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RECEIVED
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FOOD ADDITIVE SAFETY

October 22, 2021

Via FedEx & CD-ROM

Dr. Susan Carlson
Director, Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740-3835

Re: GRAS Notification for EverGrain Barley Rice Protein

Dear Dr. Carlson:

We respectfully submit the attached GRAS Notification on behalf of our client, EverGrain, LLC (EverGrain) for Barley Rice Protein to be used in a variety of conventional food and beverage products as a source of plant protein. Barley Rice Protein will be used as a substitute for, and/or in conjunction with, other sources of protein in the diet. Thus, Barley Rice Protein will not contribute any additional exposure to protein and it is not intended to be used to replace the entire daily protein intake or as the sole source of protein in the diet for consumers. More detailed information regarding product identification, intended use levels, and the manufacturing and safety of the ingredient is set forth in the attached GRAS Notification.

EverGrain has determined that their Barley Rice Protein is GRAS based on scientific procedures in accordance with 21 C.F.R. § 170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. § 170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016). Therefore, the use of the Barley Rice Protein as described in this GRAS Notification is exempt from the requirement of premarket approval as set forth in the Federal Food, Drug, and Cosmetic Act.

The analytical data, published studies, and information that are the basis for this GRAS Notification are available for FDA review and copying at reasonable times at Keller and Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001, or will be sent to FDA upon request.

We look forward to the Agency's review of this submission and would be happy to provide Agency officials with any information they may need to complete their assessment. Thank you for your attention to this matter.

Cordially yours,

Evangelia C. Pelonis

GRAS Notice for Barley Rice Protein

Prepared for: Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Dr.
College Park, Maryland 20740

Submitted by: Keller and Heckman LLP
1001 G St., NW
Suite 500W
Washington, DC 20001

On behalf of our client:

EverGrain, LLC
3205 S. 9th St
St Louis, MO 63118 USA

Date: October 22, 2021

GRAS Notice for Barley Rice Protein

Table of Contents

List of Tables

List of Figures

List of Appendices

Part 1. Signed statements and certification

I. Applicability of 21 C.F.R. part 170, subpart E

We submit this generally recognized as safe (GRAS) notice in accordance with 21 C.F.R. part 170, subpart E.

II. Name and address of the notifier

Company: EverGrain, LLC
Name: Nick Puckett
Address: 3205 S. 9th St
St. Louis, MO 63118 USA
Email: nick.puckett@everingredients.com

All communications on this matter are to be sent to Counsel for EverGrain, LLC:

Evangelia C. Pelonis
Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20005
Tel: 202-434-4106
Fax: 202-434-4646
Email: pelonis@khlaw.com

III. Name of the notified substance

The common or usual name of the notified substance will be Barley Rice Protein. It is a protein concentrate (≥85% protein) derived from brewers spent grains consisting of a mixture of barley (*Hordeum vulgare*) at levels of 40 to 100% and rice (*Oryza sativa*) at levels of 0 to 60%.

IV. Applicable conditions of use of the notified substance

EverGrain intends to market Barley Rice Protein in a variety of conventional food and beverage products as a source of plant protein. Barley Rice Protein is intended as an alternative source of protein in the foods in which the ingredient is added. It is similar to other plant protein concentrates/isolates currently in the U.S. marketplace and which have received "no questions" letters from the U.S. Food and Drug Administration (FDA) regarding their GRAS. For example, plant protein concentrates/isolates which have received no questions letters include those derived from pea (GRN 851, 804, 803, 788, 608), mung bean (GRN 684), rice (GRN 609), potato (GRN 447), hemp (GRN 771), and canola (GRN 683, 386).

V. Basis for the GRAS determination

Keller and Heckman LLP, on behalf of EverGrain LLC, hereby notifies the Agency of its determination that EverGrain's Barley Rice Protein is GRAS for its intended use, consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). This GRAS conclusion is based on scientific procedures in accordance with 21 C.F.R. §170.30(a) and (b) and conforms to the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. §170.36, 81 Fed. Reg. 54,960 (Aug. 17, 2016). The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with proposed 21 C.F.R. § 170.36. The GRAS status of Barley Rice Protein is based on data generally available in the public domain.

VI. Exclusion from premarket approval

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

VII. Availability of data and information

The information for this GRAS conclusion including analytical data, published studies, and information that are the basis for this GRAS determination are available to FDA upon request as required by 21 C.F.R. § 170.225(c)(7)(ii)(A) or (B) by contacting Keller and Heckman LLP at the below address.

Evangelia C. Pelonis
Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20005
Tel: 202-434-4106
Fax: 202-434-4646
Email: pelonis@khlaw.com

VIII. Applicability of FOIA Exemptions

EverGrain LLC is not claiming any information in Parts 2 through 7 of this document as trade secret, confidential or financial information that is privileged or confidential. Thus, none of the information and data in this submission is exempt from the Freedom of Information Act (FOIA), 5 U.S.C. Section 552.

IX. Certification

We certify on behalf of our client, EverGrain LLC, that this GRAS conclusion is based on representative data from EverGrain LLC required to support the safety and GRAS status of Barley Rice Protein. To the best of our knowledge, it is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

X. Signature and name and title of the person signing this GRAS notice:

Date: October 22, 2021

Evangelia C. Pelonis
Partner
Keller and Heckman LLP

Part 2. Identity, method of manufacture, specifications, and physical or technical effect

I. Identity

The subject of this GRAS Notice is Barley Rice Protein, which is marketed under the tradename Everpro^{BR}. Barley Rice Protein is an off-white powder protein concentrate derived from a mixture of non-wheat containing brewer's grains from the mash step of the production of beer. Specifically, the grain mixture consists of barley (*Hordeum vulgare*) at levels of 40 to 100% and rice (*Oryza sativa*) at levels of 0 to 60%. It is manufactured such that the final product is not significantly chemically changed with respect to the amino acid composition compared to the native starting materials.

