, regarding Geltor's intended uses in food of collagen polypeptide produced by *E. coli* K-12 S1988, we noted questions and points in need of clarification, which are listed below. Please provide responses to these requests within 10 business days. If you foresee any issue with this timeline or you have any other questions, please contact me as soon as possible.
>
> Thank you in advance for your attention to our comments.
>
> Sincerely,
>
> Jason
>
> **Jason Downey, Ph.D. (he/him/his)**
> *Regulatory Review Scientist*
>
> **Division of Food Ingredients**
> **Office of Food Additive Safety**
> **Center for Food Safety and Applied Nutrition**
> **U.S. Food and Drug Administration**
> jason.downey@fda.hhs.gov
>
> **FDA's Questions for the Notifier**
>
> 1. In Table 3, the composition of the collagen polypeptide produced by *E. coli* K-12 S1988 adds to around 98%. Please explain what the composition of the remaining 2% is.
> 2. Please clarify if the molecular weight of 17.915 kDa for the

collagen polypeptide produced by *E. coli* K-12 S1988 is calculated or experimentally determined.

3. Please provide the UniProt ID of the type 21 collagen polypeptide from *Gallus gallus domesticus* and discuss its amino acid sequence identity/similarity with that of the recombinant collagen polypeptide produced by *E. coli* K-12 S1988.
4. On page 13, the notifier discusses analyses of other proteins present in the final ingredient. Please briefly discuss the identity of these proteins and why they are not expected to pose a safety concern. Further, please discuss if the residual host cell proteins measured in *E. coli* strain S7555 are expected to be comparable to the production strain, *E. coli* S9188, used to produce the notified ingredient.
5. We have a few clarifying questions for the genetic modification process:
    a. On page 8, step 4 you state that "removal of all plasmids was confirmed." However, the process as described does not discuss any removal of plasmids. Please clarify.
    b. Please describe the origin and source of donor genes used in the construction of the production strain (e.g., are they *de novo* synthesized or of bacterial origin).
6. Please discuss if the final production strain is capable of genetic transfer.
7. On page 10 you state, "the culture is determined to be free of contaminants by microscopy and plate streaking." Please provide additional discussion on the use of these methods to identify potential contamination.
8. Please confirm if the final collagen polypeptide is further formulated with any additional ingredients after spray-drying or if the spray-dried, purified supernatant is the final product.
9. Please briefly discuss if any methods are used to demonstrate the absence of the production strain in the final product. If not, please discuss why this is not expected to impact safety.
10. Please confirm that all raw materials and processing aids are food grade, and that they are used in accordance with applicable U.S. regulations, are concluded to be GRAS for their intended uses, or are the subject of an effective food contact notification.
11. On page 10, you stated that isopropyl β-D-1-thiogalactopyranoside (IPTG) is used as an inducer in the production of the collagen polypeptide produced by *E. coli* K-12 S1988. Please clarify which steps described in the method of manufacture ensures the removal of IPTG. In addition, please indicate if you analyze for IPTG in the final product and indicate the method used and the limit of detection (LOD) for the method

used to confirm that IPTG is not present in the final product.

12. On page 13, you stated that culture minerals in the collagen polypeptide produced by *E. coli* K-12 S1988 were either below the LOD or present in very low concentrations (<60 mg/kg). Please specify which minerals were included and indicate the levels of each mineral in the final product. If they were present at below the LOD for the method, please provide the LOD for the method.
13. Please state whether the collagen polypeptide produced by *E. coli* K-12 S1988 is secreted into the culture medium.
14. Table 2 lists the sample size for the *Salmonella* spp. specification as “absent in 25 g; or absent in 10 g.” Please briefly discuss the rationale for the use of two sample sizes, confirm that the method used for the analysis has been validated for both sample sizes, and clarify the results (i.e., “absent in 10 g” or “absent in 25 g”) reported in your batch analyses.
15. In Table 2, the results for the batch analyses for cadmium and mercury were stated to be ND. Please clarify whether these results are below the limit of quantification (LOQ) or LOD for the method. If the actual values for the analyses for heavy metals are not below the stated LOQ or LOD, please provide the actual values.
16. In line with FDA's "Closer to Zero" initiative, we note that specifications for heavy metals should be kept as low as possible and reflect the results from the batch analyses. Please consider revising the specifications for heavy metals to be consistent with the results of the batch analyses.
17. On page 1 and 2, you stated that “Geltor intends to use Non-Animal Type 21 Collagen Polypeptide as an ingredient in selected general foods and beverage”. In other places of the notice, you stated that the ingredient is intended to substitute for other sources of protein. Please confirm that the collagen polypeptide produced by *E. coli* K-12 S1988 is intended to be used as a source of protein.
18. In Table 7, please clarify how the protein use level of 95% in nutritional bars is obtained when the specifications and results from the batch analyses for protein content of the collagen polypeptide produced by *E. coli* K-12 S1988 are stated to be 80-90%.
19. In Table 7, please clarify what would be included under the flavored beverages food category. For example, are products such as flavored milk products or hot chocolate drinks included? It may be helpful to include a list of food codes included in the dietary exposure estimate to ensure that it is clear as to the scope of the notice.

20. The dietary exposure from the intended uses of collagen polypeptide produced by *E. coli* K-12 S1988 that we estimated is significantly higher than the results in Table 8 in the notice. As noted above, it would be helpful if you could provide the food codes and use levels for the food codes included in your dietary exposure assessment in order to ensure that we understand the intended uses for the collagen polypeptide produced by *E. coli* K-12 S1988.
21. Please briefly discuss whether there will be increase in the total cumulative dietary exposure to protein in the U.S. diet or if the use of collagen polypeptide produced by *E. coli* K-12 S1988 is substitutional for other sources of protein and therefore, there would not be an increase in the total dietary exposure to protein.
22. Please provide a statement that the ingredient is not intended to be used in infant formula, products under the jurisdiction of the USDA, or in foods in which standards of identity preclude its use.
23. Please provide additional discussion for the safety narrative on the genetic modifications described on page 8, including a brief safety discussion of the donor organisms used to modify the recipient strain, *E. coli* K-12 NCM3722, a brief discussion on if any of the gene deletions described on page 8 are expected to impact safety, and if the presence of the kanamycin resistance gene is expected to impact safety.
24. Please provide the following information about the in vitro digestibility experiment:
    a. Was SDS-PAGE performed with samples collected at different time points? If yes, please provide a photograph.
    b. Because the optimum pH for pepsin activity is significantly different from that of trypsin and chymotrypsin, was the trypsin-chymotrypsin digestion carried out on the pepsin digesta or was the pepsin versus trypsin-chymotrypsin digestion carried out separately?
    c. What was the enzyme: substrate ratio (E/S) used in the experiment(s)?
    d. With the E/S ratio used, please provide a quantitative estimate of digestion over time (e.g., ~50% or ~75%, or ~90% digestion of the original starting material).
25. The notifier states that the PDCAAS score of collagen polypeptide produced by *E. coli* K-12 S1988 is 0.1. Such a low PDCAAS score is reflective of a poor protein quality. Please compare the daily essential amino acids intake from the proposed $90^{th}$ percentile consumption of collagen polypeptide produced by *E. coli* K-12 S1988 with the recommended daily intake of the respective essential amino acids. Explain why consuming collagen polypeptide produced by *E. coli* K-12 S1988

as the (potentially) primary source of protein for consumers on a low protein diet would not be of any concern.

Spherix Consulting Group

June 3, 2024

Jason Downey, Ph.D.
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 001171

Dear Dr. Downey:

Below are our responses to the Agency's requests for additional information regarding GRN 001171, as specified in your email on May 6, 2024. Your requests are in italicized text and our responses are below in plain text. Please note that the production strain is *E. coli* K-12 S9188, as specified in the GRAS Notice, not *E. coli* K-12 S1988.

1. *In Table 3, the composition of the collagen polypeptide produced by E. coli K-12 S1988 adds to around 98%. Please explain what the composition of the remaining 2% is.*

   The remaining 2% of Non-Animal Type 21 Collagen Polypeptide is carbohydrates. The carbohydrate content of three lots of Non-Animal Type 21 Collagen Polypeptide is provided in Table 1.

**Table 1. Carbohydrate Content of Non-Animal Type 21 Collagen Polypeptide**

| | Lot Number | | |
|---|---|---|---|
| | **GLT402321** | **GLT402921** | **GLT403321** |
| Carbohydrates (%)[1] | 2.0 | 2.4 | 2.5 |
| [1]per 21 CFR 101.9 | | | |

2. *Please clarify if the molecular weight of 17.915 kDa for the collagen polypeptide produced by E. coli K-12 S1988 is calculated or experimentally determined.*

   The molecular weight of 17.915 kDa was verified by liquid chromatography mass spectrometry (LC-MS).

3. *Please provide the UniProt ID of the type 21 collagen polypeptide from Gallus gallus domesticus and discuss its amino acid sequence identity/similarity with that of the recombinant collagen polypeptide produced by E. coli K-12 S1988.*

The UniProt ID for the type 21 collagen from *Gallus gallus domesticus* is A0A8V0XCL7_CHICK (https://www.uniprot.org/uniprotkb/A0A8V0XCL7/entry). The amino acid sequence of the recombinant collagen polypeptide produced by *E. coli* K-12 S9188 is 100% identical to the sequence of amino acids from position 554 to 742 of type 21 collagen from *Gallus gallus domesticus* provided in the UniProt databases (Figures 1 and 2).


*Figure 1. Alignment of the amino acid sequence of Non-Animal Type 21 Collagen Polypeptide with the amino acid sequence of type 21 collagen polypeptide from* Gallus gallus domesticus *(residues 554 to 742) provided by UniProt (A0A8V0XCL7_CHICK). "Query" denotes amino acid sequence of Non-Animal Type 21 Collagen Polypeptide.*

4. *On page 13, the notifier discusses analyses of other proteins present in the final ingredient. Please briefly discuss the identity of these proteins and why they are not expected to pose a safety concern. Further, please discuss if the residual host cell proteins measured in E. coli strain S7555 are expected to be comparable to the production strain, E. coli S9188, used to produce the notified ingredient.*

The residual *E. coli* S7555-derived proteins in Non-Animal Type 21 Collagen Polypeptide include a variety of proteins required for homeostasis, such as ribosomal subunits, transcription factors, and other cytoplasmic proteins. Because the only difference between *E. coli* S7555 and *E. coli* S9188 is the absence of ompT, which is an outer membrane protease that cleaves substrates between two basic amino acids, it is reasonable to conclude that the identity and quantity of the residual production strain-derived proteins in Non-Animal Type 21 Collagen Polypeptide manufactured with either *E. coli* S7555 and *E. coli* S9188 will be similar. Importantly, because both *E. coli* S7555 and *E. coli* S9188 are *E. coli* K-12 strains, and *E. coli* K-12 strains are non-toxigenic, non-pathogenic strains widely used to manufacture food ingredients, the presence of these residual proteins in the final ingredient is not expected to pose a safety concern. Additionally, all of these proteins will be digested and metabolized in a manner similar to other proteins consumed via the diet and/or liberated from the microbiota present in the gastrointestinal tract.

5. *We have a few clarifying questions for the genetic modification process:*

   a. *On page 8, step 4 you state that "removal of all plasmids was confirmed." However, the process as described does not discuss any removal of plasmids. Please clarify.*

      This was a typographical error. Point #4 on page 8 should state:

      "Duplication of the native *lacI* transcriptional repressor gene on the genome of the strain resulting from (3) via homologous recombination to exert greater transcriptional repression of the viral T7 RNA polymerase gene. Following this stage, all modifications to the chromosome and absence of all plasmids were confirmed by polymerase chain reaction and DNA sequencing."

   b. *Please describe the origin and source of donor genes used in the construction of the production strain (e.g., are they de novo synthesized or of bacterial origin).*

      The T7 polymerase gene was amplified from the genome of *E. coli* BL21 by polymerase chain reaction (PCR) and subsequently cloned into an expression cassette that was integrated into the genome of the production strain genome by homologous recombination. All other DNA used to engineer the production strain was de novo synthesized.

6. *Please discuss if the final production strain is capable of genetic transfer.*

   The production strain harbors an F-plasmid that does not contain any antibiotic resistance genes (Brown and Jun, 2015; Browning et al., 2023). The genome of the production strain also contains the bacteriophage λ prophage. Therefore, the host strain *E. coli* NCM 3722 is capable of transferring genetic material. However, during the genetic engineering of the production strain, the DNA recombination gene RecA was deleted by homologous recombination to improve the genetic stability of the production strain and prevent the transfer of genes within the genome or between the genome and any plasmid harbored by the strain, such as the plasmid that contains the Non-Animal Type 21 Collagen Polypeptide expression cassette or the F-plasmid. Additionally, Non-Animal Type 21 Collagen Polypeptide is manufactured under strictly controlled manufacturing conditions and no bacteriophage activity has been observed. Non-Animal Type 21 Collagen Polypeptide is also secreted into the culture medium and subsequently purified from the production strain-clarified medium via multiple diafiltration steps, which remove production strain-derived DNA residues.

   To confirm that the production process removes production strain-derived DNA from final ingredient, Geltor determined if the kanamycin gene, which is present in the Non-Animal Type 21 Collagen Peptide expression plasmid, was detectable in three batches of the final ingredient by polymerase chain reaction (PCR). The kanamycin gene was not detected, indicating that the final ingredient does not contain detectable levels of production strain-derived DNA residues.

7. *On page 10 you state, "the culture is determined to be free of contaminants by microscopy and plate streaking." Please provide additional discussion on the use of these methods to identify potential contamination.*

   Geltor samples the culture during fermentation and conducts light microscopy coupled with Gram staining and plate streaking to confirm the absence of Gram-positive bacteria (non-production strain bacteria). Geltor also evaluates the morphological characteristics of the production strain during manufacturing. The presence of Gram-positive bacteria or any bacteria not conforming to the expected morphological characteristics of the production strain indicate that the culture is contaminated, and the production run is terminated.

8. *Please confirm if the final collagen polypeptide is further formulated with any additional ingredients after spray-drying or if the spray-dried, purified supernatant is the final product.*

   No ingredients are added to the ingredient after spray-drying. The spray-dried, purified supernatant is the final product.

9. *Please briefly discuss if any methods are used to demonstrate the absence of the production strain in the final product. If not, please discuss why this is not expected to impact safety.*

   Absence of the production strain in the final ingredient is confirmed via qualification against the *E. coli* product specification of Negative/10 g, as well as screening for the presence of production strain-derived DNA by polymerase chain reaction (PCR).

10. *Please confirm that all raw materials and processing aids are food grade, and that they are used in accordance with applicable U.S. regulations, are concluded to be GRAS for their intended uses, or are the subject of an effective food contact notification.*

    All raw materials and processing aids are food grade, and either used in accordance with applicable U.S. regulations, concluded to be GRAS for their intended uses, or are the subject of an effective Food Contact Notification.

11. *On page 10, you stated that isopropyl β-D-1-thiogalactopyranoside (IPTG) is used as an inducer in the production of the collagen polypeptide produced by E. coli K-12 S1988. Please clarify which steps described in the method of manufacture ensures the removal of IPTG. In addition, please indicate if you analyze for IPTG in the final product and indicate the method used and the limit of detection (LOD) for the method used to confirm that IPTG is not present in the final product.*

    Although Geltor does not quantitate the amount of IPTG in the final ingredient, the compound is removed from the ingredient over the course of five consecutive diafiltration steps using a 3 kDa cutoff membrane. The polypeptide has a molecular weight of 17.915 kDa and IPTG has a molecular weight of 0.238 kDa. During filtration steps, the polypeptide is retained and all fermentation medium components with a molecular weight less than 3 kDa pass through the membrane and are discarded. Therefore, it is estimated that each filtration step reduces the IPTG content by 50%, resulting in a final concentration

in the ingredient of 1.25 ppm. Importantly, IPTG is used in a similar manner to produce the subjects of GRN 000745 and 001097, both of which received "no questions" letters from the Agency.

12. *On page 13, you stated that culture minerals in the collagen polypeptide produced by E. coli K-12 S1988 were either below the LOD or present in very low concentrations (<60 mg/kg). Please specify which minerals were included and indicate the levels of each mineral in the final product. If they were present at below the LOD for the method, please provide the LOD for the method.*

The mineral content of three lots of Non-Animal Type 21 Collagen Polypeptide was determined using compendial methods. Nickel, iron, selenium, molybdenum, zinc, magnesium, and manganese were detected (Table 2). Boron and sulfites were not detected, with limits of detection of 2.5 and 10 ppm, respectively.

**Table 2. Mineral Content of Non-Animal Type 21 Collagen Polypeptide**

| Element[1] | Method | LOD | Lots Number | | |
|---|---|---|---|---|---|
| | | | GLT403421 | GLT403621 | GLT403921 |
| Nickle (ppm) | AOAC OMA 2015.01 | 0.01 | 0.17 | 0.12 | 0.21 |
| Iron (ppm) | AOAC OMA 2015.01 | 0.01 | 2.53 | 1.36 | 1.51 |
| Selenium (ppm) | AOAC OMA 2015.01 | 0.01 | 0.5 | 0.88 | 0.70 |
| Molybdenum (ppm | AOAC 2011.19 (mod.) | 0.05 | 0.08 | <0.05 | <0.05 |
| Zinc (ppm) | AOAC OMA 2015.01 | 0.01 | 1.22 | 0.74 | 0.68 |
| Boron (ppm) | AOAC 984.27 (mod.), 985.01 (mod.), 2011.14 (mod.) | 2.5 | <2.5 | <2.5 | <2.5 |
| Magnesium (ppm) | AOAC OMA 2015.01 | 0.01 | 58.0 | 52.0 | 49.7 |
| Manganese (ppm) | AOAC OMA 2015.01 | 0.01 | 4.62 | 4.4 | 4.27 |
| Sulfites (as sulfur dioxide; ppm) | AOAC OMA 990.28 | 10.0 | <10.0 | <10.0 | <10.0 |

Abbreviations: AOAC Association of Official Analytical Collaboration International; OMA – Official Method of Analysis; LOD – limit of detection; ppm – parts per million.

