Generally Recognized as Safe (GRAS) Determination for the Use of Yeast Hydrolysate Peptide Complex (DNF-10) as a Food Ingredient.

November 2, 2021

Prepared for:

Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Prepared by: Chevreau Consulting LLC and Fytexia Corp.

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GRAS Notice for Yeast Hydrolysate Peptide Complex (DNF-10)

Contents

I. Part 1 - §170.225 Signed statements and Certification

In accordance to 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Fytexia Corporation hereby informs the US Food and Drug Administration (FDA) that a Yeast Hydrolysate Peptide Complex manufactured by Cremar under the trade name of DNF-10 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. This is based on Fytexia's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section I.D, below. In addition, as a responsible official of Fytexia Corp, Dr. Nathalie Chevreau hereby certifies that all data and information presented in this notice constitute a complete, representative, and balanced submission which considered all unfavorable, as well as favorable information, known to Fytexia and pertinent to the evaluation of the safety and the GRAS status of the yeast hydrolysate peptide complex for addition to foods as described herein.

Signed,

Nov 2, 2021

Nathalie Chevreau PhD, RD
Principal
Chevreau Consulting LLC
nchevreau@ncphd.net

Date

A. GRAS Notice Submission

Fytexia Corp submits this GRAS notification through its agent Dr Nathalie Chevreau PhD, RD, owner of Chevreau Consulting LLC in accordance with the requirements of 21 CFR Part 170, Subpart E.

B. Name and Address of the Notifier

Chevreau Consulting LLC
2151 E Logan Avenue
Salt Lake City, UT 84108
(801) 652 6035

C. Common or usual name of notified substance

The common name is Yeast Hydrolysate Peptide Complex
The trade name of the products is DNF-10

D. Conditions of Use

DNF-10 or yeast hydrolysate peptide complex is intended to be added as a food ingredient to a variety of food products targeted to adults. It is not intended to be added to infant formula or foods targeted to children. The intended use level is 250 mg or less o**f** DNF-10 by serving. Intended food applications include but are not limited to:

Snacks (e.g. chips, ready to eat popcorns, crackers)
Protein or nutritional bars
Dry mixes for beverage blends (e.g. smoothies, protein powder mixes, etc.)

E. Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the Code of Federal Regulations (CFR), the Yeast Hydrolysate Peptide Complex manufactured by Cremar and licensed and distributed by Fytexia Corp, has been concluded to have GRAS status for use as an ingredient for addition to specified conventional food and beverage products, as described in Part 1.D, on the basis of scientific procedures.

A comprehensive assessment of scientific (human and animal, quantitative and qualitative) literature and regulatory resources were consulted for this review. The safety of DNF-10 is supported based on its intended use. Data and information were gathered from a critical and comprehensive review of the scientific literature on the safety of Saccharomyces Cerevisiae type yeast (baker's and Brewer's yeast) and their extracts/derivatives through searches of PubMed, FDA dockets, internet searches, availability in foreign markets etc. In addition, the product was subjected to extensive physical and chemical analysis. Baker's and Brewer's yeast and their derivatives are an important part of the human diet in many countries and have been consumed since ancient times as fermenting agents, and flavoring agents. Additional yeast has a nutritional value naturally high in protein, fiber, vitamins, and minerals. Based on a

critical evaluation of the information presented below by a qualified expert, it was concluded that the proposed use of DNF-10 as a food ingredient is GRAS.

F. Premarket Exempt Status

DNF-10 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FFD&CA) based on the conclusion that the notified substance is GRAS under the conditions of intended use.

G. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of: Fytexia, c/o Pramex International Corp, 1251 Avenue of the Americas, FL 3, New York, NY 10020-1104. In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency`s review of the notice, Fytexia will supply these data and information.

H. Freedom of Information Act, 5 U.S.C. Section 552

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

I. FSIS Statement

Not applicable. Fytexia does not intend to add DNF-10 to meat or poultry products or foods that come under USDA jurisdiction.

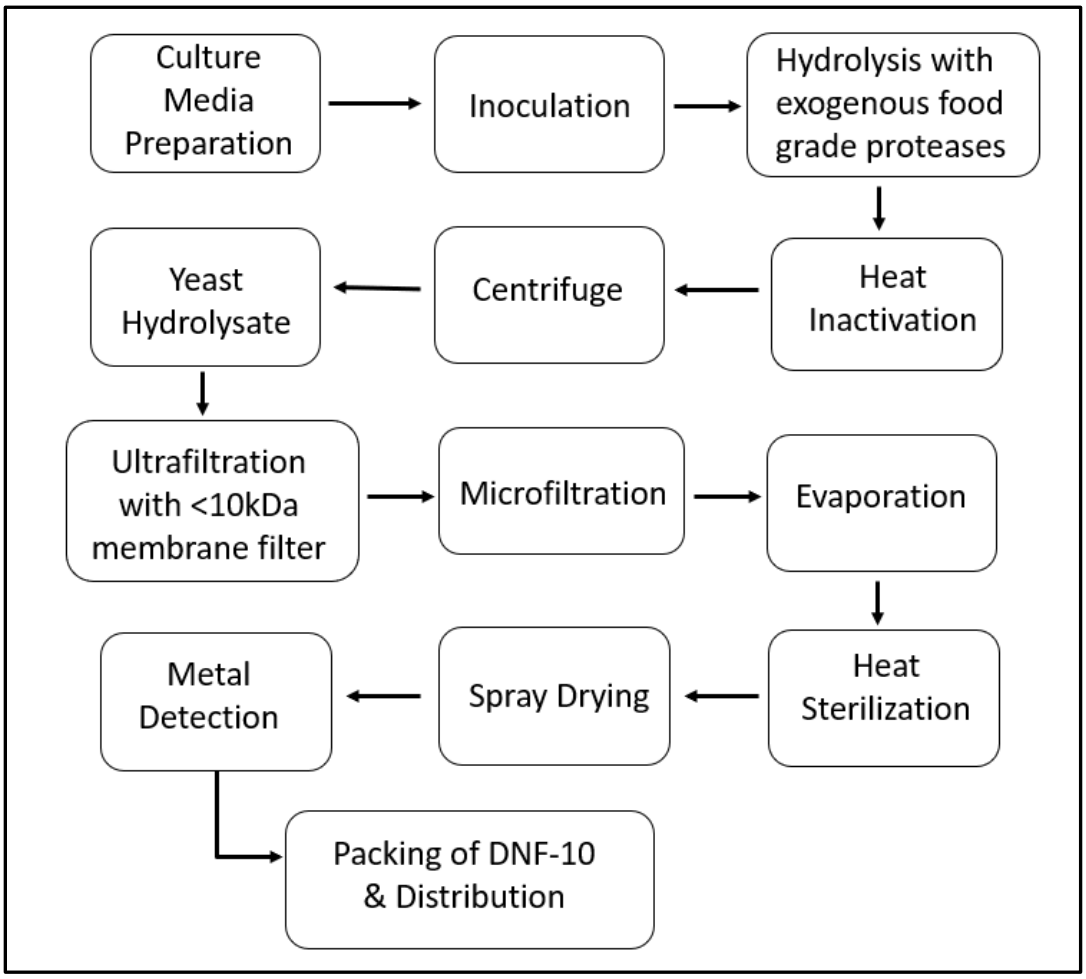
II. PART 2 - §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

A. Identity

The source of the Yeast Hydrolyzed Peptide Complex is a food grade *S*accharomyces *cerevisiae* yeast (ATCC strain 208288) which has not been genetically modified. It is referenced as Sake Yeast strain NBRC 2346 by the National Institute of Technology and Evaluation (NITE is an Incorporated Administrative Agency under the Japanese government).

B. Manufacturing Process

The yeast hydrolyzed peptide complex DNF-10 is manufactured consistent with current good manufacturing practices (cGMP) at 2 facilities located in the town of Ksan-Si, South Korea. Both facilities are ISO 22000 certified. A basic overview of the manufacturing process is illustrated on the flow chart below:



Step 1: Raw materials are received from verified suppliers. Saccharomyces cerevisiae Sake Strain NBRC 2346 is incubated in a typical liquid growth medium for 3 days at 30° C.

Step 2: Food grade proteases are added to the yeast suspension to hydrolyze the proteins contained in the yeast cells. The hydrolysis is run at 30^0C for 4 hours. The proteases are a blend of i) aminopeptidase that is made from aspergillus oryzae; ii) Subtilisin made from Bacillus licheniformis and iii) papain extracted from the latex of the immature papain plant. All the proteases have been affirmed as generally recognized as

GRAS in part 184 of the 21 CFR, Chapter I, sub chapter B. (184.1027; 184.1150; 184.1585)

Step 3: The mixture is then heat-treated at 85°C for 30 min to inactivate the yeast cells and the food enzymes.

Step 4: The mixture is centrifuged to collect the supernatant which is passed through a 10 kDa molecular weight cut-off membrane. Proteases have all a molecular weight greater than 10 kDa and will not be present in the supernatant (Papain MW=23 kDa, Subtisilin MW=27 KDa; Flavourzyme 1000L 26.5 KDa)

Step 5: The supernatant is passed through a microfilter and concentrated through an evaporator. The yeast hydrolysate peptide concentrate is then heat sterilized at 90^{o}C for 25 minutes.

Step 6: The sterilized concentrate is spray dried on a maltodextrin support to yield a yellow to light brown powder containing the final yeast hydrolysate peptide complex. The powder is run through a metal detector, through a 40-mesh sieve and finally packaged for distribution.

C. Product specifications for DNF–10 (Yeast Hydrolysate Peptide Complex)
Appropriate specification for DNF-10 to be used as a food and in foods have been established. Analyses of 3 non-consecutive lots of DNF-10 demonstrate that the product is consistently manufactured to comply with established specifications. The specification parameters comprise physical appearance, taste, crude protein level derived from total nitrogen content as well as limits for potential chemical and microbiological impurities and contaminants. The results are presented in the Table A below

Table A: Specifications and Batch Record Results for DNF-10

	Specification	**Method**	**Batch #191107**	**Batch #190610**	**Batch #180914**
Physical and Chemical Parameters					
Color/Appearance	Yellow to light brown	Visual against standard	Conforms	Conforms	Conforms
Odor	Slightly yeasty	Sensory	Conforms	Conforms	Conforms
Taste	Slightly yeasty	Organoleptic	Conforms	Conforms	Conforms
Crude Protein Content % (total nitrogen content x 6.25)	50-70	Kjeldahj	55.27	58	57.6
Carbohydrates (%)	28-33	By difference	38.4	35.1	36.2
Ash (%)	≤ 3	Residue upon ignition	2.6	2.4	2.5
Moisture (%)	≤ 8	Loss on drying at 105°C	3.92	4.5	4.3
Microbiological Parameters					
Total aerobic microbial count	≤ 3,000 CFU/g	ISO 4833-1	150	300	200
Total yeasts and mold count	≤ 50 CFU/g	ISO 21527-2	0	0	0
Coliforms	Absent in 1 g	ISO 4831	Absent	Absent	Absent
Staphylococcus aureus	Absent in 10 g	ISO 6888	Absent	Absent	Absent

Salmonella	Absent in 20 g	ISO 6579	Absent	Absent	Absent
Impurities					
Total heavy metals	< 10 ppm	Colorimetry	< 1	< 1	< 1
Arsenic (LOQ=0.07 ppm)	< 1 ppm	ICP-Inductively coupled plasma spectrometry	ND	0.01	0.07
Cadmium (LOQ=0.009 ppm)	< 1 ppm	ICP spectrometry	0.021	0.01	ND
Mercury	< 0.1 ppm	Mercury analyzer	ND	ND	ND
Lead (LOQ=0.001 ppm)	< 0.5 ppm	ICP spectrometry	ND	0.01	0.01

Peptide size, amino acids and peptide distribution in the yeast hydrolysate peptide complex were measured by size exclusion chromatography (also known as gel permeation chromatography). The results are presented in the table below (Table B)

Table B: Distribution in Yeast Hydrolysate Peptide Complex

	Percent Peptides in DNF10				
Peptides (size range)	Batch DNF181026	Batch 20170504	Batch 20170831	Batch 20180130	Average of 4 lots
Free Amino Acids	36.94	39.25	35.18	40.17	38.08
Peptides between 3 and 7 AA	9.83	6.37	9.77	6.35	8.12
Peptides between 8 and 9 AA	0.42	0.27	0.50	0.24	0.36
Peptides between 10 and 28 AA	0.52	0.27	0.59	0.28	0.42
Peptide > 29 AA	0.00	0.00	0.00	0.00	0.00

D. Amino Acids Profile

The average amino acid and nitrogen content from nucleic acid in DNF-10 was calculated using the results of the analysis of 4 batches of DNF-10, and is presented in Table C.

Table C: Amino Acid Content of Yeast Hydrolysate Peptide complex

Amino Acid	Batch 181026 (g/100 g DNF-10)	Batch 170504 (g/100 g DNF-10)	Batch 170831 (g/100 g DNF-10)	Batch 181030 (g/100 g DNF-10)	Average of 4 lots (g/100 g DNF-10)
Glutamic Acid	7.4	7.0	8.2	7.9	7.6
Aspartic Acid	5.1	5.3	5.3	5.2	5.2
Alanine	4.5	4.3	4.0	4.5	4.3
Nitrogen from nucleic acids	4.1	5.2	7.3	4.4	5.3
Lysine	3.9	3.8	4.0	4.1	3.9
Leucine	3.8	3.7	3.4	3.5	3.6
Isoleucine	2.9	3.1	3.1	3.0	2.7
Glycine	2.8	2.7	2.7	2.7	2.5

Threonine	2.4	2.5	2.7	2.5	2.4
Phenylalanine	2.5	2.8	1.9	2.4	2.2
Serine	2.4	2.2	2.1	2.1	2.07
Proline	2.2	2.3	1.7	2.2	2.0
Arginine	2.1	2.0	2.1	2.1	1.4
Valine	2.0	0.9	1.4	1.5	1.1
Histidine	1.0	1.1	1.1	1.1	1.1
Tyrosine	0.8	0.8	0.7	0.5	0.7
Methionine	0.8	0.5	0.6	0.5	0.6
Cysteine/Cystine	0.7	0.7	0.5	0.6	0.6
Tryptophan	0.5	0.6	0.6	0.6	0.5
Hydroxyproline	0.0	0.0	0.0	0.0	0.0
GABA	5.4	7.7	7.0	7.6	6.9

E. Stability

Accelerated and real-time stability studies have been conducted on 5 non-consecutive batches of DNF-10. DNF-10 (stored in paper bags with polyethylene liners) is stable for at least 36 months under standardized conditions (25°C and 60% relative humidity) and at least 36 months under warehouse (ambient) conditions. Although variations in color and moisture content were reported upon storage of DNF-10 under accelerated storage conditions (40°C and 75% relative humidity), measurements of the crude protein content, moisture and E. Coli remained within the defined specs. Together, these data support the proposed shelf-life of 36 months for DNF-10.