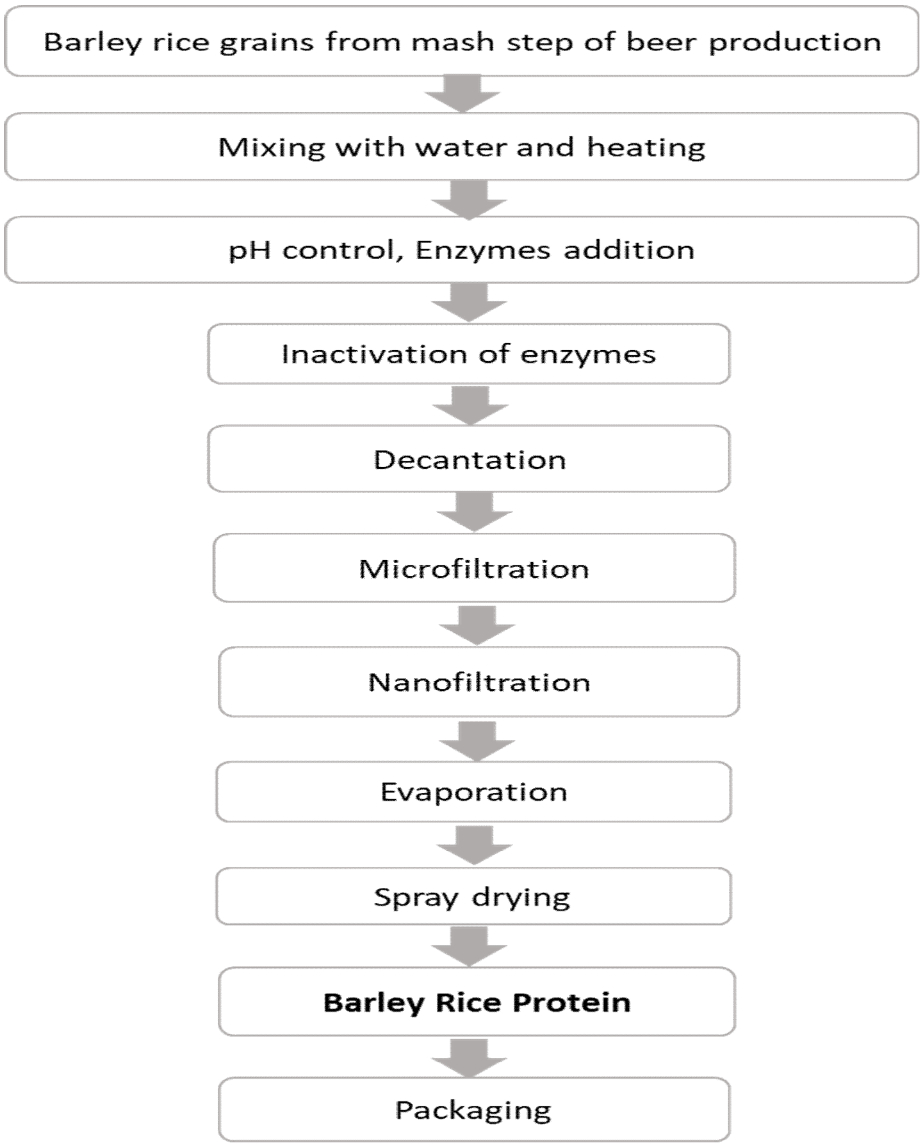
II. Method of Manufacture

In the first step of the manufacturing process occurring in the extraction vessel, the incoming grains are received into a jacketed, mixed tank with water, then heated and treated with a glucoamylase-pullulanase enzyme blend to hydrolyze the starch. After the pH is raised with sodium hydroxide and potassium hydroxide, the mixture is treated with a food-grade protease enzyme to hydrolyze the protein component. The enzymes are deactivated by heating the mixture. In the second step, the solids are separated from the liquid protein stream by pumping the slurry from the extraction tank to decanting centrifuges. The decanted solids are then mixed with water and decanted again to increase protein recovery. The washed and decanted solids are pushed through a screw press to further increase protein recovery and reduce the moisture content. The fiber content is removed in this step. Then, the combined decanted, pressed, and re-hydrolyzed liquid from hydrolysis vessels (where microfiltered retentate is recycled through the re-hydrolysis tank) are fed into a microfiltration system with diafiltration to increase protein recovery in the permeate. The retentate contains fats, fibers, and large protein molecules. Following the microfiltration step, the permeate from the microfiltration is processed in a nanofiltration system.

Barley Rice Protein is manufactured in accordance with cGMP and a food safety program that includes a Hazard Analysis and Risk-Based Preventive Controls (HARPC) plan. Furthermore, all raw materials, processing aids, additives, and food contact materials used in the production of Barley Rice Protein are food-grade or equivalent (e.g., Food Chemicals Codex, United States Pharmacopeia, or European Pharmacopeia), and are used in accordance with an applicable U.S. FDA regulation in Title 21 of the Code of Federal Regulations, have previously been determined to be GRAS, or have been the subject of an accepted food contact notification.

A schematic of the manufacturing process is presented below.

Figure 1: Manufacturing Process of Barley Rice Protein



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III. Product Specifications and Typical Values

EverGrain LLC has established various chemical, heavy metal, and microbiological specifications for Barley Rice Protein. The specifications, which have been selected to ensure a consistent food-grade product, are summarized in Table 1 together with the methods. All analytical methods have been validated for their intended use.

Table 1: Specifications for Barley Rice Protein		
	Specification	**Method of Analysis**
Chemical Parameters		
Protein (dry basis)	≥85%	AOAC 990.03; AOAC 992.15
Moisture	<8%	AOAC 925.09
Heavy Metal Parameters		
Arsenic	<0.1 ppm	AOAC 844-856 (modified)[1]
Cadmium	<0.1 ppm	AOAC 844-856 (modified)
Lead	<0.2 ppm	AOAC 844-856 (modified)
Mercury	<0.1 ppm	AOAC 844-856 (modified)
Microbiological Parameters		
Total plate count	<30,000 CFU/g	AOAC 966.23
Coliforms	<100 CFU/g	AOAC 991.14
Yeast and mold	<100 CFU/g	FDA BAM Chapter 18
Salmonella	Negative in 25 g	AOAC-RI 121501
Escherichia coli	<10 CFU/g	AOAC 991.14
Listeria spp.	Negative in 25 g	AOAC PTM 081401

AOAC = Association of Official Analytical Chemist; CFU = colony-forming units; FDA BAM = Food and Drug Administration Bacteriological Analytical Manual; ppm = parts per million.

Although not part of the specification, Barley Rice Protein also typically consists of the following quantities of fat, carbohydrates, fiber, and ash referred to as typical parameters in Table 2.

[1] The methods were modified to apply to a wider variety of sample types, to remove carbon enhancement on arsenic, and to better suit the equipment of the lab conducting the testing, Eurofins Scientific; Nutrition Analysis Center, which accredited to ISO/IEC 17025:2017.

Table 2: Typical Parameters for Barley Rice Protein		
Fat	<2%	AOAC 954.02
Total carbohydrates	<10%	Calculation
Total fiber	<5%	AOAC 991.43
Ash	<10%	AOAC 942.05

IV. Batch Analysis

The results of proximate analysis of non-consecutive batches of Barley Rice Protein are presented in Tables 3-5 below. The results demonstrate that the manufacturing process produces a consistent product that meets the establish product specifications. The tested batches of Barley Rice Protein consisted of 60 to 70% barley protein and 30 to 40% rice protein.