13. *Please state whether the collagen polypeptide produced by E. coli K-12 S1988 is secreted into the culture medium.*

Non-Animal Type 21 Collagen Polypeptide produced by *E. coli* K-12 S9188 is secreted into the culture medium. Following fermentation, *E. coli* K-12 S9188 biomass is removed from the culture medium by centrifugation. Non-Animal Type 21 Collagen Polypeptide is then purified from the clarified culture medium via multiple filtration steps.

14. *Table 2 lists the sample size for the Salmonella spp. specification as "absent in 25 g; or absent in 10 g." Please briefly discuss the rationale for the use of two sample sizes, confirm that the method used for the analysis has been validated for both sample sizes, and clarify the results (i.e., "absent in 10 g" or "absent in 25 g") reported in your batch analyses.*

    The difference in sample sizes relate to the use of either AOAC OMA 2011.3 or USP <62> during batch qualification. Specifically, AOAC OMA 2011.3 requires a sample size of 25 g whereas USP<62> requires a sample size of 10 g. The batch data presented in Table 2 represent the results of both methods. Importantly, Geltor will be using the AOAC method moving forward because it is more stringent than the USP method.

15. *In Table 2, the results for the batch analyses for cadmium and mercury were stated to be ND. Please clarify whether these results are below the limit of quantification (LOQ) or LOD for the method. If the actual values for the analyses for heavy metals are not below the stated LOQ or LOD, please provide the actual values.*

    As specified in the footer of Table 2, "ND" is the abbreviation for "not detected". Per footnote 2 in the footer of Table 2, the limits of detection for arsenic, cadmium, mercury, and lead are all 0.01 ppm. Therefore, if the heavy metal was not detected (i.e., below the limit of detection), ND was used to indicate that the level of the heavy metal was below the limit of detection in Table 2.

16. *In line with FDA's "Closer to Zero" initiative, we note that specifications for heavy metals should be kept as low as possible and reflect the results from the batch analyses. Please consider revising the specifications for heavy metals to be consistent with the results of the batch analyses.*

    Thank you for your guidance. Geltor is aware of the "Closer to Zero" initiative and will explore lowering their heavy metal specifications in the future.

17. *On page 1 and 2, you stated that "Geltor intends to use Non-Animal Type 21 Collagen Polypeptide as an ingredient in selected general foods and beverage". In other places of the notice, you stated that the ingredient is intended to substitute for other sources of protein. Please confirm that the collagen polypeptide produced by E. coli K-12 S1988 is intended to be used as a source of protein.*

    Non-Animal Type 21 Collagen Polypeptide is intended to be used as a substitute for other sources of collagen polypeptides, not as a substitute for other dietary sources of protein.

18. *In Table 7, please clarify how the protein use level of 95% in nutritional bars is obtained when the specifications and results from the batch analyses for protein content of the collagen polypeptide produced by E. coli K-12 S1988 are stated to be 80-90%.*

    Non-Animal Type 21 Collagen Polypeptide is intended to be used as a substitute for other sources of collagen polypeptides. The "Protein % (w/w)" column in Table 7 of the Notice specifies the use level of the protein component of Non-Animal Type 21 Collagen

Polypeptide, not Non-Animal Type 21 Collagen Polypeptide as a whole. Also, please note that the use level in nutrition bars is listed as 25% protein from Non-Animal Type 21 Collagen Polypeptide and the use level in protein powders is 95% protein from Non-Animal Type 21 Collagen Polypeptide.

Although the typical protein content is approximately 90%, Non-Animal Type 21 Collagen Polypeptide must contain not less than 80% protein per the product specification. If a lot of Non-Animal Type 21 Collagen Polypeptide contains 80% protein opposed to 90%, the amount of Non-Animal Type 21 Collagen Polypeptide added to the marketed product will be adjusted accordingly to deliver the targeted amount of collagen polypeptide in the finished food. Importantly, the food categories provided in Table 7 are surrogates for the foods that will contain Non-Animal Type 21 Collagen Polypeptide, allowing for the calculation of the exposure estimates to Non-Animal Type 21 Collagen Polypeptide from its intended use uses, such as a protein powder product containing predominantly collagen peptides where the collagen peptides account for approximately 95 % of the total protein content of the protein powder product. Additionally, protein powders typically contain 50-80% total protein (Gorissen et al., 2018). Therefore, if Non-Animal Type 21 Collagen Polypeptide is used in a protein powder product that contains 50% total protein, the targeted level of collagen peptide is 95% of the total protein content in the protein powder, and the lot of Non-Animal Type 21 Collagen Polypeptide used to manufacture the marketed food contains 80% protein, then Non-Animal Type 21 Collagen Polypeptide would be included at a rate of 59.4 % of the total protein content of the marketed product. If Non-Animal Type 21 Collagen Polypeptide is used in a protein powder product that contains 80% total protein, the targeted level of collagen peptide is 95% of the total protein content in the protein powder, and the lot of Non-Animal Type 21 Collagen Polypeptide used contains 80% protein, then Non-Animal Type 21 Collagen Polypeptide would be included at a rate of 95% of the total protein content of the marketed product.

19. *In Table 7, please clarify what would be included under the flavored beverages food category. For example, are products such as flavored milk products or hot chocolate drinks included? It may be helpful to include a list of food codes included in the dietary exposure estimate to ensure that it is clear as to the scope of the notice.*

The list of the food codes used in the exposure assessment is provided in Appendix 1. In brief, the beverage category includes sports drinks, flavored bottled water, and powdered fruit and sports drinks. The use level in the powdered drinks were calculated as consumed, i.e., as the reconstituted, fluid beverage.

20. *The dietary exposure from the intended uses of collagen polypeptide produced by E. coli K-12 S1988 that we estimated is significantly higher than the results in Table 8 in the notice. As noted above, it would be helpful if you could provide the food codes and use levels for the food codes included in your dietary exposure assessment in order to ensure that we understand the intended uses for the collagen polypeptide produced by E. coli K-12 S1988.*

The dietary exposure estimates submitted in the Notice were calculated on a per eating occasion basis. To estimate the daily exposure to Non-Animal Type 21 Collagen

Polypeptide, the estimated exposures have been recalculated using the 2017-2018 NHANES database and DaDIET, which is a software package that has been used to calculate the estimated daily exposures to a variety of other food ingredients that are GRAS. Consistent with FDA's estimates, the recalculated exposures were higher than those described in the Notice (Table 4). Additionally, to ensure that the exposure to Non-Animal Type 21 Collagen Polypeptide does not compromise the quality of the diet (as discussed in Chapter VI, Section D of GRN 001171), the food codes mimicking the intended uses were revised to represent only those products that currently contain collagen polypeptides. The inclusion rate in the beverage and beverage bases category was also lowered to 2% protein. The inclusion rate for ready-to-drink and instant coffee and tea was reduced to 5.5% protein to be consistent with current uses of collagen polypeptide in commercial products. The revised inclusion rates for the intended uses are provided in Table 3 and the food codes used in the revised exposure calculations are provided in Appendix 1. The updated exposure estimates are now stratified by age and sex to allow for the direct comparisons with the recommended daily allowances (RDAs) for protein intake published by the Institute of Medicine (2002) and the assessment of collagen intake that does not compromise the quality of the diet, which is based on the RDAs.

**Table 3. Intended Uses and Use Levels for Non-Animal Type 21 Collagen Polypeptide**

| Category | Application | Maximum Protein as Collagen Polypeptide % (w/w) |
|---|---|---|
| Beverages and beverage bases | Flavored beverages and beverage bases*, excluding soft drinks and energy drinks | 2 |
| Coffee and Tea | Packaged coffee & tea beverages | 5.5 |
| | Instant coffee & tea | |
| | Coffee and tea pods | 2 |
| Dairy products and substitutes | Probiotic and yogurt beverages | 2.5 |
| | Milk substitutes | 2.5 |
| Coffee creamer | Coffee creamer (liquid and powder* forms) | 20 |
| Cereal Products | Cereal bars | 12.5 |
| | Hot breakfast cereals | 12.5 |
| Nutritional foods | Nutritional beverages and beverage bases* | 4.5 |
| | Nutritional bars | 25 |
| | Protein powders | 95 |
| Confections | Gummies | 10 |
| | Chocolate confections | 10 |
| Meat, poultry, and fish substitutes | Meat, poultry, and fish substitutes | 12 |

†Range based on minimum protein purity specification of ≥80% and maximum of 100%
*Usage level reflective of that in reconstituted, ready-to-consume product

In summary, 74.4% and 75% of males and females 2+ years of age were identified as consumers of Non-Animal Type 21 Collagen Polypeptide from the intended food uses (Table 4). The mean intakes of protein from Non-Animal Type 21 Collagen Polypeptide from consumers ages 2+ from the selected food uses for males and females were 18.29 g/day and 15.52 g/day, respectively (corresponding to 0.23 g/kg body weight/day for both males and females). The 90$^{th}$ percentile intakes were 38.36 g/day and 32.1 g/day for males and females, respectively (corresponding to 0.48 g/kg body weight/day for both males and females). Please note Non-Animal Type 21 Collagen Polypeptide is intended to be substitutional for other sources of collagen polypeptides that are currently available; therefore, the use of this product will not increase the dietary exposure to collagen polypeptides. Additionally, these exposure estimates are the worst-case scenario because they assume that consumers are ingesting only products that contain Non-Animal Type 21 Collagen Polypeptide in a day.

**Table 4. Estimated Daily Intake of Protein from Non-Animal Type 21 Collagen Polypeptide in Consumers of Non-Animal Type 21 Collagen Polypeptide-Containing Products**

| Cohort | % Users | N Users | Total Population | Estimated Daily Intake | | | |
|---|---|---|---|---|---|---|---|
| | | | | g/kg body weight/day | | g/day | |
| | | | | Mean | 90th Percentile | Mean | 90th Percentile |
| *Males* | | | | | | | |
| Ages 1-2 | 45.8 | 63 | 157 | 0.31 | 0.61 | 4.08 | 9.15 |
| Ages 3-5 | 58.1 | 75 | 154 | 0.25 | 0.54 | 4.63 | 8.6 |
| Ages 6-11 | 56.5 | 166 | 328 | 0.22 | 0.53 | 7.55 | 21.31 |
| Ages 12-19 | 54.1 | 222 | 441 | 0.2 | 0.51 | 13.69 | 33.17 |
| Ages 20+ | 80.8 | 1519 | 1961 | 0.23 | 0.46 | 20.3 | 40.92 |
| Ages 2+ | 74.4 | 2011 | 2950 | 0.23 | 0.48 | 18.29 | 38.36 |
| *Females* | | | | | | | |
| Ages 1-2 | 44.8 | 55 | 141 | 0.36 | 1.05 | 4.15 | 12.98 |
| Ages 3-5 | 60.8 | 90 | 162 | 0.2 | 0.38 | 3.61 | 6.82 |
| Ages 6-11 | 53.5 | 166 | 351 | 0.15 | 0.3 | 4.85 | 11.83 |
| Ages 12-19 | 59.6 | 236 | 443 | 0.18 | 0.36 | 10.69 | 19.81 |
| Ages 20+ | 80.1 | 1632 | 2144 | 0.24 | 0.5 | 17.2 | 34.16 |
| Ages 2+ | 75 | 2158 | 3180 | 0.23 | 0.48 | 15.52 | 32.1 |

[1]Calculated using DaDIET, food codes that represent the intended uses, and user data from the 2017-2018 NHANES database.

Please note that the results from the original exposure assessment were also reproduced in Table 19, page 40 of the Notice. Because the estimated daily intakes (EDIs) were recalculated with DaDIET using revised food codes and intended use levels for some of the food categories, we have revised Table 19 below with the recomputed EDIs, stratified by age and sex (Table 5).

**Table 5. Mean Protein Consumed from Intended Uses and Use Levels of Non-Animal Type 21 Collagen Polypeptide**

| Population Group | N Users | N Population | % Users | Mean Estimated Daily Intake (g/day) |
|---|---|---|---|---|
| *Males* | | | | |
| Ages 1-2 | 63 | 157 | 45.8 | 4.08 |
| Ages 3-5 | 75 | 154 | 58.1 | 4.63 |
| Ages 6-11 | 166 | 328 | 56.5 | 7.55 |
| Ages 12-19 | 222 | 441 | 54.1 | 13.69 |
| Ages 20+ | 1519 | 1961 | 80.8 | 20.3 |
| Ages 2+ | 2011 | 2950 | 74.4 | 18.29 |
| *Females* | | | | |
| Ages 1-2 | 55 | 141 | 44.8 | 4.15 |
| Ages 3-5 | 90 | 162 | 60.8 | 3.61 |
| Ages 6-11 | 166 | 351 | 53.5 | 4.85 |
| Ages 12-19 | 236 | 443 | 59.6 | 10.69 |
| Ages 20+ | 1632 | 2144 | 80.1 | 17.2 |
| Ages 2+ | 2158 | 3180 | 75 | 15.52 |

21. *Please briefly discuss whether there will be increase in the total cumulative dietary exposure to protein in the U.S. diet or if the use of collagen polypeptide produced by E. coli K-12 S1988 is substitutional for other sources of protein and therefore, there would not be an increase in the total dietary exposure to protein.*

    The use of Non-Animal Type 21 Collagen Polypeptide will be substitutional for other collagen uses in conventional foods; therefore, there will not be an increase in the total dietary exposure to protein.

22. *Please provide a statement that the ingredient is not intended to be used in infant formula, products under the jurisdiction of the USDA, or in foods in which standards of identity preclude its use.*

    Non-Animal Type 21 Collagen Polypeptide is not intended to be used in infant formula, products under the jurisdiction of the USDA, or in foods in which standards of identity preclude its use.

23. *Please provide additional discussion for the safety narrative on the genetic modifications described on page 8, including a brief safety discussion of the donor organisms used to modify the recipient strain, E. coli K-12 NCM3722, a brief discussion on if any of the gene deletions described on page 8 are expected to impact safety, and if the presence of the kanamycin resistance gene is expected to impact safety.*

    The host or chassis strain used to generate the final production strain is the *E. coli* K-12 strain NCM3722. As summarized in Chapter 6, Section A. Safety of the Production Strain, *E. coli* K-12 strains are non-toxigenic, non-pathogenic *E. coli* strains that are widely used

in the manufacture of food ingredients that are GRAS. Also, as described in the Notice, *E. coli* NCM3722 was generated from *E. coli* K-12 NCM 196 (ntrA7) by transduction to glutamine prototrophy using a P1*vir* phage grown on the donor strain *E. coli* CGSC 6300. *E. coli* CGSC 6300 is a wild-type *E. coli* K-12 strain, also known as *E. coli* MG1655. Therefore, the genetic material introduced into the genome of *E. coli* K-12 NCM 196 (ntrA7) via transduction, generating *E. coli* NCM3722, originated from an *E. coli* K-12 strain and is not expected to confer toxigenicity or pathogenicity. Additionally, P1*vir* is a mutated form of the well-known P1 bacteriophage. The infectivity of P1*vir* is restricted to the lytic cycle, allowing for transduction of genetic material from the donor's genome to the recipient's genome, such as from the *E. coli* CGSC 6300 genome to the *E. coli* K-12 NCM 196 (ntrA7) genome (Ikeda and Tomizawa, 1965). Lytic phages also typically do not integrate their genomic DNA into the host cell's genome due to their shorter replication cycle (Ranveer et al., 2024). The remaining genetic manipulations to the *E. coli* NCM3722 genome, which occurred during the engineering of *E. coli* S9188, involved standard genetic engineering techniques and well-characterized genes with known functions. None of these additional genes confer toxigenicity or pathogenicity. Therefore, all of the genetic events that occurred during the engineering process are not expected to impact the safety of the resulting production strain.

Presence of the kanamycin resistance gene in the Non-Animal Type 21 Collagen Polypeptide expression plasmid also does not impact the safety of the production strain. As discussed in our response to Question 6, the DNA recombination gene RecA was deleted from the genome, preventing recombination between the expression plasmid and the F plasmid present in the production strain. The production strain and production strain-derived DNA residues are also removed from the ingredient during manufacturing, which has been confirmed by conformity to the *E. coli* product specification and Geltor's PCR analysis for the kanamycin gene in three lots of the final ingredient.

24. *Please provide the following information about the in vitro digestibility experiment:*

    a. *Was SDS-PAGE performed with samples collected at different time points? If yes, please provide a photograph.*

       No, SDS-PAGE was not performed with samples collected at different time points. The in vitro digestibility experiment was conducted using the Megazyme Protein Digestibility Assay Kit (https://www.megazyme.com/documents/Assay_Protocol/K-PDCAAS_DATA.pdf; accessed on May 29, 2024). Resolving the digesta via SDS-PAGE is not included in this protocol.

    b. *Because the optimum pH for pepsin activity is significantly different from that of trypsin and chymotrypsin, was the trypsin-chymotrypsin digestion carried out on the pepsin digesta or was the pepsin versus trypsin-chymotrypsin digestion carried out separately?*

Per the Megazyme Protein Digestibility Assay Kit protocol, the trypsin-chymostrysin digestion was carried out on the pepsin digesta after the pH of the pepsin digesta was adjusted to 7.4.

c. *What was the enzyme: substrate ratio (E/S) used in the experiment(s)?*

The enzyme: substrate ratios used were as follows:

1. Pepsin: 1 mg pepsin/500 mg Non-Animal Type 21 Collagen Polypeptide

2. Trypsin and Chymotrypsin: 1 mg trypsin/1 mg chymotrypsin/500 mg Non-Animal Type 21 Collagen Polypeptide

d. *With the E/S ratio used, please provide a quantitative estimate of digestion over time (e.g., ~50% or ~75%, or ~90% digestion of the original starting material).*

Per the Megazyme Protein Digestibility Assay Kit protocol, the sample was digested with pepsin for 60 min at 37°C and then the digesta were digested with typsin/chymotrypsin for 4 hr at 37°C. The protocol does not specify taking samples over time to estimate the kinetics of the reaction. The protocol also requires the use of casein as a control, which has an in vitro digestibility amino acid score of 1. Importantly, non-animal Type 21 Collagen Polypeptide had an in vitro digestibility amino acid score of 1, indicating that it was digested to a similar extent as casein over the course of the pepsin and trypsin/chymotrypsin digestion periods.