F. Labeling and storage information

1. Label declaration

The name to appear on the label will be:

Yeast hydrolysate peptide complex (from Saccharomyces cerevisiae)

2. Packaging

10 kg packages with polyethylene (PE) inner lining and aluminum outer lining.

3. Storage Conditions

Product should be stored in a cool, dry location, and in the original sealed package and avoiding direct light.

4. Shelf Life

The peptide content of this product is stable under accelerated conditions

III. Part 3 - §170.235 Dietary Exposure

A. Intended Uses for DNF-10

The yeast hydrolysate peptide complex (DNF-10) is intended to be used as an ingredient in selected conventional foods, dry beverage blends like protein powder, nutritional bars at a level of 250 mg or less per serving or less. The serving size will typically be the reference amounts customarily consumed per eating occasion (RACC) DNF-10 is not intended for use in infant formula, meat, poultry, egg products, catfish or any products that would require additional regulatory review by USDA. It is also not intended to be used in beverages containing alcohol, or in beverage intentionally marketing to children. A summary of the intended uses is shown in table D below.

Table D: Summary of the individual Proposed Food Uses and Use Levels for Yeast Hydrolysate Peptide Complex (DNF-10)

Proposed Food Use	RACC* (g)	DNF-10 level (mg/serving)	Use Level (%)
Popcorn[a]	30	250	0.83%
Salted chips	30	250	0.83%
Crackers[b]	30	250	0.83%
Nutritional bars[c] (granola, protein, breakfast)	40	250	0.63%
Protein dry powder (to be mixed with water before consumption)	20	250	1.25%

*RACC: Reference amounts customarily consumed per eating occasion (Federal Register Vol 81 No103/Friday May 27 2016/Rules and Regulations[1])

[a] 2 cups air-popped popcorn without butter weights 17 grams on average.
[b] A single serve bag of chip typically weighs 1 oz (28 g). Family size bags are typically 10 oz (283 g)
[c] Typical nutritional energy bars weight 40 g on average. Protein bars weigh 50 g on average.

B. Estimated Exposure of DNF-10 based on Selected Foods Daily Intake

The cumulative estimated exposure of DNF-10 was calculated by 1) estimating the current consumption of selected food categories, 2) applying the intended use level of

[1] https://www.govinfo.gov/content/pkg/FR-2016-05-27/pdf/2016-11865.pdf#page=42

DNF-10 to these food categories and 3) applying a probabilistic model to determine the cumulative eaters-only intake of DNF-10.

USDA NHANES[2] survey data were used to estimate current consumption of selected foods shown in table above (Table D). NHANES is a major program of the National Center for Health Statistics (NCHS) which is part of the Centers for Disease Control and Prevention (CDC). NHANES has the responsibility for producing vital and health statistics for the US and interviews about 5000 individuals per year. The database included dietary intake and food frequency surveys and can be queried for specific food categories and consumed amounts per age, gender and ethnic groups. The consumption of the selected food was estimated using a dataset that combined the NHANES surveys from 1999 to 2002, and from 2003 to 2016. The dataset contains consumption data for 92,062 individuals. Food codes were selected that represented the intended food categories and are summarized in Appendix I. The daily consumption for each food category is shown in table E below.

Table E:

Food Category	N of eaters out of 92,062 surveyed individuals (2+ years old)	% Eaters (N/92,062)	Mean consumption of food (g/p/d)	90th ile (g/p/d)
Popcorn	1153	1.25%	35	83.1
Chips	5565	6.04%	35.2	64
Crackers	3005	3.26%	26.1	52
Bars	551	0.60%	41.6	65
Protein powder	206	0.22%	60	131.4

Next, the overall dietary exposure from the use of DNF-10 in all of the proposed food categories was calculated using a probabilistic modeling. It was estimated using the method described in FDA guidance document for estimating dietary exposure (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food).

[2] NHANES: National Health and Nutrition Examination Survey. https://wwwn.cdc.gov/nchs/nhanes/Default.aspx

Briefly, in order to estimate an overall exposure from use in all food categories, the mean exposures for each food category are converted to a total sample mean intake (TSMI) by multiplying a given food category amount by the percent eaters. (Table F column 7). The total sample means for each food category can then be summed and the percent eaters for the overall exposure can be determined. A pseudo 90th percentile exposure can be estimated by multiplying the overall mean exposure by 2.

Table F:

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Foods	N of eaters (2+ years old)	% Eaters (N/92,062)	Mean consumption of food (g/p/d)	Level of DNF-10 in food (%)	Grams/p/d of DNF-10 consumed by eaters only	Total Sample Mean Intake (TSMI) in g/p/d of DNF-10
Popcorn	1153	1.25%	35	0.83%	0.2905	0.00364
Chips	5565	6.04%	35.2	0.83%	0.29216	0.01766
Crackers	3005	3.26%	26.1	0.83%	0.21663	0.00707
Bars	551	0.60%	41.6	0.63%	0.26208	0.00157
Protein powder	206	0.22%	60	1.25%	0.75	0.00168

The cumulative TSMI of DNF-10 was calculated by summing the TSMIs of DNF-10 in each food category and equaled 0.0316 grams of DNF-10 per person per day.

The percent eaters of any or all the foods in which DNF-10 is used was calculated as follows:

$$\%Eaters=\{1-[1-eaters_{popcorn})x(1-eaters_{chips})x(1-eaters_{crackers})x(1-eaters_{bars})x(1-eaters_{protein})]\}\ 100$$

where "eaters " is the fraction of the population that consumes a given food; and "(1 - eaters)" is the fraction of "non-eaters" for that food. Subtraction of the fraction of "non-eaters" of the foods from unity gives the fraction of eaters of any or all of the foods. This relationship assumes no correlation between the consumption of any or all the foods in which DNF-10 is used.

$$\%Eaters=\{1-[1-0.0125)x(1-0.0604)x(1-0.0326)x(1-0.006)x(1-0.0022)]\}x100 = 10.99\%$$

The cumulative eaters-only mean intake of DNF-10 was obtained by dividing the cumulative TSMI for DNF-10 by the percent eaters (0.0316 / 10.99% = 0.2875 g/p/d (287.5 mg/p/d)

Finally, the approximate cumulative eaters-only intake of DNF-10 at the 90th percentile was obtained by multiplying the cumulative eaters-only mean intake by two. This cumulative eaters-only pseudo-90thile intake of DNF-10 equals 0.575 g/p/d (575 mg/p/d).

C. Current Exposure to other yeast-derived products

Throughout history, yeasts from the genus Saccharomyces cerevisiae and yeast derivatives have been consumed in the human diet for many years. Yeast has been used as a leavening agent in bread making, in industries such as brewing and winemaking. Its extract has been used as a flavoring agent. Inactivated yeast cells or yeast biomass left over from beer brewing or bread making have been used for animal feed and nutritional supplements for humans.

Dried *S. cerevisiae* yeast appears in FDA regulation as food additive (21 CFR Chapter 1, subchapter B, Subpart I §172.896)[1]. It is permitted for direct addition to food for human consumption with the annotation "may be safely used in food provided the total folic acid content of the yeast does not exceed 0.04 milligram per gram of yeast" (approximately 0.008 milligram of pteroyglutamic acid per gram of yeast).[1] Baker's yeast protein is in the category of Special Dietary and Nutritional Additives permitted for direct addition to food for human consumption (21 CFR Subpart D, 172.325)[2]. An extract made from partial hydrolysis of *S. cerevisiae* yeast can be used as a flavoring agent (21 CFR Subpart B §172.590)[3]. Additionally, Baker's yeast extract, meeting appropriate food-grade specifications, is considered GRAS (Generally Recognized As Safe) and adjuvant at a level not to exceed 5 percent in food (21 CFR Subpart B, §184.1983)[4].

Table C below is a summary of the regulatory status of yeast and yeast derived products

Table C: Regulatory status of yeast and yeast derived products

Reference	Ingredient	Description	Use	Limitations
21 CFR §172.325	Baker's yeast protein	The insoluble proteinaceous material remaining after the mechanical rupture of Saccharomyces cerevisiae cells and removal of whole cells walls by centrifugation and separation of soluble cellular material	Use in food as a nutrient supplement	No limitation specified

21 CFR §172.590	Yeast malt sprout extract	Partially hydrolyzed yeast using enzymes from sprouted malt barley	Use as a flavor enhancer in food	Not in excess of that amount reasonably required to produce the intended effect
21 CFR §172.896	Dry yeast	Saccharomyces cerevisiae, saccharomyces fragilis and candida utilis	As a multipurpose additive	No limit except that folic acid content of the yeast should not exceed 0.04 mg/gram
21 CFR §172.898	Baker's yeast glycan	The comminuted washed, pasteurized and dried cell walls of Saccharomyces cerevisiae composed mainly of long chain carbohydrates (2:1 glycan:mannan), not less than 85% on a dry solid basis	An emulsifier, stabilizer, thickener or texturizer for salad dressings	Not to exceed 5% in salad dressing
21 CFR 184.1983	Baker's yeast extract	Food ingredient resulting from concentration of the solubles of mechanically ruptured cells of Saccharomyces cerevisiae	Flavoring agent and adjuvant	Not to exceed 5% in food
GRN 260 & 353	High Selenium Yeast	Saccharomyces cerevisiae strain cultivated in a selenium-enriched fermentation medium	Nutrient supplement	Baked products, non-alcoholic beverages, breakfast cereals, grain products and pastas, milk products, processed fruit & fruit juices, processed vegetables and vegetable juices, commercial soups and soup mixes, and medical foods at levels yielding **5** micrograms selenium per serving
GRN 239	Yeast Beta-glycans	Baker's yeast Saccharomyces cerevisiae cells that are lysed and beta glucans are extracted with some residual protein (<10%)	Nutrient supplement	In a variety of food products including baked goods and baking mixes, beverages and beverage bases, cereals and cereal products,

				dairy product analogs, milk and milk products, plant protein products, processed fruits and fruit juices, soft candy, soups and soup mixes at a level of up to 200 milligrams per serving
GRN 422 & 604	Enhanced Baker's yeast	Functionally enhanced Baker's Yeast that prevent acrylamide in foods and beverages	Nutrient supplement	Grain-Based Food or Bread, Cereals, Cookies, Pizza crust, Crackers etc.; Vegetable-based Foods (French-fried potatoes, Potato chips, Potato-based snack foods - Up to 5% in food

CFR: Code of Federal Regulation. GRAS: Generally Recognized As Safe. GRN: GRAS Notification.

A popular yeast derived product is a spread created in 1902 in Burton-upon-Trent, England. In the first stage of the process, the brewer's yeast is broken down into protein and amino acids. The mixture is then filtered before it is passed through a proprietary flavoring process. The final yeast extract paste contains yeast peptides, sugar, mineral salt (potassium chloride) color (caramel III), corn maltodextrin, mineral (iron), vitamins (niacin, thiamin, riboflavin, folate, B12), herbs and spice. A 5-gram serving of the yeast extract paste yields an average of almost 1 gram of protein and 35 calories. The breakdown of the protein in this yeast extract in terms of oligopeptides, smaller peptides and free amino acids has not been published. It is highly conceivable that this protein profile resembles that of DNF-10.

Other yeast derived products have been submitted to FDA and deemed GRAS. Examples are 2 high-selenium yeast-based compounds (GRAS Notification Numbers GRN 260 [5] and 353 [6]). As indicated in GRAS 260, based on a total ingestion of 100 µg Selenium per day, the user would consume 83 mg of yeast per day. This would translate in a daily consumption of about 37 to 45 mg yeast protein per day.

Another example is Baker's yeast beta-glucans. These are made from extracting the beta-glucans fraction from the cell wall of Saccharomyces cerevisiae. The final product contains at least 75% of beta-glucans and less than 10% protein. The compound has been deemed GRAS (GRN 239).[7] Under the conditions of intended use in food, total

population all-user mean and 90th percentile daily intakes have been estimated to be 413.02 mg/person/day (8.90 mg/kg body weight/day) and 827.32 mg/person/day (20.66 mg/kg body weight/day), respectively. This would correspond to a range of 41 to 83 mg of yeast-derived protein per day.

Yeast is also consumed for its nutritional value or health benefits in the form of tablets, powders, flakes or in liquid form. [8] [9-11] Inactivated Brewer's yeast cells have been available for purchase as nutrition or dietary supplements since before 1994. Nutritional Brewer's yeast is inactivated yeast with no leavening power, has a high protein profile, contains nucleic acids and is rich in fiber, B-vitamins and minerals such as zinc, chromium, iron, magnesium, folic acid, and biotin. Commercial products are widely available and may be fortified with additional B vitamins and minerals. Their serving size ranges from 8 to 20 grams of powder yielding around 9 grams of protein. There are no documented adverse events recorded on FDA MedWatch database.

In 2003, a paper entitled "A New Food Guide for North American Vegetarians" was published as a companion paper to the "Position of the American Dietetic Association and Dietitians of Canada: Vegetarian diets" publication in the same journal issue. [12]. This guide suggests that adults should "include at least three good food sources of vitamin B12 in your diet every day. These include 1 Tbsp of Red Star Vegetarian Support Formula nutritional yeast...." The guide also recommends that children obtain at least two servings of B_{12} rich foods, and pregnant or lactating women should obtain at least four servings. This level of yeast supplementation (1 Tbsp Red Star Yeast) is below the recommended safe level of nucleic acids intake. Research has shown that the consumption of up to 2 grams per day of yeast nucleic acids kept the plasma uric level within acceptable normal ranges. [13]. This would amount to 17 to 33 g yeast solid per day as the nucleic content of yeast ranges from 6 to 12%. [8]

Additionally, other credible organizations and associations recommend that vegetarians supplement their diets with yeast products as a source of nutrients that are sometimes lacking in diets void of animal products. Oral consumption of yeast products has been shown to increase markers of vitamin B_{12} in humans [14]. Some recommendations for yeast product intake include:
1) The United States Department of Agriculture (USDA) recommends nutritional yeast as a source of vitamin B_{12} for vegetarians.[15]
2) The American Heart Association recommends brewers (nutritional) yeast as a source of nutrients for vegetarians (American Heart Association);
3) The vegetarian society for the United Kingdom and the Vegetarian Resource Group

Recommends Vitamin B_{12} fortified yeast products for pregnant women who are vegetarian.[16]
4) In Canada, the Canadian Food Inspection Agency (CFIA) indicated that the intake of nutritional yeast of 14 grams/day was a reasonable daily intake in its 2003 Guide to Food Labeling and Advertising, Section 6.3.1 [17]

USDA NHANES[3] surveys were queried and 2 food codes covering yeast and yeast extract spread were identified (food codes 75236000, 75236500). Using these codes, the consumption of yeast products for individuals 2 and older averaged 12.75 grams per person per day with a 90th percentile of 18 g/person per day, even though the percentage of users was relatively low among all age groups.