Table 3: Results of Chemical Analysis of Five Non-Consecutive Batches of Barley Rice Protein

Parameter	Specification/ Typical Parameter	Manufacturing Lot Numbers				
		080318SP01	122018BRSP01	0129191EV01	060319BRSP01	060319BRSP01
Protein (dry basis)	≥85%	84.6	89.1	86.9	89.7	88.5
Moisture	<8%	4.5	2.9	4.4	4.3	5.1
Fat	<2%	0.26	0.84	1.26	1.0	0.45
Total carbohydrates	<10%	8.92	6.79	6.36	5.83	7.25
Total fiber	<5%	2.8	2.8	3.6	3.2	3.2
Soluble	-	2.0	2.0	3.1	2.3	2.8
Insoluble	-	0.8	0.8	0.5	0.9	0.4
Ash	<10%	5.51	2.97	4.85	3.06	3.37

Table 4: Heavy Metals Analysis of Five Non-Consecutive Batches of Barley Rice Protein

Parameter	Specification	Manufacturing Lot Numbers				
		080318SP01	122018BRSP01	0129191EV01	060319BRSP01	060319BRSP01
Arsenic	<0.1 ppm	0.075	0.066	0.028	0.039	0.041
Cadmium	<0.1 ppm	0.025	0.033	0.021	0.024	0.021
Lead	<0.2 ppm	0.031	<0.010	0.015	0.012	0.016
Mercury	<0.1 ppm	<0.010	<0.010	<0.010	<0.010	<0.010

Table 5: Microbiological Analysis of Four Non-Consecutive Batches of Barley Rice Protein					
Parameter	**Specification**	**Manufacturing Lot Numbers**			
		080318SP01	**122018BRSP01**	**012919EV01**	**020419SP01**
Total plate count (CFU/g)	<30,000	5,700	6,000	4,300	7,100
Coliforms (CFU/g)	<100	<10	<10	<10	<10
Yeast/Mold (CFU/g)	<100	<10	<10	<10	<10
Salmonella (/25g)	Negative	ND	ND	ND	ND
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10
Listeria spp. (/25g)	Negative	Not tested	ND	ND	ND

V. Additional Chemical Characterization

a. Mycotoxins and Secondary Metabolites

Five non-consecutive batches of Barley Rice Protein were analyzed for the presence of mycotoxins and other secondary metabolites. The results are summarized in Table 6. All mycotoxins and secondary metabolites which were analyzed were below the limit of detection in all five batches.

Table 6: Mycotoxin and Secondary Metabolite Analysis in Five Non-Consecutive Batches of Barley Rice Protein						
Mycotoxin/ Secondary Metabolite	**Method of Analysis**	**Manufacturing Lot Numbers**				
		080318SP01	**122018BRSP01**	**0129191EV01**	**060319BRSP01**	**070819BRSP01**
Aflatoxin B1	AOAC 999.07 (modified)[2]	<5 ppb	<2 ppb	<5 ppb	<5 ppb	<5 ppb

[2] The method was modified for the use of LC-MS/MS, which improves the sensitivity of the method.

Aflatoxin B2	AOAC 999.07 (modified)	<5 ppb	<2 ppb	<5 ppb	<5 ppb	<5 ppb
Aflatoxin G1	AOAC 999.07 (modified)	<5 ppb	<2 ppb	<5 ppb	NM	<5 ppb
Aflatoxin G2	AOAC 999.07 (modified)	<5 ppb	<2 ppb	<5 ppb	NM	<5 ppb
Aflatoxins Total		<5 ppb	<4 ppb	<5 ppb	<5 ppb	<5 ppb
Ochratoxin A	AOAC 999.07 (modified)	<5 µg/kg	<5 µg/kg	<5 µg/kg	<2 µg/kg	<5 µg/kg
Total fumonisins	AOAC 92(20),496	<30 µg/kg	<30 µg/kg	<30 µg/kg	<30 µg/kg	<30 µg/kg
T-2 Toxin	LC-MS/MS	<1 µg/kg	<1 µg/kg	<1 µg/kg	<1 µg/kg	<1 µg/kg
HT-2 Toxin	LC-MS/MS	<10 µg/kg	<10 µg/kg	<10 µg/kg	<10 µg/kg	<10 µg/kg
Vomitoxin (Deoxynivalenol)	LC-MS/MS	<10 µg/kg	<10 µg/kg	<10 µg/kg	<10 µg/kg	<10 µg/kg
Zearalenone	LC-MS/MS	<5 µg/kg	<5 µg/kg	<5 µg/kg	<5 µg/kg	<5 µg/kg

LC-MS/MS = liquid chromatography tandem mass spectrometry; NM = not measured; ppb = parts per billion.

b. Amino Acid Profile

The amino acid profile of four non-consecutive batches of Barley Rice Protein was characterized using a modified AOAC 982.30 method.[3] The results of the analysis

[3] Samples are hydrolyzed with 6 N HCl for 24 hours at 110 °C. The hydrolysates are filtered and diluted for ion-exchange chromatography. Post-column derivatization with ninhydrin is performed before quantitation by UV-Vis. Proline and hydroxyproline are monitored at 440 nm, and all other amino acids are detected at 570 nm.

The following differences from the official AOAC method are noted:

1. Deionized water is used instead of sodium citrate buffer for sample dilution. While sodium citrate was originally used to improve resolution, sodium citrate is destructive to

demonstrate that the amino acid profile of Barley Rice Protein is similar to that of rice and/or barley, indicating that the manufacturing process does not significantly impact the amino acid profile of the final product from the starting material. Typical amino acid profiles from literature reports on BSG are also included for comparison. The results are provided below in Table 7.

Table 7: Amino Acid Profile of Four Non-Consecutive Batches of Barley Rice Protein

Amino Acids	***Oryza sativa* L. (g/100 g protein)** [4]	***Hordeum vulgare L.* (g/100 g protein** [5]	**Brewers Spent Grains Profile** [6]	**Barley Rice Protein (g/100 g protein)**			
				Lot No. 080318BRSP01	**Lot No. 122018SP01**	**Lot No. 012919EV01**	**Lot No. 020419SP01**
Aspartic acid	9.85	6.24	6.9	9.12	9.06	8.84	9.48
Threonine	3.86	3.4	1.9-3.4	3.61	3.65	3.74	3.8
Serine	5.45	4.22	2.8-4.4	4.13	4.52	4.39	4.58
Glutamic acid	21.46	26.13	20.1-22.3	25.64	22.98	23.70	23.38
Glycine	5.19	3.62	3.3-4.7	4.20	4.12	4.25	4.42
Alanine	6.14	3.89	4.3-7.4	4.39	4.82	4.58	4.69
Valine	6.18	4.9	4.2-6.0	5.47	5.77	5.65	5.65

HPLC equipment and was removed when it was found that chromatography met or exceeded specifications for resolution and peak shape.

2. Samples are not evaporated to remove acid. Instead, higher dilutions are used.
3. Norleucine internal standard is added before the hydrolysis step not after. This provides a better indication of sample loss during hydrolysis.
4. Samples are not refluxed during the hydrolysis step. They are sealed in capped tubes to minimize acidic fumes.

[4] Based on protein content of 7.54% as reported in USDA, *Food Data Central: Rice, brown, long-grain, raw (NDB Number 20036)*, *available at* FoodData Central (usda.gov).

[5] Based on protein content of 12.5% as reported in USDA, *Food Data Central: Barley, hulled* (*NDB Number* 20004), *available at* FoodData Central (usda.gov).

[6] Essien J.P. and Udotong I.R., *Amino acid profile of biodegraded brewers spent grains (BSG)*, 12 J APPL SCI ENVIRON MANAGE. 109-111 (2008), *available at* Microsoft Word - Udotong and Essien Nigeria.doc (bioline.org.br); Connolly A. et al., *Characterization of protein-rich isolates and antioxidate phenolic extracts from pale and black brewer' spent grain*, 48 INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY. 1670-81 (2013), *available at* https://doi.org/10.1111/ijfs.12137; Kissell L.T. and Prentice N., *Protein and fiber enrichment of cookie flour with brewer's spent grain*, 56 CEREAL CHEM. 261-66 (1979), *available at* Chem56n04_261.pdf (cerealsgrains.org).