25. *The notifier states that the PDCAAS score of collagen polypeptide produced by E. coli K-12 S1988 is 0.1. Such a low PDCAAS score is reflective of a poor protein quality. Please compare the daily essential amino acids intake from the proposed 90$^{th}$ percentile consumption of collagen polypeptide produced by E. coli K-12 S1988 with the recommended daily intake of the respective essential amino acids. Explain why consuming collagen polypeptide produced by E. coli K-12 S1988 as the (potentially) primary source of protein for consumers on a low protein diet would not be of any concern.*

The recommended daily intake of essential amino acids (histidine, isoleucine, leucine, lysine, methionine plus cysteine, phenylalanine plus tyrosine, threonine, tryptophan, and valine) have been established by the Institute of Medicine for the infants 3-4 months old, children approximately 2 years old, children 10-12 years old, and adults (Institute of Medicine, 2002). Because Non-Animal Type 21 Collagen Polypeptide is not intended for foods consumed by infants, the comparison of the daily essential amino acids intake from the proposed 90$^{th}$ percentile consumption of Non-Animal Type 21 Collagen Polypeptide with the recommended daily intake of the respective essential amino acids was constrained to children 1-2 years old, children 6-11 years old, and adults (20+ years old) using estimated daily intakes of 0.89, 0.44, and 0.48 g/kg/day, respectively, which were not stratified by sex.

The comparison shows that the intake of the essential amino acids from the ingestion of Non-Animal Type 21 Collagen Polypeptide is lower, equivalent, or higher than the recommended daily intake of the corresponding amino acid depending on the amino acid and the consumer cohort (Table 6). For example, the intakes of leucine, methionine plus cysteine, phenylalanine plus tyrosine, threonine, and valine from the ingestion of Non-Animal Type 21 Collagen Polypeptide in children 1-2 are lower than the recommended daily intake for those amino acids whereas the intake of isoleucine and lysine exceed the recommended daily intakes. In children ages 6-11 years old, the intakes of all essential amino acids are lower than the recommended daily intakes. For adults, the intakes of only histidine, methionine and valine are lower than the recommended daily intakes and the intakes of isoleucine, leucine, lysine, threonine, and phenylalanine plus tyrosine all exceed the recommended daily intake. The intakes for tryptophan in all of the age groups could not be calculated because Non-Animal Type 21 Collagen Polypeptide, like all collagen, does not contain tryptophan.

Importantly, as presented in Table 9 of the Notice (page 30; reproduced below with the essential amino acids bolded), the amino acid content of Non-Animal Type 21 Collagen Polypeptide is similar to other commercially available bovine collagen peptide products. Therefore, because the Non-Animal Type 21 Collagen Polypeptide is intended to be used as a substitute for other collagen peptide-containing products that are currently available on the market, the intakes of the essential amino acids will be similar to those following the ingestion of the other collagen peptide-containing products. Additionally, the estimated daily intakes presented in our response to Question 20 represent the worst-case scenario, assuming that consumers ingest only those products that contain Non-Animal Type 21 Collagen Polypeptide in a day. They do not account for the ingestion of other products that contain other sources of protein, which would contain the essential amino acids. Comparing the results presented in Table 17 of the Notice to the EDIs presented in Table 4 above, it is apparent that consumers typically consume 2-5-fold more protein per day than the estimated 90$^{th}$ percentile daily intake of Non-Animal Type 21 Collagen Polypeptide, indicating that the ingestion of Non-Animal Type 21 Collagen Polypeptide, and for that matter any other commercially available collagen peptide-containing product, accounts for only part of the consumers total daily intake of protein. Therefore, considering that Non-Animal Type 21 Collagen Polypeptide is intended to be a substitute for other commercially-available collagen peptide-containing products, it is reasonable to conclude that consuming Non-Animal Type 21 Collagen Polypeptide as the (potentially) primary source of protein for consumers on a low protein diet will not be of concern.

**Table 6. Comparison of Essential Amino Acid Intake from Non-Animal Type 21 Collagen Polypeptide with the Required Amino Acid Intake for Children and Adults**

| Amino Acid | Amino Acid Content of Non-Animal Type 21 Collagen Polypeptide (% weight of product) [a] | Intake for Children 1-2 years old from Non-Animal Type 21 Collagen Polypeptide (mg/kg)[b] | Amino Acid Requirement for Children Ages 2 (mg/kg)[c] | Intake for Children Ages 6-11 years old from Non-Animal Type 21 Collagen Polypeptide (mg/kg)[b] | Amino Acid Requirement for Children Ages 10-12 (mg/kg)[c] | Intake for Males and Females Ages 20+ years old from Non-Animal Type 21 Collagen Polypeptide (mg/kg) [b] | Amino Acid Requirement for Adults (mg/kg)[c] |
|---|---|---|---|---|---|---|---|
| Histidine | 0.66 | 5.9 | ? | 2.9 | ? | 3.2 | 8–12 |
| Isoleucine | 3.83 | 34.1 | 31 | 16.9 | 28 | 18.4 | 10 |
| Leucine | 4.79 | 42.6 | 73 | 21.1 | 42 | 23.0 | 14 |
| Lysine | 9.45 | 84.1 | 64 | 41.6 | 44 | 45.4 | 12 |
| Methionine plus cystine | 1.21 | 10.8 | 27 | 5.3 | 22 | 5.8 | 13 |
| Phenylalanine plus tyrosine | 3.04 | 19.0 | 69 | 13.4 | 22 | 14.6 | 14 |
| Threonine | 1.85 | 16.5 | 37 | 8.1 | 28 | 8.9 | 7 |
| Tryptophan | ND | NA | 12.5 | NA | 3.3 | NA | 3.5 |
| Valine | 0.47 | 4.2 | 38 | 2.1 | 25 | 2.3 | 10 |
| Total without histidine | 24.6 | 217.2 | 352 | 108.4 | 214 | 118.3 | 84 |

Abbreviations: ND – not detected; NA – not available

[a]Obtained from Table 9 in GRN 001171.

[b]Calculated by multiplying the 90$^{th}$ percentile EDI for respective age group by the amino acid content of Non-Animal Type 21 Collagen Polypeptide. The EDIs for children 1-2 years old, children 6-11 years old, and adults (20+ years old) are 0.89, 0.44, and 0.48 g/kg/day, respectively.

[c]Obtained from Institute of Medicine (2002).

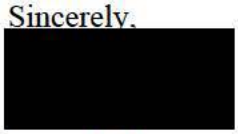
**Table 9. Protein and Amino Acid Characterization of Non-Animal Type 21 Collagen Polypeptide and Bovine Collagen Peptides**

| Parameter | Non-Animal Type 21 Collagen Polypeptide (% weight product)* | Bovine Collagen Peptides (% weight product) |
|---|---|---|
| Crude Protein | 90.52 | 92.52 |
| Alanine | 2.07 | 7.63 |
| Arginine | 5.28 | 6.31 |
| Asparagine/Aspartic acid | 4.51 | 5.12 |
| **Cysteine** | **ND** | **ND** |
| Glutamic acid/Glutamine | 16.70 | 8.86 |
| Glycine | 17.67 | 17.46 |
| **Histidine** | **0.66** | **0.48** |
| **Isoleucine** | **3.83** | **1.26** |
| **Leucine** | **4.79** | **2.55** |
| **Lysine** | **9.45** | **3.03** |
| **Methionine** | **1.21** | **0.70** |
| **Phenylalanine** | **2.14** | **1.57** |
| Proline | 12.22 | 10.60 |
| Sarcosine | ND | ND |
| Serine | 3.80 | 2.67 |
| **Threonine** | **1.85** | **1.49** |
| **Tryptophan** | **ND** | **ND** |
| **Tyrosine** | **0.90** | **0.38** |
| **Valine** | **0.47** | **1.85** |

Abbreviations: ND, not detected.
*Average of two measurements due to exclusion of an outlier.

Should you need additional information, please feel free to contact me at +1-240-367-6089 or dconze@spherixgroup.com.

Sincerely,



Dietrich B. Conze, Ph.D.
Managing Partner

## References

Brown, S. D., and Jun, S. (2015). Complete Genome Sequence of Escherichia coli NCM3722. Genome Announc *3*, e00879–15.

Browning, D. F., Hobman, J. L., and Busby, S. J. W. (2023). Laboratory strains of Escherichia coli K-12: things are seldom what they seem. Microb Genom *9*, mgen000922.

Gorissen, S. H. M., Crombag, J. J. R., Senden, J. M. G., Waterval, W. A. H., Bierau, J., Verdijk, L. B., and van Loon, L. J. C. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids *50*, 1685–1695.

Ikeda, H., and Tomizawa, J. I. (1965). Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. J Mol Biol *14*, 85–109.

Institute of Medicine (2002). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. National Academy Press: Washington, DC, USA *5*, 589–768.

Ranveer, S. A., Dasriya, V., Ahmad, M. F., Dhillon, H. S., Samtiya, M., Shama, E., Anand, T., Dhewa, T., Chaudhary, V., Chaudhary, P. et al. (2024). Positive and negative aspects of bacteriophages and their immense role in the food chain. NPJ Sci Food *8*, 1.