Food disappearance data for an ingredient can be used as a surrogate estimation of its consumption per capita. It represents the disappearance of a food in the marketing system. The data is very scarce for yeast. The only data found was a 1995 survey of industrial food additive use in the United States[18], which reported the use of baker's yeast (21 CFR §172.896) in foods to be 58,900 pounds/year. On a *per capita* basis, this amount would correspond to 3.07 mg/person/day, or 0.04 mg/kg body weight/day for a 70 kg individual. Therefore, it can be assumed that all of the components present in yeast are present in the diet as a result of the use of baker's yeast as a food additive. However, this data has only limited value in estimating dietary consumption of yeast.

IV. Part 4 – §170.240 Self-Limiting Levels of Use

The use of DNF-10 as a food ingredient is limited by the level that can be technically be added to a given food without jeopardizing its quality and consumer acceptability. The self-limiting level of use is independent of safety (toxicity, allergenic etc.) concerns.

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

DNF-10 has not been marketed prior to 1958 and the statutory basis for the conclusion that the use of DNF-10 is GRAS is through scientific procedure and equivalency, not through experience based on common use in food. Its approximate cumulative eaters-only intake of DNF-10 has been estimated at 575 mg/p/d at the 90th percentile. However, it is important to note that the main constituents of DNF-10 (amino acids and

[3] NHANES: National Health and Nutrition Examination Survey. https://wwwn.cdc.gov/nchs/nhanes/Default.aspx

small size peptides) occur following ingestion and digestion of bread, beer, nutritional yeast products and yeast extract spread and thus are not foreign compounds to the body. Therefore, the safety of DNF-10 can be supported by the facts that its main constituents have been safely consumed in the diet (discussed further in Part 6)

VI. Part 6 - §170.250 Narrative and Safety Rationale

The conclusion that DNF-10, as described herein, is GRAS under the conditions of its intended use in specified conventional food and beverage products is based on scientific procedures using generally available data and information pertaining to Baker's and Brewers' saccharomyces cerevisiae and its derived extracts rich in peptides. This includes data and information specifically for DNF-10 from preclinical and clinical studies evaluating their safety (described in Part 6.A & B below). A discussion of the findings of an independent expert requested by Fytexia to evaluate the GRAS status of DNF-10 is included in Part 6.C and a conclusion on GRAS status is presented in Part 6.D.
DNF-10 is a yeast hydrolysate peptide complex resulting from the hydrolysis of yeast cells. The hydrolysate is filtered through a 10 kDalton cuff-off membrane to collect peptide fractions smaller than 10 kDa.
Upon ingestion, the fractions that are bigger than tri-peptides are digested by pepsin in the stomach and by pancreatic proteases in the small intestine. Furthermore, the peptidases present at the brush border of the small intestine further hydrolyze the luminal peptides, converting them to free amino acids and very small peptides which are now ready for absorption. The absorbed amino acids and peptides would be similar to those obtained when consuming commercial yeast and yeast derived products.

A. Safety Studies

1. Acute and Subacute Study in Rats [19]

An acute and subacute study was conducted in male and female Sprague Dawley rats where the toxicity of DNF-10 (peptides <10 kDa) was examined. The acute dose was a single dose of 5000 mg/kg of body weight and the subacute dose was 1000 mg/kg body weight for 14 days. Biochemical, hematological and histopathological parameters were collected in the subacute toxicity study.

Animals were housed individually and placed on a 12-hour light/12-hour dark cycle. They were given a commercial chow diet and water ad libitum.

For the acute toxicity study, the animals were randomized into 2 groups. The first group received a single dose of 5000 mg/kg of yeast hydrolysate (DNF-10) orally

while the second group received an equal volume of water (Control group). The animals were observed at 1, 2, 4, and 6 hours after giving the test substance. The visual observations were recorded and included changes in the skin, fur, eyes and mucous membranes; the respiratory, circulatory, autonomic, and central nervous systems; as well as somatomotor activity and behavioral patterns. The number of survivors was noted after 24 hours and these rats were then kept another 14 days and observed once a day. On day 15, all the animals were fasted for 16 to 18 hours and then anesthetized with ethyl ether and sacrificed.

In the subacute toxicity study, the animals were divided into 4 groups and dosed every day for 14 days. Group 1 and 3 (treatment) received 1000 mg yeast hydrolysate/kg of body weight. Group 2 and 4 (controls) were given an equivalent volume of water. Group 1 and 2 were sacrificed at the end of 14 days.(subacute study) Group 3 and 4 were kept for another 14 days with no additional dosing and sacrificed at 28 days in order to assess the reversibility and the delayed occurrence of toxic effects.(Satellite study) During the first 14 days, animals were weighted and observed daily to detect signs of toxicity. Changes in the skin, fur, eyes and mucous membranes; the respiratory, circulatory, autonomic, and central nervous systems; as well as somatomotor activity and behavioral patterns were recorded systematically. Blood samples were collected before sacrificing the animals to measure hematological and biochemical parameters.
In both subacute and satellite study, liver, kidney, spleen, lungs and sex organs were removed, blotted free of blood and weighted immediately. The organ weights were expressed as a function of body weight. Liver and kidneys were processed and embedded in paraffin and sectioned. The liver was stained with hematoxylin and eosin (H&E) and the kidneys with periodic acid Schiff (PAS) for light microscopic evaluation.

Statistical analysis was performed using SPSS version 12. Difference between the control and treated groups were evaluated by Student t-test and p value less than 0.5 were considered significant.

Results:

Acute study: No sign of toxicity or death of the rats during the 14-day observation after the single dose of yeast hydrolysate (5000 mg/kg) were observed. There was no difference in weight, food and water consumption between the beginning and the end of the acute study. (Figure 1)

Subacute study: No death or sign of toxicity with regard to piloerection, alterations in

locomotor activity or diarrhea were observed during the 14 consecutive days of treatment with the yeast hydrolysate at 1000 mg/kg. The animals treated with the yeast hydrolysate gained significantly less weight compared to that of control ($p<0.05$). At the end of the satellite study, there was no difference in weight gain between the 2 groups. There were no significant differences in daily food and water intake between the treatment and the control groups.

Hematological and biochemical parameters: There was no statistical differences in the hematological parameters between the treated or the control groups in the subacute or satellite study. (Table 1 below).
Table 2 shows the biochemical profiles. There was no difference between the treatment and the control groups except for BUN in the first 14-day study. It should be noted that the BUN values of the control animals in that subacute study were slightly lower than the normal range for both genders when compared to published data. Matsuzawa et al reported normal range of BUN values in Sprague-Dawley rats between 12.9 to 19.5 mg/dl. [20] By the end of the satellite study, the animals did not show a difference between the 2 groups.

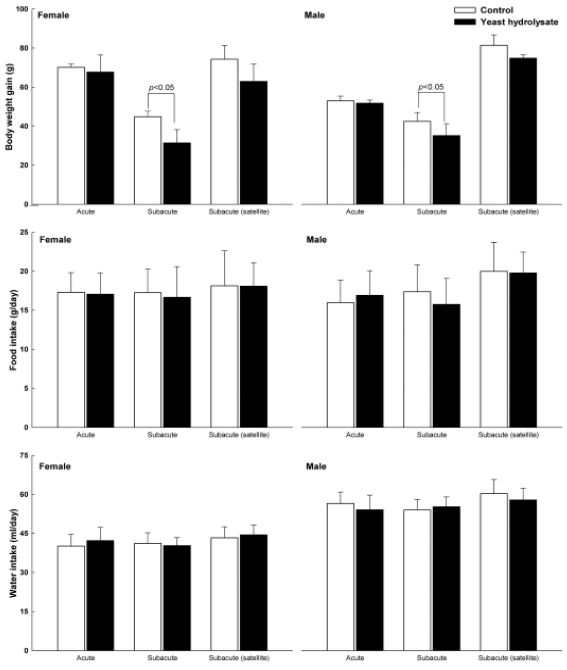


Fig. 1. Body Weight Gain and Daily Intake of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Acute and Subacute Toxicity

Bars are means ± S.D. for 5 rats/group. The group for acute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 5000 mg/kg once followed by no treatment for 14 days. The group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days. The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. The differences between the control and treated groups were evaluated by Student's *t*-test.

Table 1. Hematological Parameters of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Subacute Toxicity

Hematological parameters	Female			
	Group for subacute toxicity[a)]		Satellite group for subacute toxicity[b)]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
RBC ($\times 10^6$/μl)	7.33 ± 0.52	7.21 ± 0.19	7.47 ± 0.30	7.12 ± 0.17
WBC ($\times 10^3$/μl)	8.57 ± 1.55	8.08 ± 1.24	8.00 ± 1.34	7.20 ± 0.22
Hct (%)	43.77 ± 3.75	43.98 ± 2.27	44.73 ± 2.74	45.10 ± 1.37
Hgb (g/dl)	13.51 ± 1.52	13.49 ± 0.94	14.50 ± 0.87	14.47 ± 0.35
MCV (fl)	56.77 ± 1.45	58.42 ± 1.26	59.83 ± 1.37	58.90 ± 1.20
MCH (pg)	18.34 ± 0.58	18.21 ± 0.88	19.33 ± 0.50	19.47 ± 0.65
MCHC (g/dl)	31.12 ± 1.23	31.55 ± 1.59	32.37 ± 0.21	32.50 ± 1.00
Platelets ($\times 10^3$/μl)	845.26 ± 54.42	898.87 ± 49.99	728.00 ± 41.52	823.67 ± 38.94
Hematological parameters	Male			
	Group for subacute toxicity[a)]		Satellite group for subacute toxicity[b)]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
RBC ($\times 10^6$/μl)	7.06 ± 0.78	7.24 ± 0.86	7.96 ± 0.38	7.98 ± 0.41
WBC ($\times 10^3$/μl)	11.21 ± 1.02	11.00 ± 0.89	10.47 ± 0.31	10.82 ± 0.95
Hct (%)	45.15 ± 3.35	46.66 ± 4.32	47.25 ± 2.35	47.00 ± 4.48
Hgb (g/dl)	13.99 ± 0.98	14.28 ± 1.01	14.97 ± 0.21	15.00 ± 0.78
MCV (fl)	57.78 ± 1.24	57.62 ± 1.08	58.93 ± 1.11	58.83 ± 2.68
MCH (pg)	17.49 ± 0.52	18.00 ± 0.15	18.50 ± 0.52	18.73 ± 0.06
MCHC (g/dl)	31.07 ± 1.01	31.22 ± 1.75	31.27 ± 0.21	31.90 ± 1.44
Platelets ($\times 10^3$/μl)	721.42 ± 58.87	705.66 ± 61.41	692.67 ± 60.34	695.00 ± 60.22

Values are means ± S.D. for 5 rats/group. *a)* The group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days. *b)* The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. The differences between the control and treated groups were evaluated by Student's *t*-test.

Table 2. Blood Biochemical Parameter of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Subacute Toxicity

Blood biochemical parameter	Female			
	Group for subacute toxicity[a)]		Satellite group for subacute toxicity[b)]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Glucose (mg/dl)	89.67 ± 8.02	94.67 ± 13.65	114.00 ± 9.70	116.33 ± 10.23
BUN (mg/dl)	8.57 ± 0.50	10.37 ± 0.32**	13.40 ± 1.40	13.45 ± 1.22
Creatinine (mg/dl)	0.30 ± 0.01	0.30 ± 0.01	0.27 ± 0.05	0.28 ± 0.05
Total protein (g/dl)	5.27 ± 0.06	6.00 ± 0.10	5.81 ± 0.13	5.84 ± 0.14
Albumin (g/dl)	3.53 ± 0.12	3.80 ± 0.01	3.79 ± 0.15	3.80 ± 0.13
Total bilirubin (mg/dl)	0.47 ± 0.06	0.53 ± 0.15	0.58 ± 0.08	0.58 ± 0.09
AST (U/l)	85.33 ± 5.08	89.33 ± 6.21	90.00 ± 6.97	92.92 ± 7.80
ALT (U/l)	27.67 ± 4.16	29.33 ± 2.53	29.33 ± 4.18	30.92 ± 4.23
Blood biochemical parameter	Male			
	Group for subacute toxicity[a)]		Satellite group for subacute toxicity[b)]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Glucose (mg/dl)	102.00 ± 8.89	102.33 ± 4.93	118.67 ± 4.04	125.00 ± 6.24
BUN (mg/dl)	7.03 ± 0.32	11.80 ± 0.26**	13.00 ± 0.56	13.43 ± 0.31
Creatinine (mg/dl)	0.30 ± 0.01	0.37 ± 0.06	0.30 ± 0.01	0.30 ± 0.01
Total protein (g/dl)	5.40 ± 0.20	5.63 ± 0.15	5.60 ± 0.20	5.83 ± 0.06
Albumin (g/dl)	3.50 ± 0.01	3.60 ± 0.17	3.53 ± 0.06	3.62 ± 0.10
Total bilirubin (mg/dl)	0.47 ± 0.06	0.47 ± 0.05	0.47 ± 0.06	0.53 ± 0.06
AST (U/l)	95.00 ± 5.01	98.33 ± 13.02	96.33 ± 8.13	99.33 ± 6.79
ALT (U/l)	37.33 ± 2.16	35.00 ± 2.61	41.67 ± 2.58	39.01 ± 2.00

Values are means ± S.D. for 5 rats/group. *a)* The group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days. *b)* The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. **Significantly different from the control, $p < 0.01$. The differences between the control and treated groups were evaluated by Student's *t*-test.

Morphological parameters: Table 3 shows the relative organ weights of the treated and control animals. There was no difference measured in any of the harvested

organs.

Table 3. Relative Organ Weights of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Subacute Toxicity

Relative organ weight (g/100 g of body weight)	Group for acute toxicity[a]		Group for subacute toxicity[b]		Satellite group for subacute toxicity[c]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Female						
Liver	3.93 ± 0.35	4.02 ± 0.24	3.77 ± 0.16	3.79 ±0.14	3.72 ± 0.20	3.73 ± 0.10
Kidney	0.98 ± 0.05	0.99 ± 0.09	0.89 ± 0.05	0.87 ± 0.04	0.84 ± 0.11	0.86 ± 0.06
Spleen	0.32 ± 0.01	0.30 ± 0.01	0.26 ± 0.03	0.26 ± 0.03	0.23 ± 0.02	0.25 ±0.05
Lung	0.51 ± 0.02	0.49 ± 0.03	0.48 ± 0.03	0.47 ± 0.02	0.42 ± 0.04	0.43 ± 0.04
Heart	0.38 ± 0.01	0.36 ± 0.03	0.34 ± 0.01	0.34 ± 0.01	0.30 ± 0.01	0.32 ± 0.02
Ovary	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Male						
Liver	4.77 ± 0.38	4.88 ± 0.44	3.80 ± 0.13	3.72 ± 0.26	3.37 ± 0.10	3.31 ± 0.29
Kidney	1.06 ± 0.13	1.05 ± 0.08	0.87 ± 0.07	0.89 ± 0.01	0.82 ± 0.01	0.86 ± 0.06
Spleen	0.40 ± 0.08	0.36 ± 0.09	0.24 ± 0.01	0.20 ± 0.05	0.20 ± 0.01	0.19 ± 0.04
Lung	0.44 ± 0.01	0.45 ± 0.03	0.41 ± 0.02	0.40 ± 0.01	0.36 ± 0.04	0.38 ± 0.01
Heart	0.38 ± 0.01	0.36 ± 0.03	0.34 ± 0.01	0.34 ± 0.01	0.30 ± 0.01	0.32 ± 0.02
Testis	0.76 ± 0.09	0.74 ± 0.08	0.71 ± 0.07	0.72 ± 0.04	0.68 ± 0.05	0.69 ± 0.04

Values are means ± S.D. for 5 rats/group. *a*) The group for acute toxicity was given water vehicle or yeast hydrolysate at 5000 mg/kg once followed by no treatment for 14 days. *b*) The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1000 mg/kg daily for 14 days. *c*) The satellite group for subacute toxicity was given the water vehicle or yeast hydrolysate at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. The differences between the control and treated groups were evaluated by Student's *t*-test.