Methionine	2.37	1.92	1.1-2.3	1.82	1.88	1.95	1.93
Isoleucine	4.46	3.65	1.5-4.2	4.00	4.40	4.05	3.98
Leucine	8.71	6.79	5.8-10.8	6.97	8.10	7.27	7.38
Tyrosine	3.95	2.87	3.2-3.7	3.99	4.15	3.97	4.17
Phenylalanine	5.44	5.61	5.8-6.2	5.88	6.61	5.68	5.64
Lysine	4.02	3.65	2.1-3.2	3.43	3.23	3.33	3.39
Histidine	2.68	2.25	2.2-3.6	2.03	2.12	2.21	2.28
Arginine	7.98	5.01	1.0-6.0	4.41	4.69	4.68	4.96
Proline	4.93	11.89	4.0-12.1	11.49	10.45	10.25	9.69
Cysteine	1.27*	2.21*	0.4-1.6	1.60	1.51	1.48	1.48
Tryptophan	1.34	1.67	2.6-5.5	1.47	1.51	1.52	1.53

*Based on cysteine content.

c. Pesticides

Three non-consecutive batches of Barley Rice Protein (Lot Nos. 012919BRSP01, 060319BRSP01, 070819BRSP01) were analyzed for standard pesticides using AOAC 2007.01. No pesticide residues were detected in any batch. The complete list of pesticides that were screened for are provided in Appendix A.

Part 3. Dietary exposure

Barley Rice Protein is intended for use in a variety of conventional food and beverage products as a source of plant protein at levels ranging from 0.5 to 90 percent. Barley Rice Protein will be used as a substitute for other sources of protein in the diet. A list of examples of the proposed food categories and use levels for Barley Rice Protein is provided in Table 8 below. Food uses are organized according to the categories in 21 C.F.R. § 170.3.

Table 8: Examples of Food Applications and Use Levels for Barley Rice Protein

Food Category (21 C.F.R. § 170.3)	**Barley Rice Protein Use Levels (%)**
Baked Goods and Baking Mixes (e.g., bread, quick breads, brownies, cookies, crackers, etc.)	0.5 to 15
Beverages and Beverage Bases [e.g., ready-to-drink energy drinks, ready-to-drink (liquid) nutritional, meal replacement, and protein beverages (non-milk-based), etc]	1 to 90
Breakfast cereals (e.g., hot breakfast cereals, ready-to eat breakfast cereals, etc.)	1 to 30
Dairy Product Analogs (e.g., non-dairy milk, non-dairy milk shakes and smoothies, non-dairy cream, etc)	1 to 50
Fats and Oils (e.g., margarine/butter-type spreads, salad dressings, etc.)	1 to 10
Grain Products and Pastas (e.g., protein bars, nutrition bars, dry rice sides, etc.)	1 to 30
Gravies and Sauces (e.g., specialty types and tomato-, milk-, and buttery-based sauces)	1 to 10
Jams and Jellies	1 to 10
Nuts and Nut Products (e.g., nut-based spreads and butters)	1 to 20
Plant Protein Products (e.g., meat analogues, plant-based spreads)	1 to 30
Snack Foods	1 to 10

Soft Candy	1 to 15
Soups and Soup Mixes	1 to 15
Sweet Sauces, Toppings, and Syrups	1 to 20

The Daily Reference Value (DRV) for protein for adults and children 4 or more years of age is 50 g/day.[7] Additionally, the Institute of Medicine (IOM) used the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 to estimate the background dietary intakes of protein for the U.S. population.[8] The mean adult protein intake ranged from 56 to 104 g/day, depending on the age group, and at the 90th percentile, adult protein intake ranged from 76 g/day to 142 g/day. Based on the data, IOM established Dietary Reference Intakes (DRIs) for protein of 56 g/day for adult males and 46 g/day for adult females.

Barley Rice Protein will be added to food products as a protein substitute and therefore is not expected to contribute any additional exposure to protein for consumers. Specifically, we do not expect that the consumption of foods containing Barley Rice Protein would result in a daily consumption greater than either the DRV or DRI for protein. Most of the population's protein intake is, and will remain, in the form of unprocessed foods, including meat, poultry, fish, and legumes.

Barley Rice Protein is not intended for use in meat or poultry products that are under the jurisdiction of the U.S. Department of Agriculture (USDA).

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7 21 C.F.R. § 101.9 (c)(7)(iii).

8 IOM, *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (2005).

Part 4. Self-limiting Levels of Use

The use of Barley Rice Protein is not self-limiting. Examples of maximum use levels in various food categories are described above.

Part 5. Experience based on common use in food before 1958

The basis of this GRAS assessment is not common use in food before 1958. However, both barley and rice grains have a long history of human consumption which provides additional support for the safety of the intended use of Barley Rice Protein as discussed further below.

Part 6. Safety Narrative: Comprehensive Discussion of the Basis of GRAS Status of Barley Rice Protein

Barley Rice Protein is derived from brewers spent grains sourced form barley and rice starting materials. Both plant sources have an extensive history of safe consumption by the global population. Barley is a widely adaptable cereal crop which contains high amounts of essential nutrients, including protein, fiber, B vitamins, niacin, and minerals.[9] It was first domesticated approximately 11,000 years ago in the Middle East[10] and worldwide production has exceeded 149 million tons in 2021.[11] Similarly, rice was domesticated approximately 9,000 years ago in China[12] and global rice production has exceeded 507 million tons in 2021.[13]

The manufacturing process for Barley Rice Protein utilizes primarily mechanical processes resulting in a minimally processed ingredient that is expected to be compositionally and nutritionally similar to native rice and barley. Therefore, while the basis of the GRAS notice is not common use in food, the ancient and widespread use of the starting materials corroborates the safety of barley and rice and their respective constituent proteins.

I. Review of Toxicology Literature

A comprehensive search of the scientific literature regarding barley, rice, and brewers spent grains (BSG) generally (not necessarily exclusively composed of barley and rice) was conducted.[14] The search did not identify any relevant safety studies on barley protein or rice protein *per se,* however the search did identify studies which examined the feeding of BSG to animals and humans. These are summarized below.

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[9] *See* USDA, *supra* n. 5.

[10] Lister DL, et al., *Barley heads east: Genetic analyses reveal routes of spread through diverse Eurasian landscapes* 13 PLOS ONE (2018), *available at* https://doi.org/10.1371/journal.pone.0196652.

[11] USDA, *Crop Explorer: Barley* (Updated 08/21), *available at* Barley Explorer (usda.gov).

[12] Molina J, et al., *Molecular evidence for a single evolutionary origin of domesticated rice* 108 PNAS 8351-8356 (2011), *available at* https://doi.org/10.1073/pnas.1104686108.

[13] USDA, *Crop Explorer: Rice* (Updated 08/21), *available at* Rice Explorer (usda.gov).