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| Beverages and beverage bases | Flavored beverages and beverage bases, excluding soft drinks and energy drinks | 7104: Diet sport and energy drinks | 2.0% | 95322200 | Sports drink, low calorie (Gatorade G2) -all flavors |
| | | | | 95322500 | Sports drink, low calorie (Powerade Zero) - all flavors |
| | | | | 95323000 | Sports drink, low calorie - NS as to brand; light; diet; Zero; sugar free; fruit flavored thirst quencher beverage |
| | | 7106: Other diet drinks | | 92513010 | Slush frozen drink, no sugar added - Slurpee Lite; no dairy; light slushee or slush; light Slush Puppy; reduced sugar; no sugar added; sugar free |
| | | | | 92550400 | Vegetable and fruit juice drink, with high vitamin C, diet - Diet V8 Splash, all flavors; low calorie |
| | | | | 92550610 | Fruit flavored drink, with high vitamin C, diet - Diet Ocean Spray Juice Drinks, all flavors; Diet Ocean Spray cranberry, blueberry, or pomegranate blends |
| | | | | 92550620 | Fruit flavored drink, diet - Crystal Light; Minute Maid Light lemonade and other fruit flavored drinks; low calorie |
| | | | | 92552000 | Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet - Sugar Free Tang; Country Time Lite lemonade; Ocean Spray drink mix; low calorie |
| | | | | 92552010 | Fruit flavored drink, powdered, reconstituted, diet - Crystal Light; Sugar Free Kool-Aid; Wyler's Light; lemonade from low calorie powdered mix, NS as to brand |
| | | 7206: Sport and energy drinks | | 95320200 | Sports drink (Gatorade G) - all flavors; Gatorade, NFS |
| | | | | 95320500 | Sports drink (Powerade) - all flavors |
| | | | | 95321000 | Sports drink, NFS - All Sport; fruit flavored thirst quencher beverage; NS as to brand |
| | | 7802: Flavored or carbonated water | | 94100200 | Water, bottled, sweetened, with low calorie sweetener - flavored; Fruit 2 O; water, fruit flavored, sweetened, NS as to sweetener; Aquafina Flavor Splash; Dasani Flavored Water |
| | | | | 94100300 | Water, bottled, flavored (Capri Sun Roarin' Waters) - all flavors |
| | | 7804: Enhanced or fortified water | | 94210100 | Water, bottled, flavored (Propel Water) - all flavors; with calcium |
| | | | | 94210200 | Water, bottled, flavored (Glaceau Vitamin Water) - all flavors |
| | | | | 94210300 | Water, bottled, flavored (SoBe Life Water) - all flavors |
| | | | | 94220215 | Water, bottled, flavored, sugar free (Glaceau Vitamin Water) - Glaceau Vitamin Water 10; Glaceau Vitamin Water Zero; zero calorie |
| | | | | 94220310 | Water, bottled, flavored, sugar free (SoBe) - all flavors; 0 calories; SoBe Life Water zero calories; zero calorie |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | 9999: Not included in a food category | | 92900100 | Fruit flavored drink, with high vitamin C, powdered, not reconstituted - Tang, all flavors |
| | | | | 92900110 | Fruit flavored drink, powdered, not reconstituted - Kool-Aid, NS as to sweetener; Wylers, NS as to sweetener; Country Time lemonade; Flavor Aid; powdered lemonade, NS as to brand; store brand |
| | | | | 92900200 | Fruit flavored drink, powdered, not reconstituted, diet - Crystal Light; Sugar Free Kool-aid; Wyler's Light; lemonade from low calorie powdered mix, NS as to brand |
| | | | | 92900300 | Sports drink, dry concentrate, not reconstituted - Gatorade; fruit flavored thirst quencher beverage; NS as to brand |
| Coffee and Tea | Coffee and tea pods | 7302: Coffee | 2.0% | 92100500 | Coffee, NS as to brewed or instant - coffee singles, bags, pods, or K-cups |
| | | | | 92101000 | Coffee, brewed - coffee, brewed, NS as to regular or decaffeinated; coffee singles, bags, pods, or K-cups |
| | | | | 92101500 | Coffee, brewed, blend of regular and decaffeinated - half-caf; reduced caffeine; coffee singles, bags, pods or K-cups |
| | | | | 92101700 | Coffee, brewed, flavored - coffee singles, bags, pods, or K-cups |
| | | | | 92102400 | Iced Coffee, brewed - unsweetened; coffee singles, bags, pods, or K-cups; NS as to regular or decaffeinated |
| | | | | 92102401 | Iced Coffee, brewed, decaffeinated - unsweetened; coffee singles, bags, pods, or K-cups |
| | | | | 92111000 | Coffee, NS as to brewed or instant, decaffeinated - coffee singles, bags, pods, or K-cups |
| | | | | 92111010 | Coffee, brewed, decaffeinated - coffee singles, bags, pods, or K-cups |
| | Instant coffee and tea | | 5.5% | 92103000 | Coffee, instant, reconstituted - powdered mix; NS as to regular or decaffeinated; made from liquid concentrate |
| | | | | 92104000 | Coffee, instant, 50% less caffeine, reconstituted - powdered mix; blend of regular and decaf; half-caf; reduced caffeine |
| | | | | 92114000 | Coffee, instant, decaffeinated, reconstituted - powdered mix |
| | | | | 92121000 | Coffee, instant, pre-lightened and pre-sweetened with sugar, reconstituted - powdered mix; NS as to sweetener; Maxwell House International, flavors other than chocolate, cocoa, or mocha |
| | | | | 92121001 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with sugar, reconstituted - powdered mix; Maxwell House International, flavors other than chocolate, cocoa, or mocha |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 92121010 | Coffee, instant, pre-sweetened with sugar, reconstituted - powdered mix; NS as to sweetener |
| | | | | 92121020 | Coffee, mocha, instant, pre-lightened and pre-sweetened with sugar, reconstituted - NS as to sweetener; powdered mix; coffee and cocoa mix; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92121030 | Coffee, mocha, instant, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92121040 | Coffee, instant, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha |
| | | | | 92121041 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha |
| | | | | 92121050 | Coffee, mocha, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | 9999: Not included in a food category | | 92191100 | Coffee, instant, not reconstituted - powdered mix; NS as to regular or decaffeinated; liquid concentrate |
| | | | | 92191105 | Coffee, instant, 50% less caffeine, not reconstituted - powdered mix; blend of regular and decaf; half-caf; reduced caffeine |
| | | | | 92191200 | Coffee, instant, decaffeinated, not reconstituted - powdered mix |
| | | | | 92191400 | Coffee, instant, pre-sweetened with sugar, not reconstituted - powdered mix; NS as to sweetener |
| | | | | 92192000 | Coffee, mocha, instant, pre-lightened and pre-sweetened with sugar, not reconstituted - powdered mix; coffee and cocoa mix; NS as to sweetener; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92192030 | Coffee, mocha, instant, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92192040 | Coffee, mocha, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 92193000 | Coffee, instant, pre-lightened and pre-sweetened with sugar, not reconstituted - powdered mix; NS as to sweetener; Maxwell House International Coffee flavors other than chocolate, cocoa, or mocha |
| | | | | 92193005 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with sugar, not reconstituted - powdered mix; Maxwell House International Coffee; flavors other than chocolate, cocoa, or mocha |
| | | | | 92193020 | Coffee, instant, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha; sugar free |
| | | | | 92193025 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; sugar free; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha |
| | | | | 92291300 | Coffee substitute, dry powder |
| | | | | 92307000 | Tea, iced, instant, black, unsweetened, dry - unreconstituted powdered mix; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92307400 | Tea, iced, instant, black, pre-sweetened, dry - unreconstituted powdered mix; NS as to sweetener; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | Packaged coffee and tea beverages | 7302: Coffee | | 92100000 | Coffee, NS as to type |
| | | | | 92171000 | Coffee, bottled/canned - NS as to brand or variety; from carton; all flavors; all brands; all varieties |
| | | | | 92171010 | Coffee, bottled/canned, light - NS as to brand or variety; from carton; all flavors; all brands; all varieties |
| | | 7304: Tea | | 92305010 | Tea, iced, instant, black, unsweetened - powdered mix; liquid concentrate; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305040 | Tea, iced, instant, black, pre-sweetened with sugar - powdered mix; liquid concentrate; NS as to sweetener; sweet tea; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305050 | Tea, iced, instant, black, decaffeinated, pre-sweetened with sugar - powdered mix; liquid concentrate; NS as to sweetener; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305090 | Tea, iced, instant, black, pre-sweetened with low calorie sweetener - powdered mix; liquid concentrate; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; light, reduced calorie, diet, sugar free |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 92305110 | Tea, iced, instant, black, decaffeinated, pre-sweetened with low calorie sweetener - powdered mix; liquid concentrate; light; reduced calorie; diet; sugar free; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305180 | Tea, iced, instant, black, decaffeinated, unsweetened - powdered mix; liquid concentrate; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305900 | Tea, iced, instant, green, unsweetened - powdered mix; liquid concentrate; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated |
| | | | | 92305910 | Tea, iced, instant, green, pre-sweetened with sugar - powdered mix; liquid concentrate; white tea; herbal tea; NS as to sweetener; green and fruit, herbal, or white tea blends; decaffeinated |
| | | | | 92305920 | Tea, iced, instant, green, pre-sweetened with low calorie sweetener - powdered mix; liquid concentrate; light, reduced calorie, diet, sugar free; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated |
| | | | | 92307500 | Iced Tea/Lemonade juice drink - half and half beverage, all flavors; Arizona Half & Half; Arnold Palmer Half & Half |
| | | | | 92307510 | Iced Tea/Lemonade juice drink, light - half and half beverage, all flavors, light; Arizona Lite Half & Half; Arnold Palmer Lite Half & Half |
| | | | | 92307520 | Iced Tea / Lemonade juice drink, diet - diet half and half beverage, all flavors; Diet Snapple Half n' Half; Arnold Palmer Lite Half & Half, made from powdered mix |
| | | | | 92309000 | Tea, iced, bottled, black - can, carton, or fountain; sweet tea; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; Snapple, all flavors |
| | | | | 92309010 | Tea, iced, bottled, black, decaffeinated - can, carton, or fountain; sweet tea; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; caffeine free Snapple Tea, all flavors |
| | | | | 92309020 | Tea, iced, bottled, black, diet - light, reduced calorie, or sugar free; can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; Diet Snapple Tea, all flavors |
| | | | | 92309030 | Tea, iced, bottled, black, decaffeinated, diet - light, reduced calorie, or sugar free; can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; caffeine free Diet Snapple Tea, all flavors |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 92309040 | Tea, iced, bottled, black, unsweetened - can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92309050 | Tea, iced, bottled, black, decaffeinated, unsweetened - can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92309500 | Tea, iced, bottled, green - can, carton, or fountain; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated; Snapple |
| | | | | 92309510 | Tea, iced, bottled, green, diet - light, reduced calorie, or sugar free; can, carton, or fountain; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated; Diet Snapple |
| | | | | 92309520 | Tea, iced, bottled, green, unsweetened - can, carton, or fountain; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated |
| Dairy products and substitutes | Milk substitutes | 1404: Milk substitutes | 2.5% | 11300100 | Non-dairy milk, NFS |
| | | | | 11320000 | Soy milk - Edensoy; vanilla flavored; Silk; West Soy; Soymilk; NS as to type; Vitasoy |
| | | | | 11320100 | Soy milk, light - vanilla flavored; Silk; Vitasoy; West Soy; Soymilk |
| | | | | 11320200 | Soy milk, nonfat - vanilla flavored; West Soy; Soymilk |
| | | | | 11321000 | Soy milk, chocolate - Silk; Belsoy; Soymilk |
| | | | | 11321100 | Soy milk, light, chocolate - Silk; Vitasoy; Soymilk |
| | | | | 11321200 | Soy milk, nonfat, chocolate - Soymilk |
| | | | | 11350000 | Almond milk, sweetened - vanilla flavored; NS as to sweetened; cashew |
| | | | | 11350010 | Almond milk, sweetened, chocolate - NS as to sweetened; cashew |
| | | | | 11350020 | Almond milk, unsweetened - unsweetened vanilla; flavors other than chocolate; cashew |
| | | | | 11350030 | Almond milk, unsweetened, chocolate - Blue Diamond Almond Breeze; Silk PureAlmond; Almond Dream |
| | | | | 11360000 | Rice milk - Rice Dream beverage |
| | | | | 11370000 | Coconut milk - Silk PureCoconut beverage; So Delicious |
| | | | | 11512030 | Hot chocolate/Cocoa, ready to drink, made with non-dairy milk - almond, coconut, rice, or soy milk; chocolate espanol; chocolate caliente; Starbucks; made from syrup, tablet, or bar |
| | | | | 11512120 | Hot chocolate/Cocoa, ready to drink, made with non-dairy milk and whipped cream - almond, coconut, rice, or soy milk; Starbucks; made from syrup, tablet, or bar |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 11513310 | Chocolate milk, made from dry mix with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513375 | Chocolate milk, made from reduced sugar mix with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513385 | Chocolate milk, made from dry mix with non-dairy milk (Nesquik) - almond, coconut, rice, or soy milk |
| | | | | 11513395 | Chocolate milk, made from no sugar added dry mix with non-dairy milk (Nesquik) - almond, coconut, rice, or soy milk |
| | | | | 11513750 | Chocolate milk, made from syrup with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513805 | Chocolate milk, made from light syrup with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513855 | Chocolate milk, made from sugar free syrup with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11514150 | Hot chocolate/Cocoa, made with dry mix and non-dairy milk - almond, coconut, rice, and soy milk; cocoa mix, NFS; Swiss Miss; Hershey's; Nestle's; Carnation |
| | | | | 11514360 | Hot chocolate/Cocoa, made with no sugar added dry mix and non-dairy milk - almond, coconut, rice, and soy milk; fat free, light, diet or sugar free mix; Swiss Miss; Hershey's; Nestle's; Carnation |
| | | | | 11519215 | Strawberry milk, non-dairy - almond, coconut, rice, or soy milk |
| | | | | 42401010 | Coconut milk, used in cooking |
| | | | | 42402010 | Coconut cream, canned, sweetened - Coco Lopez |
| | Probiotic and yogurt beverages | 1002: Milk, whole | | 11115300 | Buttermilk, whole |
| | | 1004: Milk, reduced fat | | 11115200 | Buttermilk, reduced fat (2%) 2% fat, made with nonfat milk solids |
| | | 1008: Milk, nonfat | | 11115000 | Buttermilk, fat free (skim) - nonfat buttermilk |
| | | 1820: Yogurt, regular | | 11436000 | Yogurt, liquid - all types and flavors |
| Coffee Creamer | Coffee creamer (liquid and | 8008: Cream and cream substitutes | 20.0% | 12100100 | Cream, NS as to light, heavy, or half and half |
| | | | | 12110100 | Cream, light - coffee cream; table cream |
| | | | | 12120100 | Cream, half and half - plain |
| | | | | 12120106 | Cream, half and half, flavored |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | powder forms) | | | 12120110 | Cream, half and half, fat free - low fat; plain or flavored |
| | | | | 12130100 | Cream, heavy - whipping cream; NS as to heavy or light |
| | | | | 12200100 | Coffee creamer, NFS |
| | | | | 12210200 | Coffee creamer, liquid - frozen; Coffee Mate; Carnation; Coffee Rich; Dunkin Donuts; International Delight; plain |
| | | | | 12210210 | Coffee creamer, liquid, flavored - Coffee Mate; Bailey's; Bliss; Carnation; Coffee Rich; Dunkin Donuts; International Delight; Mocha Mix |
| | | | | 12210260 | Coffee creamer, liquid, fat free - low fat; light; lite; Coffee Mate; Carnation; Coffee Rich; Dunkin Donuts; International Delight; plain |
| | | | | 12210270 | Coffee creamer, liquid, fat free, flavored - light; lite; Coffee Mate; low fat; Carnation; Coffee Rich; Dunkin Donuts; International Delight; Mocha Mix |
| | | | | 12210280 | Coffee creamer, liquid, fat free, sugar free, flavored - low fat; light; lite; Coffee Mate; Carnation; International Delight; Mocha Mix |
| | | | | 12210310 | Coffee creamer, liquid, sugar free, flavored - Coffee Mate; Carnation; International Delight; Mocha Mix |
| | | | | 12210400 | Coffee creamer, powder - Coffee Mate; Cremora; Carnation; plain |
| | | | | 12210420 | Coffee creamer, powder, flavored - Coffee Mate powder, flavored; Carnation |
| | | | | 12210430 | Coffee creamer, powder, fat free - light; lite; low fat; Coffee Mate; Carnation; Cremora; plain |
| | | | | 12210440 | Coffee creamer, powder, fat free, flavored - light; lite; Coffee Mate; low fat; Carnation |
| | | | | 12210505 | Coffee creamer, powder, sugar free, flavored - fat free; light; lite; low fat; Coffee Mate; Carnation |
| | | | | 12210520 | Coffee creamer, soy, liquid - almond; Silk; So Delicious; coconut milk creamer |
| Cereal products | Cereal bars | 5202: Crackers, excludes saltines | 12.5% | 54103000 | Crackers, breakfast biscuit - BelVita, all flavors; Belvita Soft Baked |
| | | 5402: Cereal bars | | 53712000 | Snack bar, oatmeal - Quaker Oatmeal To Go, all flavors; Quaker Breakfast Cookie, all flavors |
| | | | | 53712100 | Cereal or Granola bar, NFS - with chocolate chips; New Trail Granola Bars; with oats, sugar, raisins, coconut; Sunbelt Granola Bar, all flavors |
| | | | | 53712200 | Cereal or granola bar, lowfat, NFS - all flavors; with oats, fruit and nuts, lowfat; Sunbelt Lowfat Chewy Granola Bar |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 53712210 | Cereal or granola bar, nonfat - all fruit and nut flavors; Health Valley Fat-Free Granola Bars |
| | | | | 53713000 | Cereal or granola bar, reduced sugar, NFS - all flavors |
| | | | | 53713010 | Cereal or granola bar, fruit and nut |
| | | | | 53713100 | Cereal or granola bar, peanuts , oats, sugar, wheat germ |
| | | | | 53714200 | Cereal or granola bar, chocolate coated, NFS - Kudos Granola Bars, all flavors; Nestle Sweet Success Bar |
| | | | | 53714210 | Cereal or granola bar, with coconut, chocolate coated - Little Debbie Macaroo Fudge Dipped Chewy Granola Bar; Sunbelt Fudge Dipped Chewy Granola Bar, all flavors |
| | | | | 53714220 | Cereal or granola bar with nuts, chocolate coated - Kudos Crunchy Nut Snack |
| | | | | 53714230 | Cereal or granola bar, oats, nuts, coated with non-chocolate coating - all flavors |
| | | | | 53714250 | Cereal or granola bar, coated with non-chocolate coating - all flavors; Quaker Oats Peanut Butter Whipps; Jenny's Cuisine Fruit and Nut Snack Bar |
| | | | | 53714300 | Cereal or granola bar, high fiber, coated with non-chocolate yogurt coating - lemon Fi-bar; raspberry Fi-bar; Ultra Slim Fast Vanilla Almond Crunch Snack Bar |
| | | | | 53714400 | Cereal or granola bar, with rice cereal - Kellogg's Rice Krispies Bar |
| | | | | 53714500 | Breakfast bar, NFS |
| | | | | 53714510 | Breakfast bar, date, with yogurt coating - Jenny's Cuisine Breakfast Bar |
| | | | | 53714520 | Breakfast bar, cereal crust with fruit filling, lowfat |
| | Hot breakfast cereals | 4804: Grits and other cooked cereals | | 56200300 | Cereal, cooked, NFS |
| Nutritional foods | Nutritional bars | 5404: Nutrition bars | 25.0% | 53720100 | Nutrition bar (Balance Original Bar) - all flavors |
| | | | | 53720200 | Nutrition bar (Clif Bar) - all flavors; Clif Minis |
| | | | | 53720210 | Nutrition bar (Clif Kids Organic Zbar) - all flavors |
| | | | | 53720300 | Nutrition bar (PowerBar) - all flavors; PowerBar energy bar |
| | | | | 53720400 | Nutrition bar (Slim Fast Original Meal Bar) - Slim Fast meal-on-the-go and granola meal bars, all flavors |
| | | | | 53720500 | Nutrition bar (Snickers Marathon Protein Bar) |
| | | | | 53720600 | Nutrition bar (South Beach Living Meal Bar) - all flavors |
| | | | | 53720610 | Nutrition bar (South Beach Living High Protein Bar) - all flavors |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 53720700 | Nutrition bar (Tiger's Milk) |
| | | | | 53720800 | Nutrition bar (Zone Perfect Classic Crunch) - all flavors |
| | | | | 53729000 | Nutrition bar or meal replacement bar, NFS |
| | Nutritional beverages and beverage bases | 7208: Nutritional beverages | 4.5% | 95101000 | Nutritional drink or shake, ready-to-drink (Boost) - Boost Nutritional Energy Drink |
| | | | | 95101010 | Nutritional drink or shake, ready-to-drink (Boost Plus) |
| | | | | 95102000 | Nutritional drink or shake, ready-to-drink (Carnation Instant Breakfast) - instant breakfast, ready-to-drink, NFS |
| | | | | 95103000 | Nutritional drink or shake, ready-to-drink (Ensure) - Ensure, NFS; all flavors; Bone Health; Immune Health; Muscle Health; high calcium; high protein |
| | | | | 95103010 | Nutritional drink or shake, ready-to-drink (Ensure Plus) - all flavors; Clinical Strength |
| | | | | 95104000 | Nutritional drink or shake, ready-to-drink, sugar free (Glucerna) - Glucerna shakes, all flavors |
| | | | | 95105000 | Nutritional drink or shake, ready-to-drink (Kellogg's Special K Protein) - all flavors |
| | | | | 95106000 | Nutritional drink or shake, ready-to-drink (Muscle Milk) |
| | | | | 95106010 | Nutritional drink or shake, ready-to-drink, light (Muscle Milk) |
| | | | | 95110000 | Nutritional drink or shake, ready-to-drink (Slim Fast) - all flavors; 3-2-1 Plan; Optima; meal replacement |
| | | | | 95110010 | Nutritional drink or shake, ready-to-drink, sugar free (Slim Fast) - all flavors; 3-2-1 Plan; lower carb; meal replacement |
| | | | | 95110020 | Nutritional drink or shake, high protein, ready-to-drink (Slim Fast) - all flavors; 3-2-1 Plan; meal replacement |
| | | | | 95120000 | Nutritional drink or shake, ready-to-drink, NFS - brands such as Nutrament and Equate meal replacement shake; meal replacement |
| | | | | 95120010 | Nutritional drink or shake, high protein, ready-to-drink, NFS - brands such as Monster Milk and EAS Myoplex; meal replacement, NFS |
| | | | | 95120020 | Nutritional drink or shake, high protein, light, ready-to-drink, NFS - brands such as Myoplex Lite; meal replacement |
| | | | | 95120050 | Nutritional drink or shake, liquid, soy-based - Isocal liquid nutrition; Osmolite liquid nutrition; meal replacement |
| | Protein Powders | | 95.0% | 95201000 | Nutritional powder mix (Carnation Instant Breakfast) - instant breakfast, powdered, NFS |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | 9802: Protein and nutritional powders | | 95201010 | Nutritional powder mix, sugar free (Carnation Instant Breakfast) |
| | | | | 95201200 | Nutritional powder mix (EAS Whey Protein Powder) - all flavors |
| | | | | 95201300 | Nutritional powder mix (EAS Soy Protein Powder) - all flavors |
| | | | | 95201500 | Nutritional powder mix, high protein (Herbalife) - all flavors; Healthy Meal nutritional shake mix |
| | | | | 95201600 | Nutritional powder mix (Isopure) - all flavors |
| | | | | 95201700 | Nutritional powder mix (Kellogg's Special K20 Protein Water) - all flavors |
| | | | | 95202000 | Nutritional powder mix (Muscle Milk) - all flavors |
| | | | | 95202010 | Nutritional powder mix, light (Muscle Milk) - all flavors |
| | | | | 95210000 | Nutritional powder mix (Slim Fast) - all flavors; 3-2-1 Plan; Optima |
| | | | | 95210010 | Nutritional powder mix, sugar free (Slim Fast) - all flavors; 3-2-1 Plan; lower carb |
| | | | | 95210020 | Nutritional powder mix, high protein (Slim Fast) - all flavors; 3-2-1 Plan |
| | | | | 95220000 | Nutritional powder mix, NFS - meal replacement, NFS |
| | | | | 95220010 | Nutritional powder mix, high protein, NFS - brands such as D.E.L.T.A. Enhance Formula and Joe Weider's Dynamic Protein Shake; Monster Milk; meal replacement |
| | | | | 95230000 | Nutritional powder mix, whey based, NFS - protein shake mix; brands such as Gold Standard, NOW Foods |
| | | | | 95230010 | Nutritional powder mix, protein, soy based, NFS - protein shake mix; brands such as Gold Standard, NOW Foods |
| | | | | 95230020 | Nutritional powder mix, protein, light, NFS - protein shake mix; brands such as Reliv Now, Beneprotein |
| | | | | 95230030 | Nutritional powder mix, protein, NFS - protein shake mix, NFS; brands such as NOW Foods, NFS and Gold Standard, NFS |
| Chocolate confections | Chocolate confections | 5702: Candy containing chocolate | 10.0% | 91701010 | Almonds, chocolate covered |
| | | | | 91703040 | Caramel candy, chocolate covered - Black Cow; Caramello; Chew-Its; Marathon Bar; Milk Duds; Pom Poms; Sugar Momma; Riesen Chocolate Chew; Russell Stover Caramel Heart |
| | | | | 91703050 | Caramel with nuts and cereal, chocolate covered |
| | | | | 91703060 | Caramel with nuts, chocolate covered - Goo Goo Cluster(s); Peanut Chews; Toffifay; Turtles; Reese's NutRageous |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 91703600 | Espresso coffee beans, chocolate-covered - Millstone Milk Chocolate-covered Espresso Beans |
| | | | | 91705010 | Milk chocolate candy, plain - chocolate candy, NFS; Easter Egg, NFS; Easter Egg, milk chocolate; Hershey Bar; Hershey Kiss or Star; Hershey Symphony Bar; Nestle Bar; Chocolate coins; Hershey's Hugs; Hershey's Nuggets; Easter bunnies; chocolate rose; Dove Milk Chocolate bar; chocolate balls; Cadbury Dairy Milk; Dove Promises |
| | | | | 91705020 | Milk chocolate candy, with cereal - Krackel Bar; Malted Milk Balls; Nestle Crunch; Whoppers; Hershey's Cookies 'n' Mint; Hershey's Cookies 'n' Mint Nuggets; Malted Milk Eggs |
| | | | | 91705040 | Chocolate, milk, with nuts, not almond or peanuts - Brach's Bridge Mix; Chunky with pecans; Hazelnut with chocolate (Cadbury); Ferrero Rocher Hazelnut Chocolate |
| | | | | 91705050 | Milk chocolate candy, with fruit and nuts - Chunky with fruit and nuts; Chunky, NFS; Chunky Original; Cadbury |
| | | | | 91705060 | Milk chocolate candy, with almonds - Hershey with Almonds; Nestle chocolate bar with almonds; Hershey Kisses with Almonds; Hershey's Nuggets with Almonds; Cadbury Roasted Almonds; Hershey Bites with Almonds |
| | | | | 91705070 | Chocolate, milk, with peanuts - Chunky with peanuts; Mr. Goodbar |
| | | | | 91705090 | Chocolate candy with fondant and caramel - Sky Bar |
| | | | | 91705200 | Chocolate, semi-sweet morsel - Toll House morsels; chocolate chips; Hershey's Mini Kisses Brand Milk Chocolates |
| | | | | 91705300 | Chocolate, sweet or dark - Special Dark; Dove Dark Chocolate bar; Hershey's Special Dark Kisses |
| | | | | 91705310 | Chocolate, sweet or dark, with almonds - Hershey's Special Dark with Almonds; Hershey's Special Dark with Almonds Nuggets |
| | | | | 91705500 | Mexican chocolate, tablet |
| | | | | 91706000 | Coconut candy, chocolate covered - Almond Joy; Bounty; Easter egg, chocolate covered coconut; Mounds |
| | | | | 91713010 | Fudge, chocolate, chocolate-coated - Butter Fudge, Mary Sue |
| | | | | 91713020 | Fudge, chocolate, chocolate-coated, with nuts |
| | | | | 91713030 | Fudge, chocolate - fudge, NFS; fudge, plain |
| | | | | 91713040 | Fudge, chocolate, with nuts |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 91715000 | Fudge, caramel and nut, chocolate-coated candy - Butternut Bar; Oh Henry; Powerhouse; Clark Bun Bar |
| | | | | 91718200 | Chocolate-flavored sprinkles - Jimmies |
| | | | | 91723010 | Marshmallow, chocolate covered - Easter egg, chocolate covered marshmallow; Mallo Cup; Marshmallow rabbit, chocolate covered |
| | | | | 91727010 | Nuts, chocolate covered, not almonds or peanuts - nuts, chocolate covered, NFS |
| | | | | 91728000 | Nut roll, fudge or nougat, caramel and nuts - Big Hunk; Payday; Chocolaty Payday; Pecan roll |
| | | | | 91731000 | Peanuts, chocolate covered - Goobers; Peanut Clusters |
| | | | | 91733200 | Peanut Bar, chocolate covered candy |
| | | | | 91734000 | Peanut butter, chocolate covered - peanut butter treats; peanut butter candy; Peanut Butter Meltaway Crispy Bar (Brach's); Easter egg, peanut butter, chocolate covered; Peanut Butter Meltaway Bar (Brach's); Planters Peanut Butter Chocolates |
| | | | | 91739010 | Raisins, chocolate covered - Raisinets; Raisin clusters |
| | | | | 91746010 | Sugar-coated chocolate discs - Nonpareils; Nestle Sno Caps; snow caps |
| | | | | 91760100 | Toffee, chocolate covered - Heath Bar; SKOR; Heath Sensations; Sweet Escapes Chocolate Toffee Crisp Candy Bar |
| | | | | 91760200 | Toffee, chocolate-coated, with nuts - Almond Roca |
| | | | | 91760500 | Truffles |
| | | | | 91770030 | Dietetic or low calorie candy, chocolate covered - Dietetic Chocolate TV Mix; Dietetic Chocolate Wafers; Dietetic Covered Raisins; Dietetic Crunch Bar; Dietetic Fruit and Nut Bar; Dietetic Peanut Butter Cups; Dietetic Milk Almond Bar; Dietetic Milk Chocolate Flavored Bar |
| Non-chocolate confections | Gummies | 5704: Candy not containing chocolate | 10.0% | 91708020 | Soft fruit confections - soft fruit candy; Aplets; Cotlets; Fruit Delights, all flavors; with nuts |
| | | | | 91708030 | Fruit leather and fruit snacks candy - Fruit Roll-Up; Fruit Wrinkles; Fun Fruits; Teenage Mutant Ninja Turtles Fruit Snacks; Soda-licious; Farley's Fruit Snacks; Gushers Fruit Snacks; Fruit By The Foot; Betty Crocker Fruit String Thing; Sunbelt Fruit Jammers |
| | | | | 91708100 | Fruit snacks candy, with high vitamin C - Brach's Hi-C Fruit Snacks; Kellogg's Right Bites Fruit Snacks; Welch's Fruit Snacks; Florida's Natural Pocket Fruit-to-go Stiks, all flavors |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 91708150 | Yogurt covered fruit snacks candy, with added vitamin C - Kellogg's Yogos Bits, all flavors |
| | | | | 91708160 | Yogurt covered fruit snacks candy rolls, with high vitamin C - Kellogg's Yogos Rollers, all flavors |
| | | | | 91745010 | Gumdrops - gummy bears; gummy worms; gummy fish; gummy dinosaurs; Hot tamales; Jelly beans; Jujubes; Jujy Fruits; Mike and Ike; Mint Leaves; Chuckles; Dots; Fruit slices, jellied; Good and Fruity; Brach's Rocks; spice sticks and drops; Life Savers Gummi Savers; Gummy animals/shapes |
| | | | | 91770010 | Dietetic or low calorie gumdrops |
| Meat, poultry, and fish substitutes | Meat, poultry, and fish substitutes | 2806: Processed soy products | 12.0% | 41420010 | Soybean curd - stir fried; bean curd; tofu |
| | | | | 41420050 | Soybean curd cheese |
| | | | | 41420380 | Yogurt, soy - all types and flavors |
| | | | | 41421010 | Soybean curd, deep fried - aburage; tofu; bean curd |
| | | | | 41421020 | Soybean curd, breaded, fried - tofu; bean curd |
| | | | | 41425010 | Vermicelli, made from soybeans - bean thread, cellophane, glass, or crystal noodles |
| | | | | 41440000 | Textured vegetable protein, dry |
| | | | | 41480020 | Frozen dessert, non-dairy - non-dairy ice cream; tofu, nut or rice based; Tofutti; all flavors; NS as to form |
| | | | | 41810200 | Bacon strip, meatless - Morning Star Breakfast Strip; Stripple; meat substitute; vegetarian |
| | | | | 41810400 | Breakfast link, pattie, or slice, meatless - Prosage; Morningstar; Green Giant; meat substitute; vegetarian |
| | | | | 41810600 | Chicken, meatless, NFS - meat substitute; vegetarian |
| | | | | 41810610 | Chicken, meatless, breaded, fried - Loma Linda brand; meat substitute; vegetarian |
| | | | | 41811400 | Frankfurter or hot dog, meatless - meat substitute; vegetarian; tofu dog |
| | | | | 41811600 | Luncheon slice, meatless-beef, chicken, salami or turkey - vegetarian ham; Wham; Loma Linda; Worthington; meat substitute |
| | | | | 41811800 | Meatball, meatless - meat substitute; vegetarian |
| | | | | 41811890 | Vegetarian burger or patty, meatless, no bun - veggie burger, made with soy and/or vegetables and grains; soyburger; bean burger; meat substitute; Boca burger; Gardenburger; Morningstar Farms; Worthington FriPats |
| | | | | 41812000 | Sandwich spread, meat substitute type - vegetarian |