Figures 2 and 3 show the microscopic findings in the liver and in the kidneys for the rats for the acute and subacute studies. There were no significant changes in color and texture when compared to the control group in both the males and the females. No histological alterations were noticed in the liver or in the kidneys in either groups.

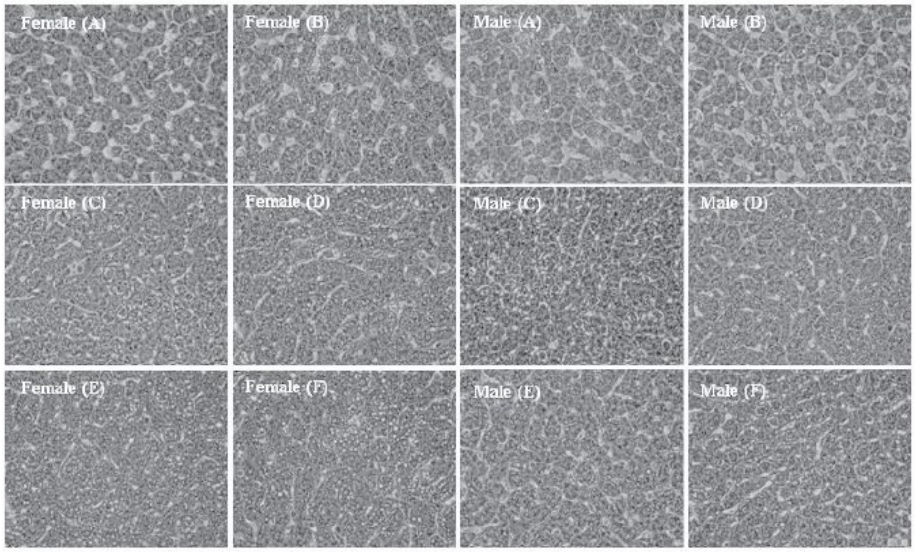


Fig. 2. Representative Microscopic Findings in the Liver of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Acute and Subacute Toxicity (Hematoxylin-eosin Stain, × 400)

(A) Water vehicle group for acute toxicity, (B) Yeast hydrolysate group for acute toxicity, (C) Water vehicle group for subacute toxicity, (D) Yeast hydrolysate group for subacute toxicity, (E) Water vehicle (satellite) group for subacute toxicity and (F) Yeast hydrolysate (satellite) group for subacute toxicity.

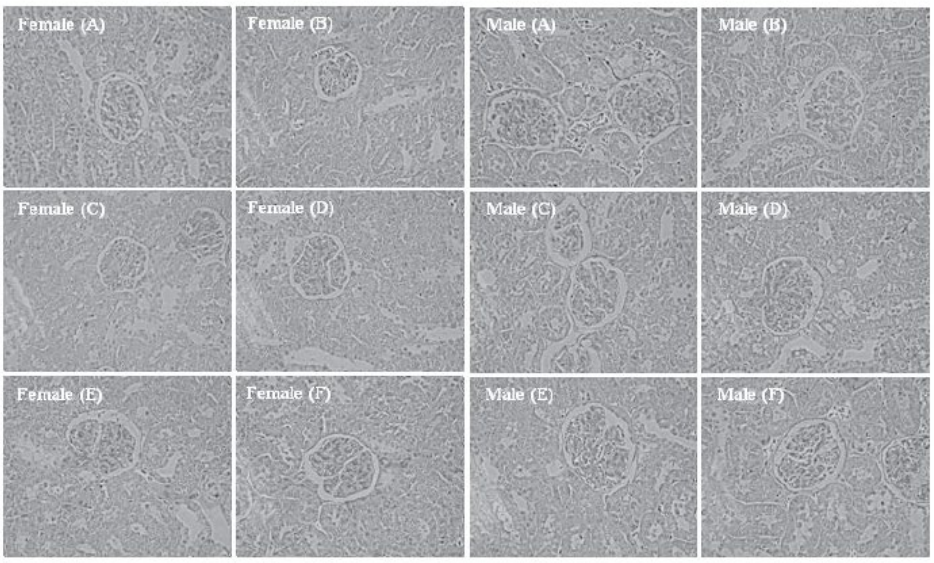


Fig. 3. Representative Microscopic Findings in Kidneys of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Acute and Subacute Toxicity (Periodic Acid Schiff Stain, × 400)

(A) Water vehicle group for acute toxicity, (B) Yeast hydrolysate group for acute toxicity, (C) Water vehicle group for subacute toxicity, (D) Yeast hydrolysate group for subacute toxicity, (E) Water vehicle (satellite) group for subacute toxicity and (F) Yeast hydrolysate (satellite) group for subacute toxicity.

This study shows that there was no observable toxicity in either male or female rats at a consumption level of 1000 mg/kg body weight. Therefore, the acute oral LD50 of the yeast hydrolysate is greater than 1000 mg/kg body weight. This suggests that the toxicity of DNF-10 yeast hydrolysate is very low. This would be expected due to the fact that this product is a derivative of nutritional yeast (commonly available in the diet) and contains low molecular weight peptides with lesser allergenic properties (see paragraph C below).

B. Clinical Studies:

Several clinical studies have been conducted during which DNF-10 was given to human subjects.[21, 22] Only one tracked safety of the yeast hydrolysate peptide complex by including liver function and electrolytes blood parameters, and hematological parameters and is described below.

1. Jung et al, 2014 [23]

In this double-blind placebo-controlled study, a yeast hydrolysate (YH) with peptide fractions smaller than 10kDa was given for 10 weeks. The subjects had a BMI greater than 25 and were specifically excluded if they had a known sensitivity or allergy to yeast. They were split into 2 groups of 27, each group having 12 men and 15 women. The intervention consisted of 500 mg of yeast hydrolysate (YH) to be taken 30 min

before breakfast and 30 min before dinner with water (total daily YH dose = 1 g). The control group took 500 mg of dextrin before the 2 meals as indicated above. (total daily placebo dose = 1 g).

Height, weight, blood pressure and heart rate were measured each week. Body fat mass and lean body mass were measured using an InBody 3.0 impedance machine. Blood samples were taken at baseline, week 5 and 10 to assess liver function, serum electrolytes and hematological biomarkers.

At the end of the study, liver function, serum electrolytes, and hematological parameters taken at baseline, weeks 5 and 10 remained within healthy ranges throughout the intervention. Resting blood pressure and heart rate remained unaffected by either treatment. No participant was removed from the study protocol for treatment-related adverse effects. This suggests that the yeast hydrolysate is safe to consume orally at a dose of 1 gram per day.

2. Use of Nutritional Yeast in clinical studies

Six studies were conducted in which nutritional Brewer's yeast was given to overweight subjects and blood sugar and lipid profiles were tracked. Upon review of the published reports, favorable results were obtained for blood sugar and lipid markers with consumption of the yeast that ranged from 800 mg to 10 grams daily. No report of adverse events was made indicating a good tolerance to the compound. [24-29]. Although the compound is different from DNF-10, it will yield to similar peptides and amino acids upon digestion by the human GI tract. Thus, it supports the concept of safety.

C. Allergenicity

S. Cerevisiae yeast is not part of the 8 major food allergens – milk, egg, peanut, tree nuts, wheat, soy, fish and crustacean shellfish that are responsible for most of the serious food allergy reactions in the US. However, a few individuals may be allergic to yeast but this is a rare problem. However, a thorough review of the scientific literature was done to explore if intact yeast cells or peptide subfractions from it consumed orally were associated with adverse events.

There are very few published reports on allergic reactions to Baker's yeast. One described the reaction of a 6-year-old boy with previously diagnosed mite-allergy and atopic dermatitis who developed urticaria and asthma upon eating fresh baked breads or pizza containing yeast. With time, he had less reaction especially if he consumed the goods at least one hour after baking. The authors could not give a reason why this happened. [30]

The 2nd one described one patient having clustered sensitivity to various fungi and yeast. However, the full paper is no longer available in PubMed and may have been retracted. [31]

Three papers by a Swedish group described an immediate hypersensitivity to bakery, brewery and wine products in 20 yeast-sensitive atopic dermatitis patients. Their later paper in 1998 reported that the yeast protein fractions with molecular weight greater than 14 kDa produced a strong IgE response. [32-34]

In 2017, Bansal et al reported a rare case of allergy to beer, wine, and cider resulting from IgE reactivity to yeasts and molds. They noted that the patient also had additional sensitivity to yeast extracts and blue cheese. [35] The authors also stated that medically confirmed IgE-mediated yeast allergy is exceptionally rare.

Thus, it appears that the incidence of allergic reaction to saccharomyces cerevisiae is low. In an allergic reaction, the protein allergen provokes an IgE antibody response. IgE binds to the specific sites (epitopes) of the surface of the protein antigen. Amino acid sequence and molecular weight of the epitopes, spatial structure and hydrophobicity of the allergenic protein, ability to withstand proteolysis during digestion for the allergenic protein play key roles in eliciting an immune response.[36] Savolainen and al reported prominent IgE binding to allergen fractions greater than 14 kDa for S. cerevisiae or other yeasts.[34].

Furthermore, we examined the database of adverse events for foods, supplements that are reported to the Center For Food Safety and Applied Nutrition (CFSAN)[37] and compiled into the Adverse Event Reporting System (AER). The reports in AER are evaluated by clinical reviewers to monitor the safety of consumer products. The searchable AER database spans from 2014 until March 2020. There were 131,258 reports of adverse events and only 5 that were related to Brewer's yeast or nutritional yeast.

The table below is the data directly extracted from the AER database.

Date of Event	Product type	Product	Description	Patient age	Gender	Medra Preferred terms	Outcomes
3/17/2007	Suspect	Red Star Nutritional Yeast	Vit/Min/Prot/ Unconv Diet (Human/Animal)			Hypersensitivity	Other outcome
10/28/2014	Suspect	PURITAN'S PRIDE Brewer's yeast powder	Vit/Min/Prot/ Unconv Diet (Human/Animal)			Hepatobiliary scan abnormal, biliary tract	Hospitalization

		Net WT 1 pound				disorder, gallbladder disorder, dyspepsia, gall bladder non-functioning	
2/4/2018	Concomitant	Brewer's Yeast	Vit/Min/Prot/ Unconv Diet (Human/Animal)	56	F	Alopecia, trichorrhexis	Other Outcome
10/1/2018	Suspect	NOW Nutritional Yeast Powder	Dietary Conventional Foods/Meal Replacements	50	F	Nasal congestion, throat tightness, hypersensitivity, throat irritation	Life Threatening
11/11/2018	Suspect	HERBAL SECRETS Brewers' yeast powder	Vit/Min/Prot/ Unconv Diet (Human/Animal)	34	F	Malaise, vomiting, abdominal pain, upper	Other Seriousness

A thorough review of the American College of Allergy, Asthma and Immunology (https://acaai.org/allergies/types/food-allergy) does not mention yeast (saccharomyces cerevisiae) as a significant allergen anywhere on the site.

Another organization that educates about food allergies (www.foodallergy.org) highlights other food allergens beyond the 8 common ones and makes no mention of yeast. (https://www.foodallergy.org/living-food-allergies/food-allergy-essentials/common-allergens/other-food-allergens).

In summary, the addition of DNF-10 to proposed foods indicated in Part 3 is expected to be safe based on published data. Intact yeast is rarely associated with allergy. DNF-10 is derived from saccharomyces cerevisiae that has been hydrolyzed and ultra-filtered to contain peptides with molecular weight smaller than 10 kDa with different spatial structure, AA sequence and hydrophobicity of native yeast cells.

Appropriate food product labeling has become a standard and appropriate regulatory practice to alert consumers of potential presence of food allergens. This type of labeling will assist consumers with their food decision-making. For example, the European Food Safety Authority recommends alerting individuals with yeast sensitivity via product labeling (European Food Safety Authority, 2008). This approach of stating that the compound is derived from yeast may be used in the various markets where DNF-10 is sold.

D. Conclusions

Based on the above data and information presented herein, Fytexia has concluded that the intended uses of a yeast hydrolysate peptide complex trademarked as DNF-10 at a level of 250 mg per serving in specified conventional foods as described in Part I.D is GRAS based on scientific procedures. The GRAS status is further supported by the evaluation of the scientific expert Dr Nathalie Chevreau, PhD, RD who is qualified by experience and safety training to evaluate the safety of food ingredients and concluded that the DNF-10 is GRAS for its intended uses in conventional foods.