[14] Scientific literature search last conducted October 21, 2021.

a. Animal BSG Studies

No long-term feeding studies in laboratory animals were identified. However, several short-term studies utilizing BSG as a component of feed were conducted to study the effect of increased fiber content on the diet. Teixeira et al. fed Wistar rats a range of plant-based diets including one containing 15% BSG for twelve days.[15] Rats were fed *ad libitum*, but food intake was restricted to 12g total/day. The authors reported no adverse events in any treatment group and that BSG was related to increased gut microbiota diversity in rats fed BSG diets.

Kanauchi et al. fed rats a series of fiber rich diets including one containing 6% BSG.[16] Rats were fed *ad libitum* for 14 days and assessed on the final 3 days of the trials. The rats were assessed for parameters related to relief of constipation, including number and mass of defecations and jejunum mucosal protein content. BSG diets resulted in significant improvements in all assessed parameters. No observed adverse effects were noted.

b. Human BSG Studies

BSG is known to be comprised in large portion of compounds which are of high value in human nutrition, including protein, fiber, and essential minerals. Because of this fact, and its ready availability as a byproduct of the brewing process, BSG has in recent years become of interest as a value-added ingredient for human nutrition, including as a source of protein.[17] Several components of BSG, particularly fiber, have been positively associated with general health, and BSG's potential as an ingredient in human food related to these positive components has been recently extensively discussed.[18]

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[15] Teixeira C., et al., *Barley products of different fiber composition selective change microbiota composition in rats,* 62 Mol. Nutr. Food Res. 1701023 (1998), *available at* https://doi.org/10.1002/mnfr.201701023.

[16] Kanauchi O. et al., *Protein and dietary fiber rich new foodstuff from brewer's spent grain increased excretion of feces and jejunum mucosal protein content in rats,* 61 BIOSCI. BIOTECH. BIOCHEM. 29-33 (1996), *available at* https://doi.org/10.1271/bbb.61.29 and Kanauchi O. et al., *Mechanism for the increased defecation and jejunum mucosal protein content in rats by feeding germinated barley foodstuff*, 61 BIOSCI. BIOTECH. BIOCHEM. 443-448 (1997), *available at* https://doi.org/10.1271/bbb.61.443.

[17] Jaegar, A. et al., *Barley Protein Properties, Extraction and Applications, with a Focus on Brewers' Spent Grain Protein*, 10 FOODS 1389 (2021), *available at* https://doi.org/10.3390/foods10061389.

[18] Steiner J. et al., *Brewer's spent grain: source of value added polysaccharides for the food industry in reference to the health claims,* 241 EUR. FOOD RES. TECHNOL. 303-315 (2015), *available at* https://doi.org/10.1007/s00217-015-2461-7; Ikram S. et al., *Composition and Nutrient Value Proposition of Brewers Spent Grain,* 82 JOURNAL OF FOOD SCIENCE 2232-2242, *available at* https://doi.org/10.1111/1750-3841.13794; Mussatto S.I. et al., *Brewer's spent*

Additionally, a few short-term studies in which human subjects were fed BSG were identified. Studies in ileostomy patients fed 62 g BSG/day to ulcerative colitis patients with previous ileostomies for 7 days.[19] These patients have significantly decreased bowel transit times, and the studies were intended to assess the effects of high fiber diets on excreted fiber, fat, and serum lipid levels. As expected, high fiber diets (*i.e.* BSG) increased excreted fiber and fat content and decreased serum lipid levels. No adverse effects were reported. Finally, Bamba et al. examined the effects of a BSG product on patients with ulcerative colitis.[20] Patients with mild to moderate active ulcerative colitis (UC) who were unresponsive to standard treatment were given 20-30g/day germinated barley foodstuff (GBF) for 28 days. All patients exhibited clinically significant improvement of UC parameters while exhibiting no observed adverse effects.

II. Compositional Analysis

The manufacturing process for Barley Rice Protein involves primarily physical processes, in which the naturally occurring barley and rice proteins are not chemically modified. The manufacturing process involves the use of pH control agents to adjust the pH to optimize the environment for enzymatic activity. However, despite the use of these pH-control agents and enzymes, the amino acid profile of Barley Rice Protein is comparable to that of native barley and rice, confirming that the manufacturing process does not chemically alter the starting material. Comparison with literature sources which describe the amino acid composition of BSG also indicates that Barley Rice Protein amino acid profiles are in line with those found in other similar products.[21] While the available literature reports do not give information on starting grain composition (*i.e.* % barley, rice, and/or corn), and not all reports assess all of the same amino acids, they nonetheless indicate that Barley Rice Protein can be expected to provide approximately the same ratios of amino acids as would be found in barley or rice alone, or in other BSG based products.

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grain: generation, characteristics and potential applications, 43 JOURNAL OF CEREAL SCIENCE 1-14 (2006), *available at* https://doi.org/10.1016/j.jcs.2005.06.001.

19 Zhang J-E. et al., *Brewer's spent grain, serum lipids and fecal sterol excretion in human subjects with ileostomies*, 121 J. NUTR. 778-784 (1991), *available at* https://doi.org/10.1093/jn/121.6.778; Aman P. et al., *Excretion and degradation of dietary fiber constituents in ileostomy subjects consuming a low fiber diet with and without brewer's spent grain*, 124 J. NUTR. 359-363 (1994), *available at* https://doi.org/10.1093/jn/124.3.359.

20 Bamba T., et al., *A new prebiotic from germinated barley for nutraceutical treatment of ulcerative colitis*, 17 JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY 818-824 (2002), *available at* https://doi.org/10.1046/j.1440-1746.2002.02709.x.

21 *See* Table 7: Amino Acid Profile of Four Non-Consecutive Batches of Barley Rice Protein.

The manufacturing process does not include any steps that would introduce a deleterious substance into the final product. Rather, the production process employs microfiltration and diafiltration steps that will remove any chemical or microbiological impurities, as well as the residues of the heat-deactivated enzymes. Analytical data for several representative batches of Barley Rice Protein demonstrate that the microbiological contaminants and heavy metals (arsenic, cadmium, lead and mercury) remain within the specified and/or acceptable levels. Specifically, the maximum level of heavy metals permitted by the specifications for Barley Rice Protein is beneath the heavy metal levels included in a number of GRAS notices for other plant protein products which have received “no questions” responses from FDA. These GRAS notices, and the heavy metals limits specified therein, are summarized in the table below.

Table 9: Heavy Metal Specification Comparison

	Estimated Dietary Exposure	Arsenic (mg/kg)	Cadmium (mg/kg)	Lead (mg/kg)	Mercury (mg/kg)
Barley Rice Protein (current GRN)	Not greater than 50 g/person/day expected (protein DRV)	<0.1	<0.1	<0.2	<0.1
GRN 851 (pea protein)	Not greater than 50 g/person/day expected (protein DRV)	<0.2	<0.2	<0.2	<0.2
GRN 803 (pea protein)	Not greater than 50 g/person/day expected (protein DRV)	≤0.2	≤0.2	≤0.5	≤0.2
GRN 804 (pea protein)	51.62 g/person/day (90^{th} percentile)	<0.1	<0.2	<0.2	<0.1
GRN 788 (pea protein concentrate)	17.3 g/person/day (90^{th} percentile)[22]	<0.1	<0.3	<0.1	<0.02
GRN 608 (pea protein concentrate)	17.3 g/person/day (90^{th} percentile)	<0.25	<0.85	<0.5	<0.1

22 We note that even though the estimated exposure to the ingredient is approximately one third of the protein DRV of 50 g, the cadmium specification is three times as high. Similar reasoning applies to the other GRAS Notices referenced in this table with estimated dietary exposures of less than 50 g.