| Appendix 1. Food Codes used to Recalculate Estimated Daily Intake | | | | | |
|---|---|---|---|---|---|
| **Food Category** | **Application** | **WWEIA Category No.: description** | **Inclusion Rate (w/w%)** | **Food code** | **Main food description - Additional food description** |
| | | | | 41812600 | Vegetarian, fillet - meat substitute; meatless |
| | | | | 41901020 | Soyburger, meatless, with cheese on bun - meat substitute; vegetarian |
| | | | | 42203200 | Soy nut butter |
| | | | | 59003000 | Meat substitute, cereal- and vegetable protein-based, fried - sauteed; Seitan; vegetarian |
| | | 3703: Frankfurter sandwiches (single code) | | 27564420 | Frankfurter or hot dog sandwich, meatless, plain, on bun - meat substitute; vegetarian; any type of bun; tofu dog |
| | | | | 27564430 | Frankfurter or hot dog sandwich, meatless, plain, on bread - meat substitute; vegetarian; any type of bread; tofu dog |
| | | | | 27564560 | Frankfurter or hot dog sandwich, meatless, on bun, with meatless chili - meat substitute; vegetarian hot dog; tofu dog; vegetarian chili; any type of bun |
| | | | | 27564570 | Frankfurter or hot dog sandwich, meatless, on bread, with meatless chili - meat substitute; vegetarian hot dog; tofu dog; vegetarian chili; any type of bread |
| Abbreviations: NFS: not further specified, WWEIA: What We Eat In America | | | | | |

**From:** Jennifer Symonds
**To:** Downey, Jason
**Cc:** Claire Kruger; Dietrich Conze; Kathy Brailer
**Subject:** Re: [EXTERNAL] GRN 001171
**Date:** Thursday, August 15, 2024 8:15:51 AM
**Attachments:** Geltor Response to FDA on GRN 001171 - 8-15-24.pdf

---



Hi Jason,

Please find attached our amendment for GRN 001171. Do not hesitate to contact me if you have any questions. Thank you!

Best regards,
Jenn Symonds

On Mon, Aug 12, 2024 at 3:40 PM Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:

> Hi Jenn,
>
> Thank you for the update!
>
> Jason
>
> ---
>
> **From:** Jennifer Symonds <jsymonds@spherixgroup.com>
> **Sent:** Monday, August 12, 2024 3:39 PM
> **To:** Downey, Jason <Jason.Downey@fda.hhs.gov>
> **Cc:** Claire Kruger <ckruger@spherixgroup.com>; Dietrich Conze <dconze@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
> **Subject:** [EXTERNAL] GRN 001171
>
> 
>
> Hello Jason,
>
> Dietz is on a well deserved vacation this week. I'll be sending in the amendment for GRN

> 001171 before the end of the week. Thank you!
>
> Best regards,
>
> Jenn Symonds
> --
>
> Jennifer M. Symonds, Ph.D.
>
> Senior Project Manager
>
> Spherix Consulting Group, Inc.
>
> 751 Rockville Pike
>
> Unit 30-B
>
> Rockville, MD 20852
>
> +1-319-621-6154

--
Jennifer M. Symonds, Ph.D.
Senior Project Manager
Spherix Consulting Group, Inc.
751 Rockville Pike
Unit 30-B
Rockville, MD 20852
+1-319-621-6154

Spherix Consulting Group

August 15, 2024

Jason Downey, Ph.D.
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 001171

Dear Dr. Downey:

Below are our responses to the Agency's requests for additional information regarding GRN 001171, as specified in your email on August 1, 2024. Your requests are in italicized text and our responses are below in plain text.

1. *In the response to question 18 in the amendment dated June 3, 2024, Geltor states "the Protein % (w/w)" column in Table 7 of the Notice specifies the use level of the protein component of Non-Animal Type 21 Collagen, not Non-Animal Type 21 Collagen Polypeptide as a whole", and "if Non-Animal Type 21 Collagen Polypeptide is used in a protein powder product that contains 50% total protein, the targeted level of collagen peptide is 95% of the total protein content in the protein powder, and the lot of Non-Animal Type 21 Collagen Polypeptide used to manufacture the marketed food contains 80% protein, then Non-Animal Type 21 Collagen Polypeptide would be included at a rate of 59.4 % of the total protein content of the marketed product."*

   *Please clarify what is represented by the use levels provided in Table 3 of the amendment. Is this the percent of the total protein that will be collagen polypeptide produced by E. coli K-12 S9188 for the given food category or is this the amount of collagen polypeptide produced by E. coli K-12 S9188 in the finished food? If this is the amount of collagen polypeptide produced by E. coli K-12 S9188 in the finished food, please indicate if it is added in addition to the protein that is in the food or if it will substitute for what is currently in the given food.*

   The use levels provided in Table 3 of the amendment should be described as the amount of Non-Animal Type 21 Collagen Polypeptide as weight per weight percent (% w/w) of the intended food uses. For clarity, this table has been revised below to reflect the intended uses and use levels for the subject of GRN 001171 (see the response to Request 2, Table 1). Please note, Non-Animal Collagen Type 21 Polypeptide is not intended to be used as a substitute for dietary protein. The subject of GRN 001171 is intended as a source of

collagen polypeptides and is considered a substitute for other sources of collagen polypeptides that are currently available.

2. *In the response to question 19 of the amendment dated June 3, 2024, Geltor states "The use level in the powdered drinks were calculated as consumed, i.e., as the reconstituted, fluid beverage." However, the use levels for the non-reconstituted powdered flavored drink, instant coffee and tea products are the same as those for a liquid beverage.*

   *We note that many of the National Health and Nutrition Examination Survey (NHANES) food codes are for the non-reconstituted powder. For these food codes, the use level that should be applied for the purposes of the dietary exposure estimate is for what is in the non-reconstituted powder, not in the final beverage when it is reconstituted. Please provide the use levels in the non-reconstituted powders and revise the dietary exposure accordingly.*

   The intended use table has been updated to indicate the intended use level of the subject of GRN 001171 in non-reconstituted powders (Table 1). The use levels in the non-reconstituted powders are ten-fold higher than the uses as consumed. For example, the use level in flavored beverages and beverages bases, as consumed, is 2% weight/weight. This corresponds to a new intended use in the non-reconstituted powdered forms of the beverages and beverage bases of 20%. The food codes chosen to represent these intended uses are listed in the Appendix to this letter.

**Table 1. Intended Uses and Use Levels for Non-Animal Type 21 Collagen Polypeptide**

| Category | Application | % (w/w) |
|---|---|---|
| Beverages and beverage bases | Flavored beverages and beverage bases, excluding soft drinks and energy drinks | 2 |
| | Flavored beverages and beverage bases, powdered, not reconstituted | 20 |
| Coffee and Tea | Packaged coffee & tea beverages | 5.5 |
| | Instant coffee & tea | |
| | Coffee, powdered, not reconstituted | 55.5 |
| | Coffee and tea pods | 2 |
| Dairy products and substitutes | Probiotic and yogurt beverages | 2.5 |
| | Milk substitutes | 2.5 |
| Coffee creamer | Coffee creamer (liquid and powder forms) | 20 |
| Nutritional foods | Nutritional beverages and beverage bases | 4.5 |
| | Nutritional bars | 25 |
| | Protein powders | 95 |
| Confections | Gummies | 10 |
| | Chocolate confections | 10 |
| Meat, poultry, and fish substitutes | Meat, poultry, and fish substitutes | 12 |

3. *Given the ambiguity regarding the intended uses, we are not able to verify the dietary exposure estimates. We further note that we have questions regarding the exclusion of certain NHANES food codes in Appendix 1. We have provided a list of individual food codes that were not included but fit in the descriptions of the intended food categories (see attached). Please indicate why these food codes were excluded, and if they should be included, please revise the dietary exposure accordingly.*

Thank you for providing this list for our review. Five of the food codes included in the FDA's list were already included in the previous dietary exposure assessment (food codes 41421020, 92307000, 92307400, 91723010, and 91701010). The food codes selected for the previous exposure assessment were based on prototypes and commercial goals of the notifier. For example, some chocolate confection food codes were excluded from the category entitled "chocolate confections" as the notifiers did not intend to use Non-Animal Type 21 Collagen Polypeptide in chocolate bars.

For a more conservative exposure assessment and greater number of uses of the subject of GRN 001171, the FDA-provided food codes were incorporated into the exposure assessment described below in Table 2. The FDA-provided food codes are indicated with an asterisk (*) in the appendix to this letter.

The updated food codes and use levels described in Table 1 were then used to revise the dietary exposure described previously. The dietary exposure assessment was performed using the most recent NHANES dataset, 2017-March 2020. The NHANES program suspended field operations in March 2020 due to the coronavirus disease 2019 (COVID-19) pandemic. As a result, data collection for the NHANES 2019-2020 cycle was not completed and the collected data are not nationally representative; therefore, data collected from 2019 to March 2020 were combined with data from the NHANES 2017-2018 cycle to form a nationally representative sample of NHANES 2017-March 2020, pre-pandemic data.

**Table 2. Estimated Daily Intake* of Non-Animal Type 21 Collagen Polypeptide in Consumers of Proposed Non-Animal Type 21 Collagen Polypeptide-containing Products in the U.S. by Age Cohort, NHANES 2017-March 2020 Pre-pandemic**

| | % Users | N Users | Total Population | EDI (g/kg body weight/day) | | EDI (g/day) | |
|---|---|---|---|---|---|---|---|
| | | | | Mean | 90th Percentile | Mean | 90th Percentile |
| *Males* | | | | | | | |
| Ages 1-2 | 45.2 | 113 | 275 | 0.44 | 0.89 | 5.42 | 10.07 |
| Ages 3-5 | 56.5 | 149 | 275 | 0.23 | 0.55 | 4.35 | 9.91 |
| Ages 6-11 | 58.7 | 346 | 602 | 0.23 | 0.54 | 7.9 | 21.31 |
| Ages 12-19 | 57.6 | 405 | 747 | 0.18 | 0.4 | 12.43 | 26.67 |
| Ages 20+ | 80.8 | 3493 | 3169 | 0.24 | 0.49 | 20.83 | 42.75 |
| *Females* | | | | | | | |
| Ages 1-2 | 45.7 | 105 | 243 | 0.33 | 0.95 | 3.93 | 10.29 |
| Ages 3-5 | 60.1 | 149 | 259 | 0.19 | 0.4 | 3.47 | 7.0 |
| Ages 6-11 | 60.7 | 327 | 596 | 0.15 | 0.3 | 4.82 | 10.78 |
| Ages 12-19 | 59.6 | 411 | 730 | 0.16 | 0.33 | 10.04 | 19.81 |
| Ages 20+ | 82.0 | 2722 | 3471 | 0.23 | 0.47 | 16.47 | 33.34 |

*Calculated using DaDIET, food codes that represent the intended uses, and user data from the 2017-March 2020 Pre-pandemic NHANES database.