DNF-10 therefore may be marketed and sold for its intended purpose in the US without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations

VII. Part 7 - §170.255 List of Supporting Data and Information

1. FDA, *Title 21-Chapter I-Subchapter B-Part 172--Food Additives Permitted for Direct Addition To Food for human Consumption. Subpart I - Multipurpose Additives - §172.896*. Current: Electronic Code of Federal Regulations. https://www.ecfr.gov/cgi-bin/text-idx?SID=08e6acb4b8cb5bce628a51d4ad8a759b&mc=true&node=pt21.3.172&rgn=div5#se21.3.172_1896.
2. FDA, *Title 21-Chapter I-Subchapter B-Part 172--Food Additives Permitted for Direct Addition To Food for human Consumption. Subpart D - Special Dietary and Nutritional Additives - §172.325*. Current: Electronic Code of Federal Regulations.
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VIII. Appendix I: Food codes used

Thirteen food codes were used for Popcorn category

54319020	Popcorn cake
54403000	Popcorn, popped in oil, unbuttered
54403010	Popcorn, air-popped (no butter or no oil added)
54403020	Popcorn, popped in oil, buttered

54403030	Popcorn, w/ cheese
54403040	Popcorn, air-popped, buttered
54403050	Popcorn, flavored
54403060	Popcorn, popped in oil, lowfat, reduced sodium
54403070	Popcorn, popped in oil, lowfat
54403090	Popcorn, popped in oil, unsalted
54403110	Popcorn, sugar syrup or caramel-coated
54403120	Popcorn, sugar syrup or caramel-coated, with nuts
54403150	Popcorn, sugar syrup or caramel-coated, fat free

35 Food Codes for the Chips Food Category

54318000	Chips, brown rice
54401010	Salty snacks, corn or cornmeal base, nuts or nuggets, toasted
54401020	Salty snacks, corn or cornmeal base, corn chips, corn-cheese chips
54401050	Salty snacks, corn or cornmeal base, corn puffs and twists; corn-cheese puffs and twists
54401080	Salty snacks, corn or cornmeal base, tortilla chips
54401090	Salty snacks, corn or cornmeal base, corn chips, corn-cheese chips, unsalted
54401100	Salty snacks, corn or cornmeal base, tortilla chips, light (baked with less oil)
54401120	Salty snacks, corn or cornmeal base, tortilla chips, fat free, made with Olean
54401150	Salty snacks, corn or cornmeal base, tortilla chips, lowfat, baked without fat
54401170	Salty snacks, corn or cornmeal base, tortilla chips, lowfat, baked without fat, unsalted
54401200	Salty snacks, corn or cornmeal base, with oat bran, tortilla chips
54401210	Salty snacks, corn based puffs and twists, cheese puffs and twists, lowfat
54402080	Salty snacks, corn or cornmeal base, tortilla chips, unsalted
54402200	Salty snack mixture, mostly corn or cornmeal based, with pretzels, without nuts
54402300	Salty snacks, wheat-based, high fiber
54402500	Salty snacks, wheat- and corn-based chips
54402600	Salty snacks, multigrain, whole grain, chips (made with whole corn, whole wheat, rice flour, and whole oat flour)
54402610	Salty snacks, multigrain and potato chips (made with rice flour, dried potatoes, corn flour, and wheat starch)
54402700	Pita chips
71201010	White potato, chips
71201015	White potato chips, regular cut
71201020	White potato chips, ruffled, rippled, or crinkle cut
71201050	White potato, chips, reduced fat
71201080	White potato, chips, fat free
71201090	White potato, chips, fat free, made with Olean
71201100	White potato, chips, restructured
71201200	White potato, chips, restructured, reduced fat and reduced sodium
71201210	White potato, chips, restructured, fat free, made with Olean
71201250	White potato, chips, restructured, baked

71201300	Potato based snacks, reduced fat, low sodium, all flavors
71202000	White potato, chips, unsalted
71202100	White potato, chips, unsalted, reduced fat
71202500	White potato chips, lightly salted
71220000	Vegetable chips
73410210	Sweet potato, chips

55 Food Codes for the Crackers Food Category

54001000	Crackers, NS as to sweet or nonsweet	54304500	Cracker, high fiber, no added fat
54101010	Cracker, animal	54307000	Crackers, matzo
54102010	Crackers, graham	54308000	Crackers, milk
54102020	Crackers, graham, chocolate covered	54309000	Crackers, oat
54102030	Crackers, graham, sugar-honey coated, cinnamon cris	54313000	Crackers, oyster
54102050	Crackers, oatmeal	54318500	Rice cake, cracker-type
54102060	Crackers, Cuban	54319000	Crackers, rice
54102070	Crackers, Cuca	54325000	Crackers, saltine
54102080	Crackers, graham, with raisins	54325010	Crackers, saltine, fat free
54102090	Cracker, graham, higher fat	54325050	Crackers, saltine, whole wheat
54102100	Crackers, graham, lowfat	54326000	Crackers, multigrain, made with whole wheat, wheat, oat, and other flours
54102110	Crackers, graham, fat free	54327950	Crackers, cylindrical, peanut-butter filled
54102200	Crackers, graham, sandwich-type, with filling	54328000	Crackers, sandwich-type, NFS
54201010	Crackers, matzo, low sodium	54328100	Cracker, sandwich-type, peanut butter filled
54202010	Crackers, saltine, low sodium	54328110	Cracker, sandwich-type, peanut butter filled, reduced fat
54202050	Crackers, saltine, fat free, low sodium	54328120	Cracker, sandwich-type, peanut butter filled, whole grain
54203010	Crackers, toast thins (rye, wheat, white flour), low sodium	54328200	Cracker, sandwich-type, cheese-filled
54204010	Cracker, 100% whole wheat, low sodium	54334000	Crackers, toast thins (rye, pumpernickel, white flour)
54205010	Cracker, snack, low sodium	54336000	Crackers, water biscuits
54205030	Cracker, cheese, low sodium	54337000	Cracker, 100% whole wheat
54205100	Cracker, snack, reduced fat, reduced sodium	54337050	Cracker, 100% whole wheat, reduced fat
54210010	Cracker, multigrain, low sodium	54337100	Crackers, whole wheat and bran
54301000	Cracker, snack	54338000	Crackers, wheat
54301100	Cracker, snack, reduced fat	54338100	Crackers, wheat, reduced fat
54301200	Cracker, snack, fat free	54339000	Crackers, corn
54304000	Cracker, cheese	54340100	Cracker, gluten free
54304100	Cracker, cheese, reduced fat	54350000	Crackers, baby food
54304150	Cracker, cheese, whole grain		

95 Food Codes for the Bars Food Category

41435000	Fiber One Fulfill Bar	53710400	Fiber One Chewy Bar
41435010	High protein bar, soy base	53710500	Kellogg's Nutri-Grain Cereal Bar
41435110	High protein bar, candy-like, soy and milk base	53710502	Kellogg's Nutri-Grain Yogurt Bar
41435120	Zone Perfect Classic Crunch nutrition bar	53710504	Kellogg's Nutri-Grain Fruit and Nut Bar
41435300	Balance Original Bar	53710600	Milk 'n Cereal bar
41435500	Clif Bar	53710700	Kellogg's Special K bar
41435700	South Beach Living High Protein Cereal Bar	53710800	Kashi GOLEAN Chewy Bars
41435710	South Beach Living Meal Replacement Bar	53710802	Kashi TLC Chewy Granola Bar
53540000	Breakfast bar, NFS	53710804	Kashi GOLEAN Crunchy Bars
53540100	Breakfast bar, cake-like	53710806	Kashi TLC Crunchy Granola Bar
53540200	Breakfast bar, cereal crust with fruit filling, lowfat	53710900	Nature Valley Chewy Trail Mix Granola Bar
53540250	Breakfast bar, cereal crust with fruit filling, fat free	53710902	Nature Valley Chewy Granola Bar with Yogurt Coating
53540300	Fiber One Chewy Bar	53710904	Nature Valley Sweet and Salty Granola Bar
53540400	Kellogg's Nutri-Grain Cereal Bar	53710906	Nature Valley Crunchy Granola Bar
53540402	Kellogg's Nutri-Grain Yogurt Bar	53711000	Quaker Chewy Granola Bar
53540404	Kellogg's Nutri-Grain Fruit and Nut Bar	53711002	Quaker Chewy 90 Calorie Granola Bar
53540500	Breakfast bar, date, with yogurt coating	53711004	Quaker Chewy 25% Less Sugar Granola Bar
53540600	Milk 'n Cereal bar	53711006	Quaker Chewy Dipps Granola Bar
53540700	Kellogg's Special K bar	53711100	Quaker Granola Bites
53540800	Kashi GOLEAN Chewy Bars	53712000	Snack bar, oatmeal
53540802	Kashi TLC Chewy Granola Bar	53712100	Granola bar, NFS
53540804	Kashi GOLEAN Crunchy Bars	53712200	Granola bar, lowfat, NFS
53540806	Kashi TLC Crunchy Granola Bar	53712210	Granola bar, nonfat
53540900	Nature Valley Chewy Trail Mix Granola Bar	53713000	Granola bar, reduced sugar, NFS
53540902	Nature Valley Chewy Granola Bar with Yogurt Coating	53713100	Granola bar, peanuts , oats, sugar, wheat germ
53540904	Nature Valley Sweet and Salty Nut Granola Bar	53714200	Granola bar, chocolate-coated, NFS
53540906	Nature Valley Crunchy Granola Bar	53714210	Granola bar, with coconut, chocolate-coated
53541000	Quaker Chewy Granola Bar	53714220	Granola bar with nuts, chocolate-coated
53541002	Quaker Chewy 90 Calorie Granola Bar	53714230	Granola bar, oats, nuts, coated with non-chocolate coating
53541004	Quaker Chewy 25% Less Sugar Granola Bar	53714250	Granola bar, coated with non-chocolate coating
53541006	Quaker Chewy Dipps Granola Bar	53714300	Granola bar, high fiber, coated with non-chocolate yogurt coating
53541100	Breakfast bar, diet meal type	53714400	Granola bar, with rice cereal
53541200	Meal replacement bar	53714500	Breakfast bar, NFS
53541300	Slim Fast Original Meal Bar	53714510	Breakfast bar, date, with yogurt coating
53542000	Snack bar, oatmeal	53714520	Breakfast bar, cereal crust with fruit filling, lowfat
53542100	Granola bar, NFS	53720100	Balance Original Bar
53542200	Granola bar, lowfat, NFS	53720200	Clif Bar
53542210	Granola bar, nonfat	53720210	Clif Kids Organic Zbar
53543000	Granola bar, reduced sugar, NFS	53720300	PowerBar
53543100	Granola bar, peanuts, oats, sugar, wheat germ	53720400	Slim Fast Original Meal Bar
53544200	Granola bar, chocolate-coated, NFS	53720500	Snickers Marathon Protein bar
53544210	Granola bar, with coconut, chocolate-coated	53720510	Snickers Marathon Energy bar
53544220	Granola bar with nuts, chocolate-coated	53720600	South Beach Living Meal Bar
53544230	Granola bar, oats, nuts, coated with non-chocolate coating	53720610	South Beach Living High Protein Bar
53544250	Granola bar, coated with non-chocolate coating	53720700	Tiger's Milk bar
53544300	Granola bar, high fiber, coated with non-chocolate yogurt coating	53720800	Zone Perfect Classic Crunch nutrition bar
53544400	Granola bar, with rice cereal	53729000	Nutrition bar or meal replacement bar, NFS

53544450	PowerBar (fortified high energy bar)			

The 6 food codes for protein powder shown below were not used because the nutritional facts for those products that I searched on the internet are based on serving size in volume (cup or 240 ml) as opposed to grams of protein powder.

As a result, those 6 categories really skew the data toward the higher end since it is reported as reconstituted volume and not the starting amount of powder. DNF-10 is not intended to be added to these kind of reconstituted products

11612000	Instant breakfast, powder, milk added
11613000	Instant breakfast, powder, sweetened with low calorie sweetener, milk added

11622010	Diet beverage, powder, reconstituted with skim milk
11631000	High calorie beverage, canned or powdered, reconstituted
11651010	Meal replacement formula, Cambridge diet, reconstituted, all flavors
41430200	Meal replacement or supplement, soy- and milk-base, powder, reconstituted with water

35 codes for the Protein Powder Category

11830800	Instant breakfast, powder, not reconstituted
11830810	Instant breakfast, powder, sweetened with low calorie sweetener, not reconstituted
11830850	High calorie milk beverage, powder, not reconstituted
11830900	Protein supplement, milk-based, powdered, not reconstituted
11830940	Meal replacement, high protein, milk based, fruit juice mixable formula, powdered, not reconstituted
11830970	Meal replacement, protein type, milk-based, powdered, not reconstituted
11830990	Nutrient supplement, milk-based, powdered, not reconstituted
11831500	Nutrient supplement, milk-based, high protein, powdered, not reconstituted

11832000	Meal replacement, protein type, milk- and soy-based, powdered, not reconstituted
11835000	Meal replacement or nutritional supplement, Cambridge diet formula, powdered, nonfat milk solids base, dry, not reconstituted
11835100	Meal replacement, Amway's Nutrilite brand Positrim Drink Mix, powdered nonfat dry milk-based, dry, not reconstituted
11835150	Dynatrim, meal replacement, powder
11835200	Lose-it (nanci), meal replacement, powder
11836000	Protein supplement, milk-based, Muscle Milk, powdered, not reconstituted
11836100	Protein supplement, milk-based, Muscle Milk Light, powdered, not reconstituted
41430000	Protein powder, NFS
41430010	Protein supplement, powdered
41430310	Protein diet powder with soy and casein
95201000	Carnation Instant Breakfast, nutritional drink mix, regular, powder
95201010	Carnation Instant Breakfast, nutritional drink mix, sugar free, powder
95201200	EAS Whey protein powder
95201300	EAS soy protein powder
95201500	Herbalife nutritional shake high protein powder
95201600	Isopure protein powder
95201700	Kellogg's Special K20 Protein Water Mix
95202000	Muscle Milk, regular, powder
95202010	Muscle Milk, light, powder
95210000	Slim Fast shake mix powder
95210020	Slim Fast shake mix high protein powder
95220000	Nutritional drink mix or meal replacement powder NFS
95220010	Nutritional drink mix or meal replacement high protein powder NFS
95230000	Protein powder whey based NFS

95230010	Protein powder soy based NFS
95230020	Protein powder light NFS
95230030	Protein powder NFS

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001033	Nov 2, 2021
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

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

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

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd):* 2020/09/03

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Matthieu Arguillere	CEO

Organization *(if applicable)*: Fytexia Corp

Mailing Address *(number and street)*: ZAE Via Europa, 3, rue d'Athenes

City	State or Province	Zip Code/Postal Code	Country
Vendres	Herault	34350	France

Telephone Number	Fax Number	E-Mail Address
+133467219098		marguillere@fytexia.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Nathalie Chevreau	Principal

Organization *(if applicable)*: Chevreau Consulting LLC

Mailing Address *(number and street)*: 2151 E Logan avenue

City	State or Province	Zip Code/Postal Code	Country
Salt Lake City	Utah	84108	United States of America

Telephone Number	Fax Number	E-Mail Address
8016526035		nchevreau@ncphd.net

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Yeast hydrolysate peptide complex

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

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

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

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

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

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

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D – INTENDED USE

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

Yeast hydrolysate peptide complex is intended to be added as a food ingredient to a variety of food products targeted to adults. It is not intended to be added to infant formula or foods targeted to children. The intended use level is 250 mg or less of the complex per serving. Intended food applications include but are not limited to snacks (crackers, ready to eat popcorn), protein or nutritional bars, dry mixes for beverage blends.