GRN 609 (rice protein)	17.3 g/person/day (90^{th} percentile)	≤0.2	≤0.3	<0.25	<0.045
GRN 771 (hemp seed protein)	13.8 g/person/day (90^{th} percentile)	≤1	≤1	≤3	≤0.1
GRN 386 (canola protein isolate and hydrolyzed canola protein isolate)	Not greater than 50 g/person/day expect (protein DRV)	<0.05 measured across all products[23]	Maximum of 0.28 measured across all products	Maximum of 0.04 measured across all products	Maximum of 0.012 measured across all products

III. Nutritional Considerations

a. Anti-Nutritional Factors

Grains such as rice and barley are reported to contain anti-nutritional factors, such as trypsin inhibitors,[24] phytic acid,[25] and phytohaemagglutinins[26] that may inhibit the absorption of vitamins, minerals, and other nutrients. The manufacturing process includes microfiltration and diafiltration processing steps that would likely remove any natural toxins and antinutritional factors that may be present in the starting materials. Considering that the starting materials are barley and rice grains obtained from the mash step of beer brewing, during which the grains are subject to milling and germination, it is likely these steps significantly reduce the antinutrient

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[23] No heavy metal specifications were established, although product was tested for the presence of heavy metals.

[24] Mikola J, Suolinna E-M, *Purification and properties of a trypsin inhibitor from barley*, 9(4) EUR J BIOCHEM 555-560 (1969), *available at* https://doi.org/10.1111/j.1432-1033.1969.tb00645.x; Tashiro M, Maki Z, *Partial purification and some properties of a trypsin inhibitor from rice bran*, 42(6) AGRIC BIOL CHEM 1119-1124 (1978), *available at* https://doi.org/10.1080/00021369.1978.10863122.

[25] FAO, *Amino Acid Content of Foods and Biological Data on Proteins* (1970), *available at* AMINO-ACID CONTENT OF FOODS AND BIOLOGICAL DATA ON PROTEINS (fao.org); Hidvégi M, Lásztity R, *Phytic acid content of cereals and legumes and interaction with proteins*, 46 PERIODICA POLYTECHNICA CHEM ENG 59-64 (2002), *available at* Periodica Polytechnica Chemical Engineering (bme.hu).

[26] FAO (1970), *supra* note 25.

content of grains, in particular phytic acid.[27] Furthermore, other processing steps, such as thermal processing will also result in further reduction of heat-labile antinutrients, such as protease inhibitors.[28] Therefore, it is not expected that Barley Rice Protein will contain any antinutritional factors at levels that would be of safety concern.

b. *In Vitro* and *In Vivo* Protein Digestibility

The structure and biological activity of most proteins is lost during the digestion process that occurs along the gastrointestinal tract.[29] This is likely due to the denaturation and degradation processes that occur. Proteins that are readily digested by these processes are less likely to pose safety concern compared to those that are resistant to digestion. Proteins that are resistant to proteolytic digestion can reach the intestinal mucosa, where they are absorbed into the systemic circulation, and may elicit an immune (allergenic) response.[30]

The protein digestibility of Barley Rice Protein was investigated in Sprague-Dawley rats.[31] Groups of male Sprague-Dawley rats (body weight 55 to 66 g; 4 to 6/group) were provided diets containing casein, protein-free, or Barley Rice Protein at levels of 15 g/day for

27 Gupta RK et al., *Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains*, 52 J FOOD SCI TECHNOL 676-684 (2015), *available at* https://doi.org/10.1007/s13197-013-0978-y.

28 Liener IE, *Plant antinutritional factors. Detoxification*, *in* ENCYCLOPEDIA OF FOOD SCIENCES AND NUTRITION, 2ND EDITION 4587-4593 (Trugo L, Finglas PM, ed. 2003), *available at* https://doi.org/10.1016/B0-12-227055-X/00936-6.

29 Delaney B et al., *Evaluation of protein safety in the context of agricultural biotechnology*, 46 FOOD CHEM TOXICOL S71-S97 (2008), *available at* https://doi.org/10.1016/j.fct.2008.01.045.

30 Toomer OT et al., *Effect of simulated gastric and intestinal digestion on temporal stability and immunoreactivity of peanut, almond, and pine nut protein allergens*, 61 J AGRIC FOOD CHEM 5903-5913 (2013), *available at* https://doi.org/10.1021/jf400953q.

31 Gallaher DD [unpublished]. *Protein Quality and Effect on Growth and Organ Weight of BereteinTM, a Protein Isolate from Barley and Rice* (2019) [Unpublished]. Prepared by Professor Gallaher, St. Paul (MN): University of Minnesota, Department of Food Science and Nutrition for Eden Prairie (MN): Zea10, LLC. While this study (the "Gallaher study") is unpublished, it merely corroborates the published literature on the digestibility of barley and rice and is not critical to the GRAS conclusion. Furthermore, it does not provide any information that would undermine the GRAS conclusion. Thus, qualified experts could conclude that Barley Rice Protein is GRAS without reviewing the Gallaher study.

9 days. Feces were collected on the last 5 days and analyzed for nitrogen content to determine the true fecal digestibility of the proteins in the diet. The authors reported the true fecal digestibility of the Barley Rice Protein to be 86.0±0.3% and 98.3±0.6% for casein.

Cooked and uncooked barley and rice were investigated in an *in vitro* digestibility model.[32] The grains were cooked by soaking for 12 hours at 4°C in water with or without 2-mercaptoethanol, and then stirred in a boiling water bath for 20 minutes. The digestibility was measured by suspending the cooked or uncooked samples in buffer containing pepsin or trypsin/chymotrypsin and incubated for 2 hours at 37°C. The *in vitro* protein digestibility of uncooked barley and rice were reported to be 93.2% and 91.1%, respectively, while the digestibility of cooked barley and rice were reported to be 80.2% and 82.1%, respectively. Further, in another study, the true digestibility of brown rice and barley were reported to be 99.7% and 88%,[33] suggesting that the rice and barley protein are readily digestible *in vivo*.