4. *In the response to question 16 in the amendment dated June 3, 2024, Geltor states "Geltor is aware of the "Closer to Zero" initiative and will explore lowering their heavy metal specifications in the future."*

   *We note that the specifications for heavy metals are higher than what is typically seen in a fermentation-derived ingredient that is produced in accordance with good manufacturing practices. We recommend that these specifications be lowered to be reflective of the batch analyses and to be as low as possible.*

   The currently available batch data for Non-Animal Type 21 Collagen allows for a reduction in the specifications for cadmium, lead, and mercury to not more than 0.125, 0.25, and 0.05, respectively.

5. *In your June 3, 2024, amendment you state that you conduct "screening for the presence of production strain-derived DNA by polymerase chain reaction (PCR)." However, this is not included as part of the specifications in your original notice. Please clarify if you intend to add this to the product specifications.*

   Geltor does not intend to include the testing for the presence of production strain-derived DNA by PCR as a product specification. The testing is conducted on a monitoring basis only to confirm that the production process is controlled.

6. *In the June 3, 2024, amendment you state that the results of batch analysis for Salmonella spp. represent both the USP and AOAC method – please clarify if you used each method to test each batch, or if the batch analysis data used different methods for different batches. If it is the latter, please provide batch analysis data using only the AOAC method or provide justification as to why these methods are comparable, as you indicate that you plan to use the AOAC method exclusively as it is "more stringent."*

   All batch data for Salmonella spp. presented in the GRAS Notice was obtained using AOAC OMA 2011.3. As specified in our response to Question 14 in the responses that were sent to FDA on June 4, 2024, Geltor will be using AOAC OMA 2011.3 moving forward because it is more stringent than the USP method.

Should you need additional information, please feel free to contact me at +1-240-367-6089 or dconze@spherixgroup.com.