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

(Check one)

☐ Yes ☒ No

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

(Check one)

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

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

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

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

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

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

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

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

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

Other Information

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

☐ Yes ☒ No

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

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Matthieu Arguillere *(name of notifier)*

has concluded that the intended use*(s)* of Yeast hydrolysate peptide complex *(name of notified substance)*

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

2. Matthieu Arguillere *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

ZAE Via Europa, 3 rue d'Athenes, 34350, Vendres, France *(address of notifier or other location)*

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

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Nathalie chevreau Digitally signed by Nathalie chevreau Date: 2021.11.02 12:58:11 -06'00'	Nathalie Chevreau Ph, RD , Principal	11/02/2021

SECTION G – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Fytexia_Documentation_supporting _GRAS_status_of_DNF-10_Oct-12-2020	Submission

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, **PRAStaff@fda.hhs.gov**. (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Viebrock, Lauren

From: Nathalie Chevreau <nachevre@gmail.com>
Sent: Thursday, July 21, 2022 6:09 PM
To: Viebrock, Lauren
Cc: Morgane JAFFRELO; Julien Cases PhD
Subject: [EXTERNAL] Re: GRN 1033 Questions
Attachments: 2022_05_20 GRN1033 Questions and partial answers from NChevreau 07-21-2022.docx

Hello Lauren,

I have not been able to work as much on the responses to your questions due to health and personal reasons.

This is how far I have gone and I will continue in the upcoming weeks.

I would like you to know that the notification has not been abandoned.

I hope this is not a problem for the FDA team

Thank you in advance.

I look forward to hearing from you

Sincerely,

Nathalie

Nathalie Chevreau Ph.D., R.D.
Chevreau Consulting Corp
2151 E Logan avenue
Salt Lake City, UT 84108
Cell (801) 652 6035
Website: www.ncphd.net

On Fri, May 20, 2022 at 10:40 AM Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov> wrote:

> Dear Dr. Chevreau
>
> During our review of GRAS Notice No. 001033, we noted questions that need to be addressed. Please find the questions attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,

Lauren

Lauren VieBrock, Ph.D.
Regulatory Review Scientist/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

May 20, 2022 from Dr Viebrock

GRN 1033 Questions:

The answers are indicated in blue ink

Regulatory:

1. Please provide the corrected the citation for the regulation for aminopeptidase that is made from *Aspergillus oryzae*. The cited regulation (21 CFR 184.1027) is for carbohydrase and protease enzymes.

Chemistry:

1. Please provide the source of the maltodextrin used in the formulation of the yeast hydrolysate peptide complex. A: Corn

2. Please state whether all analytical methods used for the batch analyses are validated for their intended purpose. A: All the analytical methods used for the batches analyses are validated for their intended purpose.

3. Please specify what is meant by "ND" in Table A (pp. 7-8) and indicate whether it refers to values below the limit of detection (LOD) or limit of quantification (LOQ). Since batch analysis results demonstrate that both arsenic and cadmium are less than 0.01 ppm, please adjust the specification of arsenic and cadmium to reflect the reality. A: there was a typo for the LOQ for arsenic. LOQ is 0.01 pm. I have the email showing the LOQ from the DNF-10 manufacturer if you want it. ND stands for Non detectable. It means the result is below the LOQ. Will change the specs for arsenic and cadmium to < 0.1 ppm

4. Please provide the LOQ for the mercury method used for the analyses in Table A (p. 7). A: LOQ of Mercury is 0.012ppm

5. Please clarify the intent of footnotes "a" and "b" of Table D (p. 10). The amounts listed in these footnotes are different than the reference amounts customarily consumed (RACC) that are used in the table and therefore, it is not clear why the footnotes are included. In addition, footnote "b" discusses chips when the intended food category is crackers. Please explain the relevance of the information for chips to that of crackers. A: The intent of footnote "a" was to help the reader visualize what a 30 g serving of popcorn represented knowing that 2 cups of popcorn weight about 17 grams. I can remove it if it does not help. Footnote "b" should indeed be for chips and not crackers. I can also remove the footnote if it does not help.

6. For the protein dry powder category in Table D (p. 10), please clarify if the provided use level is for the powder or in the beverage as consumed. If it is for the powder, please indicate how the use level was determined for the beverage as consumed. In addition, if this ingredient can be used in the powder that is used to make the reconstituted beverage, it should be included in the exposure estimate. A: it is for the protein powder. The use level was calculating by dividing 250 mg of DNF-10 by 20 g protein powder RACC. This makes a use level of 1.25%.

7. In your description of the dietary exposure estimate, you state that you used a "probabilistic model" to determine the cumulative eaters-only intake of the yeast hydrolysate (p. 11). We note that the dietary exposure estimate you present would not be a "probabilistic" model. Although this approach is described in the guidance as a way to estimate "probable" human exposure, these terms are not interchangeable in this context. For the estimate of dietary exposure, we request that you clarify:

You provided "N of eaters out of 92,062 surveyed individuals (2 years and older)" in Table E (p. 11). However, we are not able to confirm your N values using the 1999-2016 NHANES data. Please clarify if the "N" in Table E represents a number of eaters (un- weighted) over 2 years of age in the U.S. who consumed these foods. A: I looked back at the dataset that I had created back in 2020. The number of unique individuals (single SEQN) 2 years and older that were surveyed was 92,062. Datasets included in the analysis were the demographic information, dietary data (DRXIFF, DR1IFF, and DR2IFF data, FFQRAW data), and consumer behavior data (CBQ data), as available for each survey cycle. 75,544 participants had at least one item recorded for the dietary recall. N eaters were determined using dietary recall data or other applicable questionnaire variables in the datasets mentioned above for each food category. Grams of food were determined using dietary recall data. All frequencies are unweighted. Thus, I should have used 75,544 and not 92,062. I will change the N to 75,544. The number of eaters in each category will not change but the % Eaters in column 3 of Table E will change.

New Table E:

Food Category	N of eaters out of 75,544 surveyed individuals (2+ years old)	% Eaters (N/75,544)	Mean consumption of food (g/p/d)	90th ile (g/p/d)
Popcorn	1153	1.53%	35	83.1
Chips	5565	7.4%	35.2	64
Crackers	3005	4%	26.1	52
Bars	551	0.73%	41.6	65
Protein powder	206	0.27%	60	131.4

a. Further, we note that you combined the food consumption data from NHANES 1999- 2002 (a single 24-hour dietary recall) and NHANES 2003-2016 (two 24-hour dietary recalls) in your estimate. Please explain how you combined two different surveys (1-day -and 2-day dietary recalls), and what statistical software package was used to analyze the consumption data from the multiple NHANES surveys. A: For participants who only had a single 24-hour dietary recall, a single observation was used to determine consumption of each food. For participants who had two days of dietary recall data, an average of each food consumption over the two-day period was computed. All analyses were completed using SAS software.

As the result of the change of actual eaters (75,544), Table F will be changed to:

Table F

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Foods	N of eaters (2+ years old)	% Eaters (N/75,544)	Mean consumption of food (g/p/d)	Level of DNF-10 in food (%)	Grams/p/d of DNF-10 consumed by eaters only	Total Sample Mean Intake (TSMI) g/p/d of DNF-10
Popcorn	1153	1.53%	35	0.83%	0.2905	0.00443
Chips	5565	7.37%	35.2	0.83%	0.29216	0.02152
Crackers	3005	3.98%	26.1	0.83%	0.21663	0.00862
Bars	551	0.73%	41.6	0.63%	0.26208	0.00191
Protein powder	206	0.27%	60	1.25%	0.75	0.00205

The verbiage on pages 12 and 13 will be updated with the right numbers to:
The cumulative TSMI of DNF-10 was calculated by summing the TSMIs of DNF-10 in each food category and equaled 0.0385 grams of DNF-10 per person per day.

%Eaters={1-[1-0.0153)x(1-0.0737)x(1-0.0398)x(1-0.0073)x(1-0.0027)]}x100 = 13.28%

The cumulative eaters-only mean intake of DNF-10 was obtained by dividing the cumulative TSMI for DNF-10 by the percent eaters (0.0385 / 13.28% = 0.2900 g/p/d (290 mg/p/d)

Finally, the approximate cumulative eaters-only intake of DNF-10 at the 90th percentile was obtained by multiplying the cumulative eaters-only mean intake by two. This cumulative eaters-only pseudo-90thile intake of DNF-10 equals 0.580 g/p/d (580 mg/p/d).

8. Please clarify the intended technical effect (e.g., source of nutrients, protein) of the yeast hydrolysate in the finished food as it consumed.
   To provide essential amino acids and an unami taste to the food.

9. In the dietary exposure section (Part 3), you discussed the "Current Exposure to other yeast-derived products" on p. 13. Please provide a narrative indicating if the proposed use would be substitutional for the current uses of yeast-derived products or if the dietary exposure would be in addition to the current uses. If it is in addition, please provide a cumulative dietary exposure that includes all current and proposed uses.
   Consuming DNF-10 in the foods indicated above would be in addition to the current dietary exposure which is 12.75 g per day per person. With the addition of the 0.575 g/p/d, the cumulative dietary exposure would be 12.75 g + 0.575=13.32 g/p/d.

10. We note that carbohydrate (28-33%) was measured "by difference" in Table A (p. 7). Please clarify how this value was derived as the carbohydrate, ash, moisture, and protein components add up to over 100% for some batches.
    The carbohydrate formula is as follows. 100-protein-fat-water-ash = carbohydrate

11. The batch analysis results do not meet the provided specification for carbohydrate

percentage (28-33) provided in Table A. Please address this discrepancy.

Here is the updated table. I had omitted fat which is now included. The LOQ for arsenic had a typo. It should have read <0.01 ppm, not <0.07.

	Specification	Method	Batch #191107	Batch #190610	Batch #180914
Physical and Chemical Parameters					
Color/Appearance	Yellow to light brown	Visual against standard	Conforms	Conforms	Conforms
Odor	Slightly yeasty	Sensory	Conforms	Conforms	Conforms
Taste	Slightly yeasty	Organoleptic	Conforms	Conforms	Conforms
Crude Protein Content % (total nitrogen content x 6.25)	50-70	Kjeldahj	55.27	58	57.6
Carbohydrates (%)	34-40	By difference	38.2	35.1	35.6
Fat (%)	<1%	AOAC Method 98905	0.02	0.05	0.01
Ash (%)	≤ 3	Residue upon ignition	2.6	2.4	2.5
Moisture (%)	≤ 8	Loss on drying at 105°C	3.92	4.5	4.3
Microbiological Parameters					
Total aerobic microbial count	≤ 3,000 CFU/g	ISO 4833-1	150	300	200
Total yeasts and mold count	≤ 50 CFU/g	ISO 21527-2	0	0	0
Coliforms	Absent in 1 g	ISO 4831	Absent	Absent	Absent
Staphylococcus aureus	Absent in 10 g	ISO 6888	Absent	Absent	Absent
Salmonella	Absent in 20 g	ISO 6579	Absent	Absent	Absent
Impurities					
Total heavy metals	< 10 ppm	Colorimetry	< 1	< 1	< 1
Arsenic (LOQ=0.01 ppm)	< 1 ppm	ICP-Inductively coupled plasma spectrometry	ND	0.01	0.07
Cadmium (LOQ=0.009 ppm)	< 1 ppm	ICP spectrometry	0.021	0.01	ND
Mercury (LOQ=0.012 ppm)	< 0.1 ppm	Mercury analyzer	ND	ND	ND
Lead (LOQ=0.001 ppm)	< 0.5 ppm	ICP spectrometry	ND	0.01	0.01

Toxicology:

1. FDA notes that in a search of the literature pertaining to the safety of yeast hydrolysate peptide complex, an article titled "*Toxicity Evaluation by Single and Repeated Administration of Yeast Hydrolysate DNF-10*" was found.[1] This study was not discussed in the safety narrative of the notice; however, given that your article of commerce was used in the study, please summarize the findings, and discuss any relevant safety concerns.

2. On page 4 of your notice, you briefly describe your assessment of the scientific literature for the safety narrative. Please identify the search parameters used for the literature searches performed. Additionally, you did not indicate an end date in your literature search. FDA notes that a toxicology study performed with the article of commerce was published in 2021 but was not cited in this notice. Please indicate the timeframe (month and year) of the most recent comprehensive literature search performed prior to submission.

   I submitted the first draft of the notification on Nov 4, 2020. Thus, my literature search end date was September 2020.

3. In Table C (page 8-9), you list the amino acid composition for your article of commerce, and include the non-protein amino acid, Gamma-amino Butyric Acid (GABA). GABA is a potent inhibitory neurotransmitter with important regulatory roles throughout the body, including in the heart and brain.[2] Please discuss how exposure to GABA from the intended use is not a safety concern if it exceeds current levels of exposure from other natural sources of GABA.

4. On pages 23-24, you reference several clinical studies that examined the appetite suppression effects associated with yeast hydrolysate consumption.[3] FDA notes that several papers elucidating the mechanisms that mediate these appetite suppressive effects have been published. These studies, done with your article of commerce, suggest that yeast hydrolysate can modulate the expression levels of several orexigenic factors, such as neurotransmitters

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[1] Ahn Y., et al., *Toxicity Evaluation by Single and Repeated Administration of Yeast Hydrolysate DNF-10*. Prev Nutr Food Sci, 2021. **26**(1):75-81.

[2] Oketch-Rabah HA., et al., *United States Pharmacopeia (USP) Safety Review of Gamma-Aminobutyric Acid (GABA)*. Nutrients, 2021. **13**(8):2742.

[3] Fytexia referenced three human clinical studies in the safety narrative. Please see references 21-23 on page 28 of the notice.

involved in energy homeostasis and the hormone ghrelin.[4,5] Ghrelin is a potent orexigenic signaling hormone. Importantly, it also functions within the cardiovascular, immune, and reproductive systems and plays a critical role in energy and glucose homeostasis.[6] In the brain ghrelin has noted effects on mood, memory, and sleep.[7] Thus, although the scientific merit behind these effects has not been established, there appears to be plausible modes of action for the purported physiological effects attributable to yeast hydrolysate. In light of these pleiotropic effects, please provide a discussion of the safety of yeast hydrolysate peptide complex as it relates to its functional effect on ghrelin. Please include in this discussion populations that may be particularly susceptible to ghrelin modulation, including diabetic patients who are susceptible to insulin-induced hypoglycemia, and individuals with mood or neurodegenerative disorders.

[4] Jung EY., et al., *Effects of Yeast Hydrolysate on Neuropeptide Y (NPY) and Tryptophan Hydroxylase (TPH) Immunoreactivity in Rats*. Phytotherapy Research, 2009. **23**: 619-623.
[5] Hong, KB., et al., *Yeast Hydrolysate as a Functional Anti-obesity Ingredient: Appetite Suppressive Effects of Yeast Hydrolysate in Food-deprived Mice*. Progress in Nutrition, 2015. **17**(3): 262-264.
[6] Pradhan G., et al., *Ghrelin: much more than a hunger hormone*. Curr Opin Clin Nutr Metab Care, 2013. **16**(6): 619-624.
[7] Shi L., et al., *Ghrelin and Neurodegenerative Disorders – a Review*. Mol Neurobiol, 2017. **54**(2): 1144-1155.