In a study investigating the protein quality of barley and rice, large White gilts (body weight 50±3.5 kg; N=6/group) were fitted with ileal T-cannulas and allowed to recovery for 14 days, and allocated to an experimental diet containing either barley or rice at concentrations of 972 g/kg feed or 964 g/kg feed respectively.[34] Animals were provided the test diets for 7 days, and ileal digesta and feces were collected on the last day of the study period. The authors reported true fecal digestibility of crude protein of 95.5±3.2% in the rice group and 86.5±2.6 in the barley group. The standardized ileal digestibility for rice and barley were reported to be 92.9±2.1% and 80.7±3.6%, respectively. The results of this study are consistent with the digestibility of brown rice and barley reported by the FAO (1999) and Hamaker et al.[35]

In another study investigating the apparent ileal digestibility and standardized ileal digestibility of cereal grains, including dehulled barley and polished white (Jasmine) rice, growing barrow pigs (body weight 30.2±3.2 kg; N=9/group) were provided dehulled barley or polished white rice as a protein source for 14 days[36] Ileal digesta was collected on study days

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32 Hamaker BR et al., *Improving the in vitro protein digestibility of sorghum with reducing agents*, 84(3) PROC NATL ACAD SCI USA 626-628 (1987), https://doi.org/10.1073/pnas.84.3.626, *available at* https://doi.org/10.1073/pnas.84.3.626.

33 FAO, *Nutritional quality of cereals*, FERMENTED CEREALS: A GLOBAL PERSPECTIVE (FAO Agricultural Services Bulletin No. 138) (1999), *available at* http://www.fao.org/3/x2184e/x2184e05.htm.

34 Nitrayová, et al., *Comparison of two methods of protein quality evaluation in rice, rye and barley as food protein sources in human nutrition*, 12 Potravinarstvo 762-766 (2018), *available at* http://dx.doi.org/10.5219/991.

35 *See supra* notes 32 and 33.

36 Cervantes-Pahm et al., *Digestible indispensable amino acid score and digestible amino acids in eight cereal grains*, 111(9) Br J Nutr 1663-1672 (2014), *available at* https://doi.org/10.1017/s0007114513004273.

13 and 14 for 10 hours. The apparent ileal digestibility of dehulled barley and polished white rice were reported to be 61.50% and 70.59%, respectively, while the standardized ileal digestibility was reported to be 74.49% and 88.07%, respectively.

Based on the available *in vitro* and *in vivo* data, it is anticipated that Barley Rice Protein would be readily digested, yielding individual amino acids and small peptides that would be absorbed and handled by the body in normal metabolic processes, similar to that of other dietary protein sources.

c. Nutritional Value and Protein Quality Evaluation

Evaluation of protein quality is intended to determine the capacity of food protein sources to meet protein and essential amino acid requirements and to satisfy metabolic requirements for amino acids and nitrogen. Several methods are commonly used to assess the quality and nutritional value of a protein, including the protein efficiency ratio, net protein utilization, biological value, protein digestibility corrected amino acid score, and digestible indispensable amino acid score.

Considering that Barley Rice Protein is minimally processed and based on the similarities of the amino acid profile of the barley and rice protein with that of native barley and rice (see Part 2,V,b), Barley Rice Protein is compositionally and nutritionally similar to the protein component of the native starting materials.

1. Protein Efficiency Ratio

The protein efficiency ratio (PER) is the amount of weight gain per gram of protein consumed using rats from a single strain, fed isonitrogenous diets of the protein to be examined or casein for 28 days. The PER of casein is commonly set to 2.5 and is used as a reference value. For rice and barley, the PER values of 1.5 and 1.7 were reported, respectively.[37]

2. Biological Value

The biological value (BV) of a protein represents the proportion of ingested protein that is absorbed and utilized. FAO reported average BV of 74% and 70% for rice and barley, respectively,[38] which is comparable with the net protein utilization values for rice and barley (see below).

3. Protein Ratio and Net Protein Utilization

The net protein ratio (NPR) or net protein utilization (NPU) values are measures of the percentage of amino acids consumed that are converted into proteins and utilized by the body.

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37 Canadian Food Inspection Agency (CFIA), *Elements within the Nutrition Facts Table*, *available at* Elements within the Nutrition Facts table - Canadian Food Inspection Agency (canada.ca).

38 FAO, *supra* note 33.

The NPR is calculated by the mean weight gain of the animals given the test diet plus the average weight loss of animals given the basal, protein-free diet, divided by the mean amount of protein consumed by the animals given the test diet. In comparison, the NPU is calculated based on the amount of protein absorbed and available for metabolic use by multiplying the BV of the test protein by its digestibility and dividing by 100. FAO reported NPU values of approximately 74% and 62% for rice and barley, respectively, suggesting that approximately 74% or 62% of rice protein or barley protein consumed are absorbed and retained, and, in theory, utilized within the body.[39]

4. *FAO Recommended Amino Acid Scoring Patterns Compared*

A comparison of the amino acid profile of Barley Rice Protein with the recommended scoring patterns proposed by FAO for various population groups, including infants (birth to 6 months), children (6 months to 3 years), older child and adolescent (4 to 18 years), and adults (>18 years) are presented in Table 10.[40] Based on the amino acid profile of Barley Rice Protein, with the exception of lysine content, Barley Rice Protein meets or exceeds the amino acid requirements of children (6 months to 3 years) and older children/adolescents (4 to 18 years) and adults (>18 years).

Table 10: Comparison of the Recommended Amino Acid Scoring Patterns for Various Population Groups with the Amino Acid Profile of Barley Rice

Essential Amino Acids (mg/g protein)	Barley Rice Protein	Infant (Birth to 6 Months)	Child (6 Months to 3 Years)	Older Child and Adolescent (4 to 18 Years), and Adult (>18 Years)
Histidine	21.6	21	20	16
Isoleucine	41.1	55	32	30
Leucine	74.3	96	66	61
Lysine	33.5	69	57	48
Methionine + Cysteine	34.1	33	27	23

39 *Id.*

40 FAO, *Dietary Protein Quality Evaluation in Human Nutrition. Report of an FAO Expert Consultation* (*FAO Food and Nutrition Paper 92*) (2013), *available at* Dietary protein quality evaluation in human nutrition (fao.org).

Phenylalanine + Tyrosine	100.2	94	52	41
Threonine	37.0	44	31	25
Tryptophan	15.1	17	8.5	6.6
Valine	56.4	55	43	40

5. *Protein Digestibility-Corrected Amino Acid Score*

The PDCAAS rating proposed by the FAO and adopted by the U.S. FDA as "the preferred best" method to evaluate protein quality is a measure of the bioavailability of nutritionally essential amino acids from a given protein source in comparison to a reference protein.[41] The PDCAAS approach to evaluating protein quality is based on the principle that the nutritive value of a protein is dependent on its capacity to provide nitrogen and amino acids in sufficient amounts to meet the essential amino acid requirements of humans. The quality of some proteins can be assessed directly using amino acid score values, while others cannot due to poor digestibility and/or bioavailability. The PDCAAS approach utilizes both the amino acid composition of a protein and its digestibility profile to accurately predict the protein quality of foods for human diets. The PDCAAS rating relates the content of the first limiting essential amino acid of the protein of interest (*i.e.,* Barley Rice Protein) to the content of the same amino acid in a reference pattern of essential amino acids (*i.e.,* amino acid score), corrected for fecal digestibility, which is often measured using a rat balance assay. As shown in Table 11, Barley Rice Protein has a balanced amino acid composition, with the limiting amino acid being lysine.