Sincerely,

Dietrich B. Conze, Ph.D.
Managing Partner

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| Beverages and beverage bases | Flavored beverages and beverage bases - not reconstituted | 9999: Not included in a food category | 0.2 | 92900100 | Fruit flavored drink, with high vitamin C, powdered, not reconstituted - Tang, all flavors |
| | | | | 92900110 | Fruit flavored drink, powdered, not reconstituted - Kool-Aid, NS as to sweetener; Wylers, NS as to sweetener; Country Time lemonade; Flavor Aid; powdered lemonade, NS as to brand; store brand |
| | | | | 92900200 | Fruit flavored drink, powdered, not reconstituted, diet - Crystal Light; Sugar Free Kool-aid; Wyler's Light; lemonade from low calorie powdered mix, NS as to brand |
| | | | | 92900300 | Sports drink, dry concentrate, not reconstituted - Gatorade; fruit flavored thirst quencher beverage; NS as to brand |
| | | | | 11830400* | Strawberry beverage powder, dry mix, not reconstituted - flavors other than chocolate |
| | Flavored beverages and beverage bases, excluding soft drinks and energy drinks | 7104: Diet sport and energy drinks | 0.02 | 95322200 | Sports drink, low calorie (Gatorade G2) - all flavors |
| | | | | 95322500 | Sports drink, low calorie (Powerade Zero) - all flavors |
| | | | | 95323000 | Sports drink, low calorie - NS as to brand; light; diet; Zero; sugar free; fruit flavored thirst quencher beverage |
| | | 7106: Other diet drinks | | 92513010 | Slush frozen drink, no sugar added - Slurpee Lite; no dairy; light slushee or slush; light Slush Puppy; reduced sugar; no sugar added; sugar free |
| | | | | 92550400 | Vegetable and fruit juice drink, with high vitamin C, diet - Diet V8 Splash, all flavors; low calorie |
| | | | | 92550610 | Fruit flavored drink, with high vitamin C, diet - Diet Ocean Spray Juice Drinks, all flavors; Diet Ocean Spray cranberry, blueberry, or pomegranate blends |
| | | | | 92550620 | Fruit flavored drink, diet - Crystal Light; Minute Maid Light lemonade and other fruit flavored drinks; low calorie |
| | | | | 92552000 | Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet - Sugar Free Tang; Country Time Lite lemonade; Ocean Spray drink mix; low calorie |
| | | | | 92552010 | Fruit flavored drink, powdered, reconstituted, diet - Crystal Light; Sugar Free Kool-Aid; Wyler's Light; lemonade from low calorie powdered mix, NS as to brand |
| | | 7206: Sport and energy drinks | | 95320200 | Sports drink (Gatorade G) - all flavors; Gatorade, NFS |
| | | | | 95320500 | Sports drink (Powerade) - all flavors |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 95321000 | Sports drink, NFS - All Sport; fruit flavored thirst quencher beverage; NS as to brand |
| | | 7802: Flavored or carbonated water | | 94100200 | Water, bottled, sweetened, with low calorie sweetener - flavored; Fruit 2 O; water, fruit flavored, sweetened, NS as to sweetener; Aquafina Flavor Splash; Dasani Flavored Water |
| | | | | 94100300 | Water, bottled, flavored (Capri Sun Roarin' Waters) - all flavors |
| | | 7804: Enhanced or fortified water | | 94210100 | Water, bottled, flavored (Propel Water) - all flavors; with calcium |
| | | | | 94210200 | Water, bottled, flavored (Glaceau Vitamin Water) - all flavors |
| | | | | 94210300 | Water, bottled, flavored (SoBe Life Water) - all flavors |
| | | | | 94220215 | Water, bottled, flavored, sugar free (Glaceau Vitamin Water) - Glaceau Vitamin Water 10; Glaceau Vitamin Water Zero; zero calorie |
| | | | | 94220310 | Water, bottled, flavored, sugar free (SoBe) - all flavors; 0 calories; SoBe Life Water zero calories; zero calorie |
| | | 7204: Fruit drinks | | 92541010* | Fruit flavored drink, powdered, reconstituted - Kool-Aid, NS as to sweetener; Wylers, NS as to sweetener; Country Time lemonade, made from dry mix; Flavor Aid; lemonade from powdered mix, NS as to brand; store brand |
| Coffee and Tea | Coffee and tea pods | 7302: Coffee | 0.02 | 92100500 | Coffee, NS as to brewed or instant - coffee singles, bags, pods, or K-cups |
| | | | | 92101000 | Coffee, brewed - coffee, brewed, NS as to regular or decaffeinated; coffee singles, bags, pods, or K-cups |
| | | | | 92101500 | Coffee, brewed, blend of regular and decaffeinated - half-caf; reduced caffeine; coffee singles, bags, pods or K-cups |
| | | | | 92101700 | Coffee, brewed, flavored - coffee singles, bags, pods, or K-cups |
| | | | | 92102400 | Iced Coffee, brewed - unsweetened; coffee singles, bags, pods, or K-cups; NS as to regular or decaffeinated |
| | | | | 92102401 | Iced Coffee, brewed, decaffeinated - unsweetened; coffee singles, bags, pods, or K-cups |
| | | | | 92111000 | Coffee, NS as to brewed or instant, decaffeinated - coffee singles, bags, pods, or K-cups |
| | | | | 92111010 | Coffee, brewed, decaffeinated - coffee singles, bags, pods, or K-cups |
| | Instant coffee - not reconstituted | 9999: Not included in a food category | 0.55 | 92191100 | Coffee, instant, not reconstituted - powdered mix; NS as to regular or decaffeinated; liquid concentrate |
| | | | | 92191105 | Coffee, instant, 50% less caffeine, not reconstituted - powdered mix; blend of regular and decaf; half-caf; reduced caffeine |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 92191200 | Coffee, instant, decaffeinated, not reconstituted - powdered mix |
| | | | | 92191400 | Coffee, instant, pre-sweetened with sugar, not reconstituted - powdered mix; NS as to sweetener |
| | | | | 92192000 | Coffee, mocha, instant, pre-lightened and pre-sweetened with sugar, not reconstituted - powdered mix; coffee and cocoa mix; NS as to sweetener; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92192030 | Coffee, mocha, instant, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92192040 | Coffee, mocha, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92193000 | Coffee, instant, pre-lightened and pre-sweetened with sugar, not reconstituted - powdered mix; NS as to sweetener; Maxwell House International Coffee flavors other than chocolate, cocoa, or mocha |
| | | | | 92193005 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with sugar, not reconstituted - powdered mix; Maxwell House International Coffee; flavors other than chocolate, cocoa, or mocha |
| | | | | 92193020 | Coffee, instant, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha; sugar free |
| | | | | 92193025 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted - powdered mix; sugar free; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha |
| | Instant coffee and tea | 7302: Coffee | 0.055 | 92103000 | Coffee, instant, reconstituted - powdered mix; NS as to regular or decaffeinated; made from liquid concentrate |
| | | | | 92104000 | Coffee, instant, 50% less caffeine, reconstituted - powdered mix; blend of regular and decaf; half-caf; reduced caffeine |
| | | | | 92114000 | Coffee, instant, decaffeinated, reconstituted - powdered mix |
| | | | | 92121000 | Coffee, instant, pre-lightened and pre-sweetened with sugar, reconstituted - powdered mix; NS as to sweetener; Maxwell House International, flavors other than chocolate, cocoa, or mocha |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 92121001 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with sugar, reconstituted - powdered mix; Maxwell House International, flavors other than chocolate, cocoa, or mocha |
| | | | | 92121010 | Coffee, instant, pre-sweetened with sugar, reconstituted - powdered mix; NS as to sweetener |
| | | | | 92121020 | Coffee, mocha, instant, pre-lightened and pre-sweetened with sugar, reconstituted - NS as to sweetener; powdered mix; coffee and cocoa mix; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92121030 | Coffee, mocha, instant, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | | | 92121040 | Coffee, instant, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha |
| | | | | 92121041 | Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Sugar Free Coffee; flavors other than chocolate, cocoa, or mocha |
| | | | | 92121050 | Coffee, mocha, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted - powdered mix; sugar free; Maxwell House International Coffee flavors, Chocolate, Cocoa, or Mocha |
| | | 9999: Not included in a food category | | 92291300 | Coffee substitute, dry powder |
| | | | | 92307000 | Tea, iced, instant, black, unsweetened, dry - unreconstituted powdered mix; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92307400 | Tea, iced, instant, black, pre-sweetened, dry - unreconstituted powdered mix; NS as to sweetener; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | Packaged coffee and tea beverages | 7302: Coffee | | 92100000 | Coffee, NS as to type |
| | | | | 92171000 | Coffee, bottled/canned - NS as to brand or variety; from carton; all flavors; all brands; all varieties |
| | | | | 92171010 | Coffee, bottled/canned, light - NS as to brand or variety; from carton; all flavors; all brands; all varieties |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | 7304: Tea | | 92305010 | Tea, iced, instant, black, unsweetened - powdered mix; liquid concentrate; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305040 | Tea, iced, instant, black, pre-sweetened with sugar - powdered mix; liquid concentrate; NS as to sweetener; sweet tea; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305050 | Tea, iced, instant, black, decaffeinated, pre-sweetened with sugar - powdered mix; liquid concentrate; NS as to sweetener; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305090 | Tea, iced, instant, black, pre-sweetened with low calorie sweetener - powdered mix; liquid concentrate; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; light, reduced calorie, diet, sugar free |
| | | | | 92305110 | Tea, iced, instant, black, decaffeinated, pre-sweetened with low calorie sweetener - powdered mix; liquid concentrate; light; reduced calorie; diet; sugar free; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305180 | Tea, iced, instant, black, decaffeinated, unsweetened - powdered mix; liquid concentrate; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92305900 | Tea, iced, instant, green, unsweetened - powdered mix; liquid concentrate; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated |
| | | | | 92305910 | Tea, iced, instant, green, pre-sweetened with sugar - powdered mix; liquid concentrate; white tea; herbal tea; NS as to sweetener; green and fruit, herbal, or white tea blends; decaffeinated |
| | | | | 92305920 | Tea, iced, instant, green, pre-sweetened with low calorie sweetener - powdered mix; liquid concentrate; light, reduced calorie, diet, sugar free; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated |
| | | | | 92307500 | Iced Tea / Lemonade juice drink - half and half beverage, all flavors; Arizona Half & Half; Arnold Palmer Half & Half |
| | | | | 92307510 | Iced Tea / Lemonade juice drink, light - half and half beverage, all flavors, light; Arizona Lite Half & Half; Arnold Palmer Lite Half & Half |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 92307520 | Iced Tea / Lemonade juice drink, diet - diet half and half beverage, all flavors; Diet Snapple Half n' Half; Arnold Palmer Lite Half & Half, made from powdered mix |
| | | | | 92309000 | Tea, iced, bottled, black - can, carton, or fountain; sweet tea; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; Snapple, all flavors |
| | | | | 92309010 | Tea, iced, bottled, black, decaffeinated - can, carton, or fountain; sweet tea; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; caffeine free Snapple Tea, all flavors |
| | | | | 92309020 | Tea, iced, bottled, black, diet - light, reduced calorie, or sugar free; can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; Diet Snapple Tea, all flavors |
| | | | | 92309030 | Tea, iced, bottled, black, decaffeinated, diet - light, reduced calorie, or sugar free; can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea; caffeine free Diet Snapple Tea, all flavors |
| | | | | 92309040 | Tea, iced, bottled, black, unsweetened - can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92309050 | Tea, iced, bottled, black, decaffeinated, unsweetened - can, carton, or fountain; black and fruit, green, herbal, white, or other tea blends; NS as to type of tea |
| | | | | 92309500 | Tea, iced, bottled, green - can, carton, or fountain; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated; Snapple |
| | | | | 92309510 | Tea, iced, bottled, green, diet - light, reduced calorie, or sugar free; can, carton, or fountain; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated; Diet Snapple |
| | | | | 92309520 | Tea, iced, bottled, green, unsweetened - can, carton, or fountain; white tea; herbal tea; green and fruit, herbal, or white tea blends; decaffeinated |
| Coffee Creamer | Coffee creamer (liquid and powder forms) | 8008: Cream and cream substitutes | 0.2 | 12100100 | Cream, NS as to light, heavy, or half and half |
| | | | | 12110100 | Cream, light - coffee cream; table cream |
| | | | | 12120100 | Cream, half and half - plain |
| | | | | 12120106 | Cream, half and half, flavored |
| | | | | 12120110 | Cream, half and half, fat free - low fat; plain or flavored |
| | | | | 12130100 | Cream, heavy - whipping cream; NS as to heavy or light |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 12200100 | Coffee creamer, NFS |
| | | | | 12210200 | Coffee creamer, liquid - frozen; Coffee Mate; Carnation; Coffee Rich; Dunkin Donuts; International Delight; plain |
| | | | | 12210210 | Coffee creamer, liquid, flavored - Coffee Mate; Bailey's; Bliss; Carnation; Coffee Rich; Dunkin Donuts; International Delight; Mocha Mix |
| | | | | 12210260 | Coffee creamer, liquid, fat free - low fat; light; lite; Coffee Mate; Carnation; Coffee Rich; Dunkin Donuts; International Delight; plain |
| | | | | 12210270 | Coffee creamer, liquid, fat free, flavored - light; lite; Coffee Mate; low fat; Carnation; Coffee Rich; Dunkin Donuts; International Delight; Mocha Mix |
| | | | | 12210280 | Coffee creamer, liquid, fat free, sugar free, flavored - low fat; light; lite; Coffee Mate; Carnation; International Delight; Mocha Mix |
| | | | | 12210310 | Coffee creamer, liquid, sugar free, flavored - Coffee Mate; Carnation; International Delight; Mocha Mix |
| | | | | 12210400 | Coffee creamer, powder - Coffee Mate; Cremora; Carnation; plain |
| | | | | 12210420 | Coffee creamer, powder, flavored - Coffee Mate powder, flavored; Carnation |
| | | | | 12210430 | Coffee creamer, powder, fat free - light; lite; low fat; Coffee Mate; Carnation; Cremora; plain |
| | | | | 12210440 | Coffee creamer, powder, fat free, flavored - light; lite; Coffee Mate; low fat; Carnation |
| | | | | 12210505 | Coffee creamer, powder, sugar free, flavored - fat free; light; lite; low fat; Coffee Mate; Carnation |
| | | | | 12210520 | Coffee creamer, soy, liquid - almond; Silk; So Delicious; coconut milk creamer |
| Confections | Chocolate confections | 5702: Candy containing chocolate | 0.1 | 91701010 | Almonds, chocolate covered |
| | | | | 91703040 | Caramel candy, chocolate covered - Black Cow; Caramello; Chew-Its; Marathon Bar; Milk Duds; Pom Poms; Sugar Momma; Riesen Chocolate Chew; Russell Stover Caramel Heart |
| | | | | 91703050 | Caramel with nuts and cereal, chocolate covered |
| | | | | 91703060 | Caramel with nuts, chocolate covered - Goo Goo Cluster(s); Peanut Chews; Toffifay; Turtles; Reese's NutRageous |
| | | | | 91703600 | Espresso coffee beans, chocolate-covered - Millstone Milk Chocolate-covered Espresso Beans |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 91705010 | Milk chocolate candy, plain - chocolate candy, NFS; Easter Egg, NFS; Easter Egg, milk chocolate; Hershey Bar; Hershey Kiss or Star; Hershey Symphony Bar; Nestle Bar; Chocolate coins; Hershey's Hugs; Hershey's Nuggets; Easter bunnies; chocolate rose; Dove Milk Chocolate bar; chocolate balls; Cadbury Dairy Milk; Dove Promises |
| | | | | 91705020 | Milk chocolate candy, with cereal - Krackel Bar; Malted Milk Balls; Nestle Crunch; Whoppers; Hershey's Cookies 'n' Mint; Hershey's Cookies 'n' Mint Nuggets; Malted Milk Eggs |
| | | | | 91705040 | Chocolate, milk, with nuts, not almond or peanuts - Brach's Bridge Mix; Chunky with pecans; Hazelnut with chocolate (Cadbury); Ferrero Rocher Hazelnut Chocolate |
| | | | | 91705050 | Milk chocolate candy, with fruit and nuts - Chunky with fruit and nuts; Chunky, NFS; Chunky Original; Cadbury |
| | | | | 91705060 | Milk chocolate candy, with almonds - Hershey with Almonds; Nestle chocolate bar with almonds; Hershey Kisses with Almonds; Hershey's Nuggets with Almonds; Cadbury Roasted Almonds; Hershey Bites with Almonds |
| | | | | 91705070 | Chocolate, milk, with peanuts - Chunky with peanuts; Mr. Goodbar |
| | | | | 91705090 | Chocolate candy with fondant and caramel - Sky Bar |
| | | | | 91705200 | Chocolate, semi-sweet morsel - Toll House morsels; chocolate chips; Hershey's Mini Kisses Brand Milk Chocolates |
| | | | | 91705300 | Chocolate, sweet or dark - Special Dark; Dove Dark Chocolate bar; Hershey's Special Dark Kisses |
| | | | | 91705310 | Chocolate, sweet or dark, with almonds - Hershey's Special Dark with Almonds; Hershey's Special Dark with Almonds Nuggets |
| | | | | 91705500 | Mexican chocolate, tablet |
| | | | | 91706000 | Coconut candy, chocolate covered - Almond Joy; Bounty; Easter egg, chocolate covered coconut; Mounds |
| | | | | 91713010 | Fudge, chocolate, chocolate-coated - Butter Fudge, Mary Sue |
| | | | | 91713020 | Fudge, chocolate, chocolate-coated, with nuts |
| | | | | 91713030 | Fudge, chocolate - fudge, NFS; fudge, plain |
| | | | | 91713040 | Fudge, chocolate, with nuts |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 91715000 | Fudge, caramel and nut, chocolate-coated candy - Butternut Bar; Oh Henry; Powerhouse; Clark Bun Bar |
| | | | | 91718200 | Chocolate-flavored sprinkles - Jimmies |
| | | | | 91723010 | Marshmallow, chocolate covered - Easter egg, chocolate covered marshmallow; Mallo Cup; Marshmallow rabbit, chocolate covered |
| | | | | 91727010 | Nuts, chocolate covered, not almonds or peanuts - nuts, chocolate covered, NFS |
| | | | | 91728000 | Nut roll, fudge or nougat, caramel and nuts - Big Hunk; Payday; Chocolaty Payday; Pecan roll |
| | | | | 91731000 | Peanuts, chocolate covered - Goobers; Peanut Clusters |
| | | | | 91733200 | Peanut Bar, chocolate covered candy |
| | | | | 91734000 | Peanut butter, chocolate covered - peanut butter treats; peanut butter candy; Peanut Butter Meltaway Crispy Bar (Brach's); Easter egg, peanut butter, chocolate covered; Peanut Butter Meltaway Bar (Brach's); Planters Peanut Butter Chocolates |
| | | | | 91739010 | Raisins, chocolate covered - Raisinets; Raisin clusters |
| | | | | 91746010 | Sugar-coated chocolate discs - Nonpareils; Nestle Sno Caps; snow caps |
| | | | | 91760100 | Toffee, chocolate covered - Heath Bar; SKOR; Heath Sensations; Sweet Escapes Chocolate Toffee Crisp Candy Bar |
| | | | | 91760200 | Toffee, chocolate-coated, with nuts - Almond Roca |
| | | | | 91760500 | Truffles |
| | | | | 91770030 | Dietetic or low calorie candy, chocolate covered - Dietetic Chocolate TV Mix; Dietetic Chocolate Wafers; Dietetic Covered Raisins; Dietetic Crunch Bar; Dietetic Fruit and Nut Bar; Dietetic Peanut Butter Cups; Dietetic Milk Almond Bar; Dietetic Milk Chocolate Flavored Bar |
| | | | | 91700500* | M&M's Almond Chocolate Candies |
| | | | | 91703070* | Rolo |
| | | | | 91703150* | Toblerone, milk chocolate with honey and almond nougat |
| | | | | 91703250* | TWIX Chocolate Fudge Cookie Bars - TWIX Cookies-n-Creme candy bar |
| | | | | 91703400* | Whatchamacallit - Sweet Escapes Caramel and Peanut Butter Crispy Bar |
| | | | | 91705005* | Chocolate candy, other, NFS |
| | | | | 91705012* | Chocolate candy with nuts, other, NFS |
| | | | | 91705030* | Kit Kat - Kit Kat Bites |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 91705080* | Chocolate candy, cookie filled - Kit Kat; Twix |
| | | | | 91705290* | Dark chocolate candy, other, NFS |
| | | | | 91705312* | Dark chocolate candy with nuts, other, NFS |
| | | | | 91705315* | Dark chocolate candy with nuts - Dove; Hershey bar, kiss, or nugget |
| | | | | 91705440* | Chocolate candy, fudge - all varieties |
| | | | | 91705450* | Chocolate candy, caramel filled - Heath; Milk Duds |
| | | | | 91705460* | Chocolate candy, caramel filled with nuts - turtles |
| | | | | 91705470* | Chocolate candy, coconut filled - Almond Joy; Mounds |
| | | | | 91705480* | Chocolate candy, cream filled - chocolate covered cherries; chocolate mint; truffles; peppermint patty |
| | | | | 91705510* | Chocolate candy, nougat filled - Milky Way; Three Musketeers |
| | | | | 91705520* | Chocolate candy, nougat filled with nuts - Baby Ruth; Snickers; Whatchamacallit |
| | | | | 91705530* | Chocolate candy, peanut butter filled - peanut butter cup; Butterfinger; Reese's peanut butter cup |
| | | | | 91705550* | Chocolate candy with dried fruit - raisins; Raisinettes |
| | | | | 91706010* | Chocolate candy, sugar free |
| | | | | 91707010* | Fondant, chocolate covered - chocolate covered boxed candy; homemade mints, chocolate-covered; Cadbury's creme egg; Butter creams; Cherries, chocolate covered; Chocolate Jots; Easter egg, chocolate covered creme; Irish Cream Mints; Mints (Brach's); Mint Jots; Peppermint Pattie; Thin Mints; Junior Mints |
| | | | | 91715100* | SNICKERS Bar |
| | | | | 91715200* | Baby Ruth |
| | | | | 91715300* | 100 GRAND Bar - $ 100,000 Bar |
| | | | | 91718100* | Butterfinger - Butterfinger BB's; Bittyfingers |
| | | | | 91718110* | Butterfinger Crisp |
| | | | | 91726130* | MILKY WAY Bar |
| | | | | 91726140* | MILKY WAY MIDNIGHT Bar |
| | | | | 91726150* | MARS Almond Bar |
| | | | | 91726410* | Nougat, chocolate covered - Charleston Chew |
| | | | | 91726420* | 3 MUSKETEERS Bar |
| | | | | 91731010* | M&M's Peanut Chocolate Candies |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 91731060* | M&M's Peanut Butter Chocolate Candies |
| | | | | 91734100* | Reese's Peanut Butter Cup - Reese's Bites |
| | | | | 91734450* | Reese's Crispy Crunchy Bar |
| | | | | 91746100* | Chocolate candy, candy shell - M&Ms |
| | | | | 91746110* | Chocolate candy, candy shell with nuts - M&Ms; Reese's pieces |
| | | | | 91746120* | Sixlets |
| | | | | 91746150* | Easter egg, candy coated chocolate - Cadbury's Mini Eggs |
| | | | | 91746200* | M&M's Pretzel Chocolate Candies |
| | Gummies | 5704: Candy not containing chocolate | 0.1 | 91708020 | Soft fruit confections - soft fruit candy; Aplets; Cotlets; Fruit Delights, all flavors; with nuts |
| | | | | 91708030 | Fruit leather and fruit snacks candy - Fruit Roll-Up; Fruit Wrinkles; Fun Fruits; Teenage Mutant Ninja Turtles Fruit Snacks; Soda-licious; Farley's Fruit Snacks; Gushers Fruit Snacks; Fruit By The Foot; Betty Crocker Fruit String Thing; Sunbelt Fruit Jammers |
| | | | | 91708100 | Fruit snacks candy, with high vitamin C - Brach's Hi-C Fruit Snacks; Kellogg's Right Bites Fruit Snacks; Welch's Fruit Snacks; Florida's Natural Pocket Fruit-to-go Stiks, all flavors |
| | | | | 91708150 | Yogurt covered fruit snacks candy, with added vitamin C - Kellogg's Yogos Bits, all flavors |
| | | | | 91708160 | Yogurt covered fruit snacks candy rolls, with high vitamin C - Kellogg's Yogos Rollers, all flavors |
| | | | | 91745010 | Gumdrops - gummy bears; gummy worms; gummy fish; gummy dinosaurs; Hot tamales; Jelly beans; Jujubes; Jujy Fruits; Mike and Ike; Mint Leaves; Chuckles; Dots; Fruit slices, jellied; Good and Fruity; Brach's Rocks; spice sticks and drops; Life Savers Gummi Savers; Gummy animals/shapes |
| | | | | 91770010 | Dietetic or low calorie gumdrops |
| | | | | 91745010* | Candy, gummy - all shapes; gumdrops; jelly beans; Hot Tamales, Mike and Ike, Sour Patch |
| | | | | 91746300* | Candy, fruit snacks - tamarind candy |
| | | | | 91746350* | Candy, fruit leather - fruit roll-up |
| Dairy products | Milk substitutes | 1404: Milk substitutes | 0.025 | 11300100 | Non-dairy milk, NFS |
| | | | | 11320000 | Soy milk - Edensoy; vanilla flavored; Silk; West Soy; Soymilk; NS as to type; Vitasoy |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| and substitutes | | | | 11320100 | Soy milk, light - vanilla flavored; Silk; Vitasoy; West Soy; Soymilk |
| | | | | 11320200 | Soy milk, nonfat - vanilla flavored; West Soy; Soymilk |
| | | | | 11321000 | Soy milk, chocolate - Silk; Belsoy; Soymilk |
| | | | | 11321100 | Soy milk, light, chocolate - Silk; Vitasoy; Soymilk |
| | | | | 11321200 | Soy milk, nonfat, chocolate - Soymilk |
| | | | | 11350000 | Almond milk, sweetened - vanilla flavored; NS as to sweetened; cashew |
| | | | | 11350010 | Almond milk, sweetened, chocolate - NS as to sweetened; cashew |
| | | | | 11350020 | Almond milk, unsweetened - unsweetened vanilla; flavors other than chocolate; cashew |
| | | | | 11350030 | Almond milk, unsweetened, chocolate - Blue Diamond Almond Breeze; Silk PureAlmond; Almond Dream |
| | | | | 11360000 | Rice milk - Rice Dream beverage |
| | | | | 11370000 | Coconut milk - Silk PureCoconut beverage; So Delicious |
| | | | | 11512030 | Hot chocolate/Cocoa, ready to drink, made with non-dairy milk - almond, coconut, rice, or soy milk; chocolate espanol; chocolate caliente; Starbucks; made from syrup, tablet, or bar |
| | | | | 11512120 | Hot chocolate/Cocoa, ready to drink, made with non-dairy milk and whipped cream - almond, coconut, rice, or soy milk; Starbucks; made from syrup, tablet, or bar |
| | | | | 11513310 | Chocolate milk, made from dry mix with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513375 | Chocolate milk, made from reduced sugar mix with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513385 | Chocolate milk, made from dry mix with non-dairy milk (Nesquik) - almond, coconut, rice, or soy milk |
| | | | | 11513395 | Chocolate milk, made from no sugar added dry mix with non-dairy milk (Nesquik) - almond, coconut, rice, or soy milk |
| | | | | 11513750 | Chocolate milk, made from syrup with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513805 | Chocolate milk, made from light syrup with non-dairy milk - almond, coconut, rice, or soy milk |
| | | | | 11513855 | Chocolate milk, made from sugar free syrup with non-dairy milk - almond, coconut, rice, or soy milk |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 11514150 | Hot chocolate/Cocoa, made with dry mix and non-dairy milk - almond, coconut, rice, and soy milk; cocoa mix, NFS; Swiss Miss; Hershey's; Nestle's; Carnation |
| | | | | 11514360 | Hot chocolate/Cocoa, made with no sugar added dry mix and non-dairy milk - almond, coconut, rice, and soy milk; fat free, light, diet or sugar free mix; Swiss Miss; Hershey's; Nestle's; Carnation |
| | | | | 11519215 | Strawberry milk, non-dairy - almond, coconut, rice, or soy milk |
| | | | | 42401010 | Coconut milk, used in cooking |
| | | | | 42402010 | Coconut cream, canned, sweetened - Coco Lopez |
| | Probiotic and yogurt beverages | 1002: Milk, whole | | 11115300 | Buttermilk, whole |
| | | 1004: Milk, reduced fat | | 11115200 | Buttermilk, reduced fat (2%) - 2% fat, made with nonfat milk solids |
| | | 1008: Milk, nonfat | | 11115000 | Buttermilk, fat free (skim) - nonfat buttermilk |
| | | 1820: Yogurt, regular | | 11436000 | Yogurt, liquid - all types and flavors |
| Meat, poultry, and fish substitutes | Meat, poultry, and fish substitutes | 2806: Processed soy products | 0.12 | 41420010 | Soybean curd - stir fried; bean curd; tofu |
| | | | | 41420050 | Soybean curd cheese |
| | | | | 41420380 | Yogurt, soy - all types and flavors |
| | | | | 41421010 | Soybean curd, deep fried - aburage; tofu; bean curd |
| | | | | 41421020 | Soybean curd, breaded, fried - tofu; bean curd |
| | | | | 41425010 | Vermicelli, made from soybeans - bean thread, cellophane, glass, or crystal noodles |
| | | | | 41440000 | Textured vegetable protein, dry |
| | | | | 41480020 | Frozen dessert, non-dairy - non-dairy ice cream; tofu, nut or rice based; Tofutti; all flavors; NS as to form |
| | | | | 41810200 | Bacon strip, meatless - Morning Star Breakfast Strip; Stripple; meat substitute; vegetarian |
| | | | | 41810400 | Breakfast link, pattie, or slice, meatless - Prosage; Morningstar; Green Giant; meat substitute; vegetarian |
| | | | | 41810600 | Chicken, meatless, NFS - meat substitute; vegetarian |
| | | | | 41810610 | Chicken, meatless, breaded, fried - Loma Linda brand; meat substitute; vegetarian |
| | | | | 41811400 | Frankfurter or hot dog, meatless - meat substitute; vegetarian; tofu dog |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 41811600 | Luncheon slice, meatless-beef, chicken, salami or turkey - vegetarian ham; Wham; Loma Linda; Worthington; meat substitute |
| | | | | 41811800 | Meatball, meatless - meat substitute; vegetarian |
| | | | | 41811890 | Vegetarian burger or patty, meatless, no bun - veggie burger, made with soy and/or vegetables and grains; soyburger; bean burger; meat substitute; Boca burger; Gardenburger; Morningstar Farms; Worthington FriPats |
| | | | | 41812000 | Sandwich spread, meat substitute type - vegetarian |
| | | | | 41812600 | Vegetarian, fillet - meat substitute; meatless |
| | | | | 41901020 | Soyburger, meatless, with cheese on bun - meat substitute; vegetarian |
| | | | | 42203200 | Soy nut butter |
| | | | | 59003000 | Meat substitute, cereal- and vegetable protein-based, fried - sauteed; Seitan; vegetarian |
| | | 3703: Frankfurter sandwiches (single code) | | 27564420 | Frankfurter or hot dog sandwich, meatless, plain, on bun - meat substitute; vegetarian; any type of bun; tofu dog |
| | | | | 27564430 | Frankfurter or hot dog sandwich, meatless, plain, on bread - meat substitute; vegetarian; any type of bread; tofu dog |
| | | | | 27564560 | Frankfurter or hot dog sandwich, meatless, on bun, with meatless chili - meat substitute; vegetarian hot dog; tofu dog; vegetarian chili; any type of bun |
| | | | | 27564570 | Frankfurter or hot dog sandwich, meatless, on bread, with meatless chili - meat substitute; vegetarian hot dog; tofu dog; vegetarian chili; any type of bread |
| | | 3102: Bean, pea, legume dishes | | 41812400* | Pot pie, no meat - beef-like and chicken-like pot pies; meat substitute |
| | | | | 41812450* | Vegetarian chili, made with meat substitute - meatless |
| | | 3744: Vegetable sandwiches/burgers | | 41901010* | Veggie burger, on bun - vegetarian; soy or bean burger; on bread or roll |
| | | | | 41901030* | Falafel sandwich |
| Nutritional foods | Nutritional bars | 5404: Nutrition bars | 0.25 | 53720100 | Nutrition bar (Balance Original Bar) - all flavors |
| | | | | 53720200 | Nutrition bar (Clif Bar) - all flavors; Clif Minis |
| | | | | 53720210 | Nutrition bar (Clif Kids Organic Zbar) - all flavors |
| | | | | 53720300 | Nutrition bar (PowerBar) - all flavors; PowerBar energy bar |
| | | | | 53720400 | Nutrition bar (Slim Fast Original Meal Bar) - Slim Fast meal-on-the-go and granola meal bars, all flavors |
| | | | | 53720500 | Nutrition bar (Snickers Marathon Protein Bar) |
| | | | | 53720600 | Nutrition bar (South Beach Living Meal Bar) - all flavors |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 53720610 | Nutrition bar (South Beach Living High Protein Bar) - all flavors |
| | | | | 53720700 | Nutrition bar (Tiger's Milk) |
| | | | | 53720800 | Nutrition bar (Zone Perfect Classic Crunch) - all flavors |
| | | | | 53729000 | Nutrition bar or meal replacement bar, NFS |
| | | | | 53710800 | Cereal or granola bar (Kashi Chewy) - chocolate coated; all flavors; GOLEAN; TLC |
| | | | | 53710802 | Cereal or granola bar (Kashi Crunchy) - all flavors; GOLEAN; TLC |
| | Nutritional beverages and beverage bases | 7208: Nutritional beverages | 0.045 | 95101000 | Nutritional drink or shake, ready-to-drink (Boost) - Boost Nutritional Energy Drink |
| | | | | 95101010 | Nutritional drink or shake, ready-to-drink (Boost Plus) |
| | | | | 95102000 | Nutritional drink or shake, ready-to-drink (Carnation Instant Breakfast) - instant breakfast, ready-to-drink, NFS |
| | | | | 95103000 | Nutritional drink or shake, ready-to-drink (Ensure) - Ensure, NFS; all flavors; Bone Health; Immune Health; Muscle Health; high calcium; high protein |
| | | | | 95103010 | Nutritional drink or shake, ready-to-drink (Ensure Plus) - all flavors; Clinical Strength |
| | | | | 95104000 | Nutritional drink or shake, ready-to-drink, sugar free (Glucerna) - Glucerna shakes, all flavors |
| | | | | 95105000 | Nutritional drink or shake, ready-to-drink (Kellogg's Special K Protein) - all flavors |
| | | | | 95106000 | Nutritional drink or shake, ready-to-drink (Muscle Milk) |
| | | | | 95106010 | Nutritional drink or shake, ready-to-drink, light (Muscle Milk) |
| | | | | 95110000 | Nutritional drink or shake, ready-to-drink (Slim Fast) - all flavors; 3-2-1 Plan; Optima; meal replacement |
| | | | | 95110010 | Nutritional drink or shake, ready-to-drink, sugar free (Slim Fast) - all flavors; 3-2-1 Plan; lower carb; meal replacement |
| | | | | 95110020 | Nutritional drink or shake, high protein, ready-to-drink (Slim Fast) - all flavors; 3-2-1 Plan; meal replacement |
| | | | | 95120000 | Nutritional drink or shake, ready-to-drink, NFS - brands such as Nutrament and Equate meal replacement shake; meal replacement |
| | | | | 95120010 | Nutritional drink or shake, high protein, ready-to-drink, NFS - brands such as Monster Milk and EAS Myoplex; meal replacement, NFS |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | | | 95120020 | Nutritional drink or shake, high protein, light, ready-to-drink, NFS - brands such as Myoplex Lite; meal replacement |
| | | | | 95120050 | Nutritional drink or shake, liquid, soy-based - Isocal liquid nutrition; Osmolite liquid nutrition; meal replacement |
| | Protein Powders | 9802: Protein and nutritional powders | 0.95 | 95201000 | Nutritional powder mix (Carnation Instant Breakfast) - instant breakfast, powdered, NFS |
| | | | | 95201010 | Nutritional powder mix, sugar free (Carnation Instant Breakfast) |
| | | | | 95201200 | Nutritional powder mix (EAS Whey Protein Powder) - all flavors |
| | | | | 95201300 | Nutritional powder mix (EAS Soy Protein Powder) - all flavors |
| | | | | 95201500 | Nutritional powder mix, high protein (Herbalife) - all flavors; Healthy Meal nutritional shake mix |
| | | | | 95201600 | Nutritional powder mix (Isopure) - all flavors |
| | | | | 95201700 | Nutritional powder mix (Kellogg's Special K20 Protein Water) - all flavors |
| | | | | 95202000 | Nutritional powder mix (Muscle Milk) - all flavors |
| | | | | 95202010 | Nutritional powder mix, light (Muscle Milk) - all flavors |
| | | | | 95210000 | Nutritional powder mix (Slim Fast) - all flavors; 3-2-1 Plan; Optima |
| | | | | 95210010 | Nutritional powder mix, sugar free (Slim Fast) - all flavors; 3-2-1 Plan; lower carb |
| | | | | 95210020 | Nutritional powder mix, high protein (Slim Fast) - all flavors; 3-2-1 Plan |
| | | | | 95220000 | Nutritional powder mix, NFS - meal replacement, NFS |
| | | | | 95220010 | Nutritional powder mix, high protein, NFS - brands such as D.E.L.T.A. Enhance Formula and Joe Weider's Dynamic Protein Shake; Monster Milk; meal replacement |
| | | | | 95230000 | Nutritional powder mix, whey based, NFS - protein shake mix; brands such as Gold Standard, NOW Foods |
| | | | | 95230010 | Nutritional powder mix, protein, soy based, NFS - protein shake mix; brands such as Gold Standard, NOW Foods |
| | | | | 95230020 | Nutritional powder mix, protein, light, NFS - protein shake mix; brands such as Reliv Now, Beneprotein |
| | | | | 95230030 | Nutritional powder mix, protein, NFS - protein shake mix, NFS; brands such as NOW Foods, NFS and Gold Standard, NFS |
| | | | | 11830900* | Protein supplement, milk-based, powdered, not reconstituted - Meritene |