Viebrock, Lauren

From: Nathalie Chevreau <nachevre@gmail.com>
Sent: Wednesday, September 7, 2022 11:02 AM
To: Viebrock, Lauren
Cc: Julien Cases PhD; Morgane JAFFRELO
Subject: Re: [EXTERNAL] Re: GRN 001033
Attachments: 2022_05_20 GRN1033 Questions and Answers from NChevreau 09-06-2022.docx

Hello Lauren,

Thank you for allowing more time to complete the request. Please find all the answers to the follow up questions for GRN 001033.

Let me know if you have any questions or comments.

We look forward to hearing from the committee soon.

Sincerely,

Nathalie

Nathalie Chevreau Ph.D., R.D.
Chevreau Consulting Corp
2151 E Logan avenue
Salt Lake City, UT 84108
Cell (801) 652 6035
Website: www.ncphd.net

On Wed, Aug 31, 2022 at 8:09 AM Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov> wrote:

> Hi Nathalie,
>
> I wanted to check in with you regards to the responses to our questions for GRN 1033. We request a complete response by Monday, September 12, 2022. I am happy to talk by phone if we can provide any clarification.
>
> Best,
> Lauren

From: Viebrock, Lauren
Sent: Tuesday, August 23, 2022 3:23 PM
To: Nathalie Chevreau <nachevre@gmail.com>
Subject: RE: [EXTERNAL] Re: GRN 001033

Hi Nathalie,

The email regarding 90 days was to inform you that we are extending our evaluation of GRN 1033 by 90 days. This indicates the timeframe within which we anticipate providing a response to your GRAS notice. In order to do that, we need the responses to the questions well before that. We typically request a response to our questions within 10 business days. I understand you have had some extenuating circumstances that require a little more time to provide the information. Please provide the responses as soon as you are able.

Thanks,

Lauren

From: Nathalie Chevreau <nachevre@gmail.com>
Sent: Monday, August 15, 2022 12:32 PM
To: Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov>
Subject: [EXTERNAL] Re: GRN 001033

Hello Lauren,

I am working this week on the GRAS notification questions that were sent to me on May 22 2022. Do I understand well that I have 90 days after July 30, 2022 to send you the answers?

I would appreciate clarification to make sure that I do not miss the deadline

Thank you.

I look forward to hearing from you

Sincerely,

Nathalie

Nathalie Chevreau Ph.D., R.D.
Chevreau Consulting Corp

2151 E Logan avenue
Salt Lake City, UT 84108
Cell (801) 652 6035
Website: www.ncphd.net

On Tue, Aug 2, 2022 at 8:18 PM Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov> wrote:

> Dear Dr. Chevreau,
>
> This email is to inform you that, in accordance with 21 CFR 170.265 (b)(2), FDA is extending the normal 180 day review timeframe by 90 days. The original 180 day date for GRN 001033 is 7/30/22.
>
> Regards,
>
> Lauren
>
> **Lauren VieBrock, Ph.D.**
> *Regulatory Review Scientist/Microbiology Reviewer*
>
> **Center for Food Safety and Applied Nutrition**
> **Office of Food Additive Safety**
> **U.S. Food and Drug Administration**
> Tel: 301-796-7454
> lauren.viebrock@fda.hhs.gov

May 20, 2022 from Dr Viebrock

GRN 1033 Questions:

The answers are indicated in blue ink

Regulatory:

1. Please provide the corrected the citation for the regulation for aminopeptidase that is made from *Aspergillus oryzae*. The cited regulation (21 CFR 184.1027) is for carbohydrase and protease enzymes. A: the aminopeptidase is a protease that was sold for human consumption before the passage of DSHEA in 1994. It is listed in the UNPA list of ingredients that were sold in the US market prior to 1994. It is considered GRAS as it complies with the food grade specifications of the Food Chemical Codex (FCC). This ingredient also complies with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Chemistry:

1. Please provide the source of the maltodextrin used in the formulation of the yeast hydrolysate peptide complex. A: Corn

2. Please state whether all analytical methods used for the batch analyses are validated for their intended purpose. A: All the analytical methods used for the batches analyses are validated for their intended purpose.

3. Please specify what is meant by "ND" in Table A (pp. 7-8) and indicate whether it refers to values below the limit of detection (LOD) or limit of quantification (LOQ). Since batch analysis results demonstrate that both arsenic and cadmium are less than 0.01 ppm, please adjust the specification of arsenic and cadmium to reflect the reality. A: there was a typo for the LOQ for arsenic. LOQ is 0.01 pm. I have the email showing the LOQ from the DNF-10 manufacturer if you want it. ND stands for Non detectable. It means the result is below the LOQ. Will change the specs for arsenic and cadmium to < 0.1 ppm.

4. Please provide the LOQ for the mercury method used for the analyses in Table A (p. 7). A: LOQ of Mercury is 0.012ppm

5. Please clarify the intent of footnotes "a" and "b" of Table D (p. 10). The amounts listed in these footnotes are different than the reference amounts customarily consumed (RACC) that are used in the table and therefore, it is not clear why the footnotes are included. In addition, footnote "b" discusses chips when the intended food category is crackers. Please explain the relevance of the information for chips to that of crackers. A: The intent of footnote "a" was to help the reader visualize what a 30 g serving of popcorn represented knowing that 2 cups of popcorn weight about 17 grams. I can remove it if it does not help. Footnote "b" should indeed be for chips and not crackers. I can also remove the footnote if it does not help.

6. For the protein dry powder category in Table D (p. 10), please clarify if the provided use level is for the powder or in the beverage as consumed. If it is for the powder, please indicate how the use level was determined for the beverage as consumed. In addition, if this ingredient can be used in the powder that is used to make the reconstituted beverage,

it should be included in the exposure estimate. A: it is for the protein powder. The use level was calculating by dividing 250 mg of DNF-10 by 20 g protein powder RACC. This makes a use level of 1.25%.

7. In your description of the dietary exposure estimate, you state that you used a "probabilistic model" to determine the cumulative eaters-only intake of the yeast hydrolysate (p. 11). We note that the dietary exposure estimate you present would not be a "probabilistic" model. Although this approach is described in the guidance as a way to estimate "probable" human exposure, these terms are not interchangeable in this context. For the estimate of dietary exposure, we request that you clarify:

   You provided "N of eaters out of 92,062 surveyed individuals (2 years and older)" in Table E (p. 11). However, we are not able to confirm your N values using the 1999-2016 NHANES data. Please clarify if the "N" in Table E represents a number of eaters (un- weighted) over 2 years of age in the U.S. who consumed these foods. A: I looked back at the dataset that I had created back in 2020. The number of unique individuals (single SEQN) 2 years and older that were surveyed was 92,062. Datasets included in the analysis were the demographic information, dietary data (DRXIFF, DR1IFF, and DR2IFF data, FFQRAW data), and consumer behavior data (CBQ data), as available for each survey cycle. 75,544 participants had at least one item recorded for the dietary recall. N eaters were determined using dietary recall data or other applicable questionnaire variables in the datasets mentioned above for each food category. Grams of food were determined using dietary recall data. All frequencies are unweighted. Thus, I should have used 75,544 and not 92,062. I will change the N to 75,544. The number of eaters in each category will not change but the % Eaters in column 3 of Table E will change.

   New Table E:

Food Category	N of eaters out of 75,544 surveyed individuals (2+ years old)	% Eaters (N/75,544)	Mean consumption of food (g/p/d)	90th ile (g/p/d)
Popcorn	1153	1.53%	35	83.1
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Crackers	3005	4%	26.1	52
Bars	551	0.73%	41.6	65
Protein powder	206	0.27%	60	131.4

   a. Further, we note that you combined the food consumption data from NHANES 1999- 2002 (a single 24-hour dietary recall) and NHANES 2003-2016 (two 24-hour dietary recalls) in your estimate. Please explain how you combined two different surveys (1-day -and 2-day dietary recalls), and what statistical software package was used to analyze the consumption data from the multiple NHANES surveys. A: For participants who only had a single 24-hour dietary recall, a single observation was used to determine consumption of each food. For participants who had two days of dietary recall data, an average of each food consumption over the two-day period was computed. All analyses were completed using SAS software.

As the result of the change of actual eaters (75,544), Table F will be changed to:

Table F

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Foods	N of eaters (2+ years old)	% Eaters (N/75,544)	Mean consumption of food (g/p/d)	Level of DNF-10 in food (%)	Grams/p/d of DNF-10 consumed by eaters only	Total Sample Mean Intake (TSMI) g/p/d of DNF-10
Popcorn	1153	1.53%	35	0.83%	0.2905	0.00443
Chips	5565	7.37%	35.2	0.83%	0.29216	0.02152
Crackers	3005	3.98%	26.1	0.83%	0.21663	0.00862
Bars	551	0.73%	41.6	0.63%	0.26208	0.00191
Protein powder	206	0.27%	60	1.25%	0.75	0.00205

The verbiage on pages 12 and 13 will be updated with the right numbers to:
The cumulative TSMI of DNF-10 was calculated by summing the TSMIs of DNF-10 in each food category and equaled 0.0385 grams of DNF-10 per person per day.

%Eaters={1-[1-0.0153)x(1-0.0737)x(1-0.0398)x(1-0.0073)x(1-0.0027)]}x100 = 13.28%

The cumulative eaters-only mean intake of DNF-10 was obtained by dividing the cumulative TSMI for DNF-10 by the percent eaters (0.0385 / 13.28% = 0.2900 g/p/d (290 mg/p/d)

Finally, the approximate cumulative eaters-only intake of DNF-10 at the 90th percentile was obtained by multiplying the cumulative eaters-only mean intake by two. This cumulative eaters-only pseudo-90thile intake of DNF-10 equals 0.580 g/p/d (580 mg/p/d).

8. Please clarify the intended technical effect (e.g., source of nutrients, protein) of the yeast hydrolysate in the finished food as it consumed.
   To provide essential amino acids and an unami taste to the food.

9. In the dietary exposure section (Part 3), you discussed the "Current Exposure to other yeast-derived products" on p. 13. Please provide a narrative indicating if the proposed use would be substitutional for the current uses of yeast-derived products or if the dietary exposure would be in addition to the current uses. If it is in addition, please provide a cumulative dietary exposure that includes all current and proposed uses.
   Consuming DNF-10 in the foods indicated above would be in addition to the current dietary exposure which is 12.75 g per day per person. With the addition of the 0.575 g/p/d, the cumulative dietary exposure would be 12.75 g + 0.575=13.32 g/p/d.

10. We note that carbohydrate (28-33%) was measured "by difference" in Table A (p. 7). Please clarify how this value was derived as the carbohydrate, ash, moisture, and protein components add up to over 100% for some batches.

The carbohydrate formula is as follows. 100-protein-fat-water-ash = carbohydrate

11. The batch analysis results do not meet the provided specification for carbohydrate percentage (28-33) provided in Table A. Please address this discrepancy.
Here is the updated table. I had omitted fat which is now included. The LOQ for arsenic had a typo. It should have read <0.01 ppm, not <0.07.

	Specification	Method	Batch #191107	Batch #190610	Batch #180914
Physical and Chemical Parameters					
Color/Appearance	Yellow to light brown	Visual against standard	Conforms	Conforms	Conforms
Odor	Slightly yeasty	Sensory	Conforms	Conforms	Conforms
Taste	Slightly yeasty	Organoleptic	Conforms	Conforms	Conforms
Crude Protein Content % (total nitrogen content x 6.25)	50-70	Kjeldahj	55.27	58	57.6
Carbohydrates (%)	34-40	By difference	38.2	35.1	35.6
Fat (%)	<1%	AOAC Method 98905	0.02	0.05	0.01
Ash (%)	≤ 3	Residue upon ignition	2.6	2.4	2.5
Moisture (%)	≤ 8	Loss on drying at 105°C	3.92	4.5	4.3
Microbiological Parameters					
Total aerobic microbial count	≤ 3,000 CFU/g	ISO 4833-1	150	300	200
Total yeasts and mold count	≤ 50 CFU/g	ISO 21527-2	0	0	0
Coliforms	Absent in 1 g	ISO 4831	Absent	Absent	Absent
Staphylococcus aureus	Absent in 10 g	ISO 6888	Absent	Absent	Absent
Salmonella	Absent in 20 g	ISO 6579	Absent	Absent	Absent
Impurities					
Total heavy metals	< 10 ppm	Colorimetry	< 1	< 1	< 1
Arsenic (LOQ=0.01 ppm)	< 1 ppm	ICP-Inductively coupled plasma spectrometry	ND	0.01	0.07
Cadmium (LOQ=0.009 ppm)	< 1 ppm	ICP spectrometry	0.021	0.01	ND

Mercury (LOQ=0.012 ppm)	< 0.1 ppm	Mercury analyzer	ND	ND	ND
Lead (LOQ=0.001 ppm)	< 0.5 ppm	ICP spectrometry	ND	0.01	0.01

Toxicology:

1. FDA notes that in a search of the literature pertaining to the safety of yeast hydrolysate peptide complex, an article titled "*Toxicity Evaluation by Single and Repeated Administration of Yeast Hydrolysate DNF-10*" was found.[1] This study was not discussed in the safety narrative of the notice; however, given that your article of commerce was used in the study, please summarize the findings, and discuss any relevant safety concerns. A: Indeed, I did not discuss this paper since it was published after I submitted the 1st GRAS notification on Nov 4, 2020. Here is the summary that I will add to the notification document.

   Single dose Acute and Repeated-Dose Chronic Toxicity Study (Ahn 2021)
   The study was conducted in male and female Sprague Dawley rats where the toxicity of DNF-10 (peptides <10 kDa) was examined. The acute dose was a single oral dose of 5,000 mg/kg of body weight and the animals were observed for 14 days. The repeated dose was 1,000 mg/kg body weight given daily for 90 days. The control animals in both cases received water. DNF-10 toxicity was evaluated by collecting blood biochemical, hematological parameters and organ weights at 14 days for the single dose and at 90 days for the repeated dose portion and comparing the data between control and treatment groups.

   No death was observed in any groups, at 14 or at 90 days. No fur loss, nor diarrhea or edema and no decreased motor activity were observed in any of the groups.
   During the 14 days following the single dose of DNF-10, all animals gained weight as expected and there was no difference between control and treatment groups for their respective gender. During the 90 days sub-chronic study, the males gained statistically less weight on DNF-10 compared to their controls from week 6 until the end of the study. The females gained slightly less gain on DNF-10 but the difference was not statistically significant compared to their corresponding control group.

   No pathological abnormalities were found in the organs of the animals except for the brain of the males in the treatment group. However, the increased brain weight was still within normal range. The hematological and the blood biochemical parameters (liver function, kidney and lipid profiles all remained within normal range.

   This study confirms previous results indicating no toxicity of DNF-10.