| Appendix. Food Codes used for Revised Estimated Daily Intake (EDI) Calculation for Non-Animal Type 21 Collagen Polypeptide | | | | | |
|---|---|---|---|---|---|
| **Intended Use Food Category** | **Application** | **WWEIA Category No.: Description** | **Inclusion Rate (w/w)** | **Food code** | **Main Food Description - Additional Food Description** |
| | | 9999: Not included in a food category | | 11832000* | Meal replacement, protein type, milk- and soy-based, powdered, not reconstituted - D.E.L.T.A. Enhance Formula; Joe Weider's Dynamic Protein Shake |
| | | | | 11836000* | Protein supplement, milk-based, Muscle Milk, powdered, not reconstituted - all flavors |
| | | | | 11836100* | Protein supplement, milk-based, Muscle Milk Light, powdered, not reconstituted - all flavors |

**From:** Dietrich Conze
**To:** Downey, Jason
**Cc:** Claire Kruger; Kathy Brailer; Jennifer Symonds
**Subject:** [EXTERNAL] Re: GRN 001171 - Questions for the Notifier
**Date:** Friday, September 6, 2024 2:31:24 PM
**Attachments:** Geltor Response to FDA on GRN 001171 - 9-6-24.pdf



Hi Jason,

Attached are our responses to your questions and points that need clarification.

Regards and have hope you have a nice weekend.

Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

> On Sep 3, 2024, at 10:28 AM, Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:
>
> Hi Dietz,
>
> During our evaluation of GRN 001171 and its amendments, regarding Geltor's intended uses in food of collagen polypeptide produced by *E. coli* K-12 S1988, we noted questions and points in need of clarification, which are listed below. Please provide responses to these requests within 10 business days. If you foresee any issue with this timeline or have any questions, please contact me as soon as possible.
>
> Thank you in advance for your attention to our comments.
>
> Sincerely,
>
> Jason

Spherix Consulting Group

September 6, 2024

Jason Downey, Ph.D.
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Clarification Questions Regarding GRN 001171

Dear Dr. Downey:

Below are our responses to the Agency's requests for additional information regarding GRN 001171, as specified in your email on September 3, 2024. Your requests are in italicized text and our responses are below in plain text.

1. *In the response to question 2 in the amendment dated August 15, 2024, Geltor provides updated intended uses of collagen polypeptide produced by E. coli K-12 S9188 in various foods in Table 1.*

   a. *In Table 1, the level for "Coffee, powdered, not reconstituted" is indicated as 55.5%, but in the Appendix, this level is stated to be 55%. Please clarify which one is correct.*

      The correct inclusion rate for coffee, powdered, not reconstituted is 55%.

   b. *In Table 1, non-reconstituted powdered tea is not included as an intended use, However, in the Appendix, two dry instant iced teas, National Health and Examination Survey (NHANES) food codes 92307000 and 92307400, are included. Please clarify if this is an intended use.*

      The collagen polypeptide produced by *E. coli* K-12 S9188 is intended to be used as an ingredient in both coffee and teas, including non-reconstituted, powdered tea, which includes dry instant iced teas.

   c. *In Table 1, the level for "Coffee, powdered, not reconstituted" is indicated as 55.5%, but in the Appendix, the use level for powdered coffee and tea products for NHANES food codes 92291300, 92307000 and 92307400, is stated to be 5.5%. Please clarify this discrepancy. If the use level of 5.5% is incorrect, please provide an updated dietary exposure estimate using the correct use level.*

751 Rockville Pike, Unit 30-B
Rockville, MD 20852
301-557-0375; info@spherixgroup.com
http://www.spherixgroup.com

Food code 92291300 is for "Coffee substitute, dry powder." This description does not state that the consumption of this food is as unreconstituted powder, as explicitly stated for food codes 9219110, 92191200, 92191400, 92192000, 92912030, 92192040, 92193000, 92193005, 92103020, and 92193025. The use level for this food code is correctly listed as 5.5%, corresponding to the category "Instant coffee and tea," in the updated Table 1, shown in the response to Question 2.

Food codes 92307000 and 92307400 are for unreconstituted, powdered tea mixes. Both food codes should have a use level of 55%; the previous use level of 5.5% specified in the Appendix was incorrect. Although the previous exposure assessment was calculated with the incorrect inclusion rate of 5.5%, an updated exposure estimates using the correct inclusion rate of 55% yielded similar results to the estimates provided in Table 2 of the amendment dated August 15, 2024. The updated exposure estimates are provided in the revised Table 2 below.

**Table 2. Estimated Daily Intake* of Non-Animal Type 21 Collagen Polypeptide in Consumers of Proposed Non-Animal Type 21 Collagen Polypeptide-containing Products in the U.S. by Age Cohort, NHANES 2017-March 2020 Pre-pandemic**

| | % Users | N Users | Total Population | EDI (g/kg body weight/day) | | EDI (g/day) | |
|---|---|---|---|---|---|---|---|
| | | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Males | | | | | | | |
| Ages 1-2 | 45.2 | 113 | 275 | 0.44 | 0.89 | 5.42 | 10.07 |
| Ages 3-5 | 56.5 | 149 | 275 | 0.23 | 0.55 | 4.35 | 9.91 |
| Ages 6-11 | 58.7 | 346 | 602 | 0.23 | 0.54 | 7.91 | 21.31 |
| Ages 12-19 | 57.6 | 405 | 747 | 0.18 | 0.4 | 12.47 | 26.67 |
| Ages 20+ | 80.8 | 3493 | 3169 | 0.24 | 0.49 | 20.84 | 42.75 |
| Ages 2+ | 74.9 | 3451 | 4918 | 0.24 | 0.5 | 18.63 | 39.85 |
| Females | | | | | | | |
| Ages 1-2 | 45.7 | 105 | 243 | 0.33 | 0.95 | 3.93 | 10.29 |
| Ages 3-5 | 60.1 | 149 | 259 | 0.19 | 0.4 | 3.52 | 7.0 |
| Ages 6-11 | 60.7 | 327 | 596 | 0.15 | 0.3 | 4.83 | 10.78 |
| Ages 12-19 | 59.6 | 411 | 730 | 0.16 | 0.33 | 10.06 | 19.81 |
| Ages 20+ | 82.0 | 2722 | 3471 | 0.23 | 0.47 | 16.49 | 33.34 |
| Ages 2+ | 77.0 | 3677 | 5198 | 0.22 | 0.45 | 14.84 | 31.27 |

*Calculated using DaDIET, food codes that represent the intended uses, and user data from the 2017-March 2020 Pre-pandemic NHANES database.

2. *In the response to question 2 in the amendment dated August 15, 2024, Geltor provides the updated intended uses of collagen polypeptide produced by E. coli K-12 S9188 in various foods in Table 1. In the response to question 3 in the amendment dated August 15, 2024, Geltor states "some chocolate confection food codes were excluded from the category entitled 'chocolate confections'". In addition, in the appendix of the amendment dated August 15, 2024, Geltor provides beverage food codes, which are in agreement with the description in the response to question 19 in the amendment dated June 3, 2024, i.e., "the beverage category includes sports drinks, flavored bottled water, and powdered fruit and sports drinks".*

*a. Based on our understanding of this information, we have modified Table 1 as below. Please confirm whether Table 1 captures the intended uses of your ingredient.*

We confirm that the revised Table 1 captures the intended uses of the ingredient.

**Table 1. Intended Food Categories and Maximum Use Levels of Collagen Polypeptide Produced by *E. coli* K-12 S9188**

| Food Category | Maximum Use Levels (%, w/w) |
|---|---|
| Flavored beverages and beverage bases, including sports drinks, flavored bottled water, and powdered fruit and sports drinks | 2 |
| Flavored beverages and beverage bases, powdered, not reconstituted | 20 |
| Packaged coffee & tea beverages | 5.5 |
| Instant coffee & tea | 5.5 |
| Coffee and tea, powdered, not reconstituted | 55 |
| Coffee and tea pods | 2 |
| Probiotic and yogurt beverages | 2.5 |
| Milk substitutes | 2.5 |
| Coffee creamer (liquid and powder forms) | 20 |
| Nutritional beverages and beverage bases | 4.5 |
| Nutritional bars | 25 |
| Protein powders | 95 |
| Gummies | 10 |
| Chocolate confections (excluding chocolate bars) | 10 |
| Meat, poultry, and fish substitutes | 12 |

3. *In the response to question 3 in the amendment dated August 15, 2024, Geltor provides an updated dietary exposure estimate for collagen polypeptide produced by E. coli K-12 S9188 in different age groups in Table 2. However, Geltor did not specify whether the results are a per capita or an eaters-only estimate. In addition, Geltor did not provide an "eaters-only" estimate of dietary exposure at the mean and 90$^{th}$ percentile for the U.S. population aged 2 years and older. Please clarify the dietary exposure estimate and provide a dietary exposure for the U.S. population aged 2 years and older.*

The exposure estimates provided in Table 2 of the amendment dated August 15, 2024, are for both the mean and 90$^{th}$ percentile consumers of foods containing non-animal type 21 collagen polypeptide, i.e. "eaters-only," as indicated in the title of Table 2, "Estimated Daily Intake of Non-Animal Type 21 Collagen Polypeptide in Consumers of Proposed Non-Animal Type 21 Collagen Polypeptide-containing Products in the U.S. by Age Cohort, NHANES 2017-March 2020 Pre-pandemic."

The mean and 90$^{th}$ percentile consumer estimates described in revised Table 2 included above in our response to Question 1c are for "eaters-only."

Should you need additional information, please feel free to contact me at +1-240-367-6089 or dconze@spherixgroup.com.

Dietrich B. Conze, Ph.D.
Managing Partner

**Spherix Consulting Group, Inc.**
751 Rockville Pike, Unit 30-B, Rockville, MD 20852
301-557-0375; info@spherixgroup.com

**From:** Dietrich Conze
**To:** Downey, Jason
**Cc:** Claire Kruger; Kathy Brailer; Jennifer Symonds
**Subject:** [EXTERNAL] Re: GRN 001171 - Questions for the Notifier
**Date:** Friday, September 27, 2024 1:48:22 PM
**Attachments:** Geltor Response to FDA on GRN 001171 - 9-27-24.docx

---



Hi Jason,

Attached are our responses.

Regards.

Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

> On Sep 25, 2024, at 9:35 AM, Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:
>
> Hi Dietz,
>
> During our evaluation of GRN 001171 and its amendments, regarding Geltor's intended uses in food of collagen polypeptide produced by *E. coli* K-12 S1988, we noted questions and points in need of clarification, which are listed below. Please provide responses to these requests within 10 business days. If you foresee any issue with this timeline or have any questions, please contact me as soon as possible.
>
> Thank you in advance for your attention to our comments.
>
> Sincerely,
>
> Jason
>
> **Jason Downey, Ph.D. (he/him/his)**

*Regulatory Review Scientist*

**Division of Food Ingredients**
**Office of Food Additive Safety**
**Center for Food Safety and Applied Nutrition**
**U.S. Food and Drug Administration**
jason.downey@fda.hhs.gov

## FDA's Questions to the Notifier

1. In the response to question 4 in the amendment dated August 15, 2024, Geltor provides updated heavy metal specifications for cadmium, lead, and mercury to be not more than 0.125, 0.25, and 0.05 mg/kg, respectively. The arsenic specification was kept at 0.25 mg/kg. We typically see levels around 0.1 mg/kg for heavy metals for fermentation-derived ingredients. Please consider lowering the specifications for lead, arsenic, and cadmium to be consistent with other ingredients produced in a similar manner.

2. Geltor provides a separate dietary exposure for females and males aged 2 years and older in the response to question 3 in the amendment dated September 6, 2024, but did not provide a dietary exposure for the overall population aged 2 years and older. Please provide an eaters-only dietary exposure at the mean and $90^{th}$ percentile for the U.S. population aged 2 years and older.

**From:** Dietrich Conze
**To:** Downey, Jason
**Cc:** Kathy Brailer; Claire Kruger
**Subject:** [EXTERNAL] Re: GRN 001171 quick question
**Date:** Tuesday, October 15, 2024 11:32:22 AM



Hi Jason,

Yes, the intended uses are substitutional for “added" collagen peptides in conventional food.

Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

> On Oct 15, 2024, at 10:43 AM, Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:
>
> Hi Dietz,
>
> We have one quick request for clarification on GRN 001171. In the June 3 amendment, the notifier says, “… Collagen Polypeptide is intended to be substitutional for other sources of collagen polypeptides that are currently available; therefore, the use of this product will not increase the dietary exposure to collagen polypeptides.” Can you clarify that the notifier’s intended uses are substitutional for ***added*** collagen polypeptides in conventional food and that it is not intended as substitutional for collagen polypeptides in food generally, e.g., collagen naturally present in meat? The food categories don’t seem to be categories that typically “naturally” collagen, but these are statements that we tend to quote in response letters, and we didn’t want to assume the “added” bit.
>
> Thank you!

Jason

**Jason Downey, Ph.D. (he/him/his)**
*Regulatory Review Scientist*

**Office of Food Chemical Safety, Dietary Supplements, and Innovation**
**Human Foods Program**
**U.S. Food and Drug Administration**
jason.downey@fda.hhs.gov