2. On page 4 of your notice, you briefly describe your assessment of the scientific literature for the safety narrative. Please identify the search parameters used for the literature searches performed. Additionally, you did not indicate an end date in your literature search. FDA notes that a toxicology study performed with the article of commerce was published in 2021 but was not cited in this notice. Please indicate the timeframe (month and year) of the most recent

[1] Ahn Y., et al., Toxicity Evaluation by Single and Repeated Administration of Yeast Hydrolysate DNF-10. Prev Nutr Food Sci, 2021. **26**(1):75-81.

comprehensive literature search performed prior to submission.

I submitted the first draft of the notification on Nov 4, 2020. Thus, my literature search end date was September 2020. I ran additional literature search until August 2022. I did extensive literature search. The terms that I used were as follow and also included combination of those: yeast peptide, yeast hydrolysate, saccharomyces, cerevisiae, DNF-10, marmite, Brewer's yeast, baker's yeast, nutritional yeast, yeast allergy, yeast protein, satiety, yeast, diabetes, appetite suppressant, ghrelin, weight loss, weight control, obesity, hypersensitivity, toxicity, safety, yeast nucleic acids, uric acid, food grade, yeast amino acids, chromium yeast, selenium yeast, GRAS, yeast composition, metabolic syndrome, lipid profile, GABA, gamma-amino butyric acid, neurotransmitter, hormones, spent yeast, ghrelin receptors, hormones receptors, post-prandial glucose, yeast supplement, yeast supplementation, oral yeast, yeast fermentation, neuropeptide.

3. In Table C (page 8-9), you list the amino acid composition for your article of commerce, and include the non-protein amino acid, Gamma-amino Butyric Acid (GABA). GABA is a potent inhibitory neurotransmitter with important regulatory roles throughout the body, including in the heart and brain.[2] Please discuss how exposure to GABA from the intended use is not a safety concern if it exceeds current levels of exposure from other natural sources of GABA.

GABA is an ubiquitous non-protein amino acid that is present in almost every plant. The amount of GABA synthetized by a plant depends on plant species, plant variety, severity of stress experienced during growth or storage and post-harvest processing. (Table below). In common crops like tomato, potato, spinach, wheat and rice, GABA contents tend to be above 100 mg per 100 g. GABA has also been measured in livestock animals but the results are from tissues that are NOT included in human diet. There are detectable but small amounts of GABA in honey, eggs and milk. Another source of GABA in the diet is through foods that have been processed using microorganisms and as the result contain a higher amount of GABA than the standard starting product.

Food Item	GABA Content (mg/100 g food)	References
Green mustard leaf	97	Kim 2013
Traditional Korean Kimchi made of green mustard leaf	Anywhere between 10 to 100 based on fermenting organism, length of fermentation, start vegetable and precursor (MSG)	Kim 2013; Lee 2018; Seok 2008; Paramithiotis 2022
Lychee pulp	170 - 350	Ramoz-Ruiz 2018
Green tea leaf dried (oxidized)	100 - 200	Tsushida 1987, Oketch-Rabah 2021
Green tea leaves -fresh	3 - 9	Sawai 2001
Ripe tomatoes	84 -189 (depending of the varieties	Akihiro 2008, Saito 2008
Mulberry	86 - 185	Ramoz-Ruiz 2018
Barley	26 - 89	Kihara 2007
Brown rice	1 - 15	Oh 2003, Choi 2004, Kim 2012
Thai rice	7 - 29	Kittibunchakul 2017

[2] Oketch-Rabah HA., et al., *United States Pharmacopeia (USP) Safety Review of Gamma-Aminobutyric Acid (GABA)*. Nutrients, 2021. **13**(8):2742.

Japanese rice	7 - 14	Kamatsuzaki 2007
70% dark chocolate	16	Dala-Paula 2020
Rice germ	10.75 - 18.62	Wang 2022
Fava beans	0.30 - 20.60	Yang 2013
Potato tuber	54 - 342	Steward 1949; Zazzeroni 2009
Sweet potato	14	Oh 2004
Radish	28	Kato 2015
Pumpkin	371 - 1553	Qi 2012
Fresh cucumber	5.5	Paramithiotis 2022
Fresh broccoli florets	3	Oh 2003
Green cabbage	3 - 7	Park 2014
Red cabbage	2 - 35	Park 2014
Spinach	43 - 267	Oh 2003, Yoon 2017
Just harvested strawberries	1.5 – 3.6	Ramoz-Ruiz 2018
Soybean	5 - 50	Zazzeroni 2009; Abe 2005
Heat dry soybean	447	Takahashi 2013
Soybean sprouts	122 - 500	Martinez-Villaluenga 2006; Tianwawang 2016
Wheat	65 - 79	Kim 2007
Fresh grapefruit	14	Ramoz-Ruiz 2018
White button mushroom	125 - 360	Chen 2012, Oka 1981
Ripe Tomato juice	82.2 mg/100 ml	Inaba 1980
Navel orange juice	24 mg/100 ml	Ramoz-Ruiz 2018
Soy sauce from germinated soy bean	0.683 mg/100 ml	Zhao 2021
Soy sauce from soy bean	0.242 mg/100 ml	Zhao 2021

A healthy diet based on plant products (cereals, fruits and vegetables) following the USDA dietary guidelines will provide significant amount of GABA as a natural nutrient.

In 1987, the per capita consumption of GABA from natural sources in the US was estimated at 136 mg/person/day (Stofberg and Grundschober 1987). It is likely to be a low estimate as the content of GABA in food is not always listed in the Food composition tables. A recent study in Japan proves this point.[3] (Ito 2021) Standardized meals that were provided in a hospital in Okinawa were analyzed for the content of GABA. GABA was also calculated using the Standard Tables of Food Composition in Japan. The actual amount of GABA in the meals was greater by a factor of 2 than the estimate from the Food Tables (67.3 mg versus 30 mg)

The intended use of DNF-10 based on the cumulative eaters-only pseudo-90thile intake of 0.580

[3] Ito S. Comparison of Analyzed and Calculated Values of Gamma-Aminobutyric Acid (GABA) Intake from Hospital Diet. J Nutr Sci Vitaminol. 2021. **67**:139-42.

g/p/d (580 mg/p/d) would provide an additional 40 mg of GABA per day. (0.67 mg/kg for a 60 kg individual). This represents a small fraction of the amount that is estimated to be consumed in a healthy diet, especially if an individual follows a healthy diet based on plant products (cereals, fruits and vegetables) that follows the USDA dietary guidelines.

GABA has been extensively studied in animals for toxicity and has been deemed to be of low oral toxicity. Acute toxicity studies in mice showed that the oral median lethal dose (LD50) ranges from >1 to 12 g/kg pf body weight (Oshima 1965, Frey and Loscher 1980). Sauchi et al (2009) reported a no-observed-adverse-effect level (NOAEL) of 200 mg/kg body weight/day when administered to male and female Wistar rats in the diet over a 28-day period. No toxicological relevant effects were reported in a 90-day study in Sprague-Dawley rats that received doses up to 2,500 mg/kg body weight /day by oral gavage (Takeshima 2014). Kato et al (2005) conducted a study of 28- day and 90-day with rats and the highest dose given was only 5 mg GABA/kg body weight/day. They concluded that the (NOAEL) was 5 mg/kg body weight/day. The dose of GABA tested in this latest study was considerably lower than the dose tested in the studies cited above (Sauchi 2009 200 mg/kg bw).

A review conducted by the EPA indicated that studies in the literature involving prolonged chronic administration of large doses of GABA to rats and dogs (up to 1 g/kg/day) reported no signs of toxicity or untoward effects (Federal Register Volume 62, Number 209 - Wednesday, 29 October 1997). Taken all together, these studies support the safety of oral exogenous GABA.

GABA supplementation also has been the subject of clinical studies for treatment of insomnia, high blood pressure and stress. Oketch-Rabah et al (2021) at the US Pharmacopeia and the Office of Dietary Supplements did a review of the clinical trials and concluded that GABA is relatively safe in short term studies of doses up to 18 grams per day (up to 4 days) and in longer term studies (12 weeks) at doses up to 120 mg/d.

Thus, the weight of the evidence supports the safety of an additional 40 mg of GABA per day when consumed in foods that contains DNF-10.

4. On pages 23-24, you reference several clinical studies that examined the appetite suppression effects associated with yeast hydrolysate consumption.[4] FDA notes that several papers elucidating the mechanisms that mediate these appetite suppressive effects have been published. These studies, done with your article of commerce, suggest that yeast hydrolysate can modulate the expression levels of several orexigenic factors, such as neurotransmitters involved in energy homeostasis and the hormone ghrelin.[5,6] Ghrelin is a potent orexigenic signaling hormone. Importantly, it also functions within the cardiovascular, immune, and reproductive systems and plays a critical role in energy and glucose homeostasis.[7] In the brain ghrelin has noted effects on mood, memory, and sleep.[8] Thus, although the scientific merit

[4] Fytexia referenced three human clinical studies in the safety narrative. Please see references 21-23 on page 28 of the notice.

[5] Jung EY., et al., *Effects of Yeast Hydrolysate on Neuropeptide Y (NPY) and Tryptophan Hydroxylase (TPH) Immunoreactivity in Rats*. Phytotherapy Research, 2009. **23**: 619-623.

[6] Hong, KB., et al., *Yeast Hydrolysate as a Functional Anti-obesity Ingredient: Appetite Suppressive Effects of Yeast Hydrolysate in Food-deprived Mice*. Progress in Nutrition, 2015. **17**(3): 262-264.

[7] Pradhan G., et al., *Ghrelin: much more than a hunger hormone*. Curr Opin Clin Nutr Metab Care, 2013. **16**(6): 619-624.

[8] Shi L., et al., *Ghrelin and Neurodegenerative Disorders – a Review*. Mol Neurobiol, 2017. **54**(2): 1144-1155.

behind these effects has not been established, there appears to be plausible modes of action for the purported physiological effects attributable to yeast hydrolysate. In light of these pleiotropic effects, please provide a discussion of the safety of yeast hydrolysate peptide complex as it relates to its functional effect on ghrelin. Please include in this discussion populations that may be particularly susceptible to ghrelin modulation, including diabetic patients who are susceptible to insulin-induced hypoglycemia, and individuals with mood or neurodegenerative disorders.

The animal and human studies carried out with the DNF-10 added to the diet seem to indicate a reduction of food intake and a subsequent weight and fat loss. However, it is unclear whether these effects are due to a significant change of ghrelin levels. Some studies suggest that DNF-10's effect is mediated through a reduction of ghrelin whereas other studies do not observe the ghrelin reduction.[9,10] The lipolytic effect of YH may be independent from YH effect on ghrelin. By the same token, it is unclear whether ghrelin inhibits insulin secretion or not. (Padhan 2013). Thus, it is unlikely that DNF-10 consumption would be unsafe for individuals who are susceptible to insulin-induced hypoglycemia or those with mood or neurodegenerative disorders.

[9] Jung EY, Hong YH, Kim JH, Park Y, Bae SH, Chang UJ, et al. Effects of yeast hydrolysate on hepatic lipid metabolism in high-fat-diet-induced obese mice: yeast hydrolysate suppresses body fat accumulation by attenuating fatty acid synthesis. Ann Nutr Metab. 2012;61(2):89-94.

[10] Hong KB, Jung EY, Kim JH, Chang UJ, Suh HJ. Yeast hydrolysate as a functional anti-obesity ingredient: appetite suppressive effects of yeast hydrolysate in food-deprived mice. Progress in Nutrition. 2015;17(3):262-4.

Viebrock, Lauren

From: Nathalie Chevreau <nachevre@gmail.com>
Sent: Tuesday, February 14, 2023 8:59 PM
To: Viebrock, Lauren
Subject: [EXTERNAL] Re: GRN 1033 Questions
Attachments: 2023_02_07 GRN 1033 questions with NC answers Feb-14-2023.docx; GRN1033 DNF10-Feb 14-2023 Fytexia-clean version-flat references.pdf

Hello Dr Viebrok,

Please find the answers to the questions that you sent on Feb 17 in the attached word document.

I also attached the updated notification GRN1033 with the various changes and edits that were made based on the input of your group and I signed it.

I will assume that when the committee is good with all the answers, I can upload the latest version using the secure Webtrader or do you pass the updated notification to your administrative group directly? please let me know.

I look forward to hearing from you.

Sincerely,

Nathalie

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On Tue, Feb 7, 2023 at 1:19 PM Viebrock, Lauren <Lauren.Viebrock@fda.hhs.gov> wrote:

> Dear Dr. Chevreau
>
> During our review of GRAS Notice No. 001033, we noted additional questions that need to be addressed. Please find the questions attached to this email.
>
> We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,

Lauren

Lauren VieBrock, Ph.D.
Regulatory Review Scientist/Microbiology Reviewer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 301-796-7454
lauren.viebrock@fda.hhs.gov

February 7, 2022:

GRN 1033 Questions:

1. We note that the specification limit for lead is < 0.5 mg/kg (Table A on p. 7 of the notice) while the results of batch analyses demonstrate that the levels of lead are consistently below 0.01 mg/kg, i.e., at least 50 times lower than the established limit for lead. We request that, similar to lowering the limits for arsenic and cadmium, you lower the specification limit for lead to better reflect the batch analyses.
   NC Answer: the specs for arsenic and cadmium had been lowered as indicated in the response sent on Sept 6, 2023. I will enclose the updated notification document to this email, so you are all looking at the most updated version.

2. The specifications for your ingredient include a limit for total heavy metals of < 10 mg/kg (Table A on p. 7 of the notice). We note that typically we do not require notifiers to establish a limit for total heavy metals, but for the individual limits for arsenic, lead, cadmium, and mercury, as appropriate. We would not expect other heavy metals to be present in an ingredient manufactured under controlled conditions in accordance with good manufacturing practices. Please confirm that you do not expect other heavy metals to be present in your ingredient or provide a justification for your high total heavy metal limit of < 10 mg/kg.
   NC Answer: We removed the specs for total heavy metals since it is not required.

3. Please note that the uses of the enzymes listed in your GRAS notice are for uses as enzymes in production of conventional foods, and therefore status for uses in dietary supplements are not relevant. We note that the enzymes used in production of your ingredient meet FCC and JECFA specifications. Please confirm that the enzymes used are from safe and suitable sources.

   NC Answer: Two of the proteases used in the enzymatic hydrolysis of the yeast, aminopeptidase made from aspergillus oryzae (Flavourzyme 1000 L), and subtilisin made from Bacillus licheniformis are provided by Novozymes. Novozymes guarantees that their enzymes comply with the recommended purity specifications for food-grades enzymes of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC). (see attached specs for the enzymes). Specifically, the FCC indicates that protease enzyme preparations that are derived from any of the following source organisms are acceptable for use in food processing: *Bacillus licheniformis, Bacillus subtilis, Aspergillus niger, or Aspergillus oryzae* (FCC, 7th Ed., pl 185). The supplier of the third enzyme, papain is Enzybel and guarantees that the enzyme complies with the recommended purity for food grade enzyme (See attached specs). Therefore these 3 food grade proteases are suitable and safe for the enzymatic preparation of DNF-10.