Table 11: Calculation of Amino Acid Scores for Barley Rice Protein

Essential Amino Acids	Barley Rice Protein (mg/g protein)[42]	Older Child and Adolescent (4 to 18 Years), and Adult (>18 Years) (mg/g protein)[43]	Calculated Amino Acid Scores Using FAO Reference Requirements
Histidine	21.6	16	1.4

[41] FAO/WHO, *Protein Quality Evaluation. Report of Joint FAO/WHO Expert Consultation* (*FAO Food and Nutrition Paper 51*) (1991), *available at* Wayback Machine (archive.org); 58 Fed. Reg. 20792205 (Jan. 6, 1993).

[42] Based on the averages of amino acid values provided in Table 7.

[43] Reference amounts determined by FAO (2013). *Supra* note 40

Isoleucine	41.1	30	1.4
Leucine	74.3	61	1.2
Lysine	33.5	48	0.7
Methionine + Cysteine	34.1	23	1.5
Phenylalanine + Tyrosine	100.2	41	2.4
Threonine	37.0	25	1.5
Tryptophan	15.1	6.6	2.3
Valine	56.4	40	1.4

The PDCAAS rating can be calculated as follows:[44]

PDCAAS (%) = (mg of limiting amino acid in 1 g of test protein ÷ mg of same amino acid in 1 g of reference protein) × fecal digestibility × 100%

Considering the reported digestibility of 86% for the Barley Rice Protein, using the above formula, a PDCAAS value of 60% was calculated for Barley Rice Protein. The calculated PDCAAS values for rice and barley were reported to be 81% and 61%, respectively[45] In comparison, the PDCAAS score for the gold standard protein, casein, is 100%. Overall, this data suggests that Barley Rice Protein is of comparable protein quality to barley, although results may vary depending on the starting grain composition of the Barley Rice Protein.[46]

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44 The reference protein pattern of essential amino acids is based on the amino acid requirements of 2- to 5-year-old preschool-aged children as determined by FAO/WHO/UNU, *Energy and Protein Requirements. Report of a Joint FAO/WHO/UNU Expert Consultation (WHO Technical Report Series 724)* (1985), *available at* WHO_TRS_724_(chp1-chp6).pdf.

45 Nitrayová, et al., *supra* note 34.

46 As noted above, batch analysis (including amino acid composition) was conducted on batches containing between 60 and 70% barley and between 30 and 40% rice.

6. *Digestible Indispensable Amino Acid Score*

More recently, FAO has recommended the Digestible Indispensable Amino Acid Score (DIAAS) as the preferred method for assessing the quality of dietary proteins on the basis that it provides a more accurate measure for digestion of amino acids rather than the crude protein levels measured by PDCAAS. PDCAAS rates protein sources against the amino acid reference pattern of a 2- to 5-year-old child, whereas DIAAS differentiates between the needs of infants and children with 3 reference patterns: 0 to 6 months, 6 months to 3 years, and greater than 3 years of age.[47]

Accordingly, the DIAAS method was used to estimate the protein quality of Barley Rice Protein. DIAAS defines protein quality by the amino acid with the lowest ratio of digestible indispensable amino acid (IAA) in 1 g of the protein to the amount of the same IAA in 1 g of the reference protein. Accordingly, the formula for calculating the protein quality of Barley Rice Protein would be:

DIAAS (%) = (mg of digestible IAA in 1 g of barley Rice Protein ÷ mg of the same IAA in 1 g of the reference protein) × 100%

The digestible IAA is the amount of the amino acid in Barley Rice Protein (in mg per 1 g of protein) adjusted by the true ileal IAA digestibility. The calculation of digestible IAA in Barley Rice Protein is provided in Table 12.

Table 12: Calculation of Digestible Indispensable Amino Acid Score of Barley Rice Protein

IAA	**IAA Content in Barley Rice Protein (mg AA/g protein)[48]**	**True Ileal Digestibility of IAA in Dehulled Barley (%)[49]**	**Digestible IAA in Barley Rice Protein (%)[50]**
Histidine	21.60	81.18	17.5
Isoleucine	41.08	76.44	31.4
Leucine	74.30	79.24	58.9
Lysine	33.45	73.34	24.7
Methionine + Cysteine	34.13	79.06	27.0

47 FAO (2013), *supra* note 40.

48 Again, these are based on the averages of amino acid values provided in Table 7.

49 Based on the true ileal digestibility of IAA in dehulled barley provided in Cervantes-Pahm, et al., *supra* note 36.

50 Calculated by multiplying "IAA Content in Barley Rice Protein" by "True Ileal Digestibility of IAA in Dehulled Barley" (*i.e.*, column 2 × column 3).

Phenylalanine + Tyrosine	100.23	79.52	79.7
Threonine	37.00	71.78	26.6
Tryptophan	15.08	84.11	12.7
Valine	56.35	77.16	43.5

The amount of IAA in the reference protein is also known as the amino acid pattern of the reference protein and is based on the amino acid requirements of the different age groups. The calculation of DIAAS of Barley Rice Protein based on the FAO recommended amino acid scoring patterns of different age groups is provided in Table 13.

Table 13: Calculation of Digestible Indispensable Amino Acid Score of Barley Rice Protein

IAA	**Digestible IAA in Barley Rice Protein (%)[51]**	**IAA in the Reference Protein (mg IAA per g of protein)[52]**			**DIAAS (%)[53]**		
		Infant	**Child**	**Older Children**	**Infant**	**Child**	**Older Child**
Histidine	17.5	21	20	16	83.5	87.7	109.6
Isoleucine	31.4	55	32	30	57.1	98.1	104.7
Leucine	58.9	96	66	61	61.3	89.2	96.5
Lysine	**24.7**	**69**	**57**	**48**	**35.7**	**43.3**	**51.4**
Methionine + Cysteine	27.0	33	27	23	81.8	99.9	117.3
Phenylalanine + Tyrosine	79.7	94	52	41	84.8	153.3	194.4
Threonine	26.6	44	31	25	60.4	85.7	106.2
Tryptophan	12.7	17	8.5	6.6	74.6	149.2	192.1
Valine	43.5	55	43	40	79.1	101.1	108.7

As presented in Table 13, and consistent with the PDCAAS analysis, lysine is the limiting digestible IAA present in Barley Rice Protein. The DIAAS of Barley Rice Protein is

51 Based on the digestible IAA score in Barley Rice Protein as calculated in Table 12.

52 Based on the amino acid scoring pattern recommended by FAO. Amino acid scoring patterns for "infant," "child," and "older child" represents the amino acid requirements of 0 to 6 months, 6 months to 3 years of age, and 3 to 10 years of age, respectively. FAO (2013), *supra* note 38. The amino acid scoring pattern for older children is used to estimate the DIAAS of adolescents and adults.

53 Calculated by dividing "Digestible IAA in Barley Rice Protein" by "IAA in the Reference Protein" (*i.e.*, dividing column 2 by column 3).

calculated as 35.7%, 43.3%, and 51.4% for infants, children, and older children, adolescents, or adults, respectively. In comparison, Nitrayová, et al.[54] reported DIAAS values of 55% for barley and 79% for rice, while Cervantes-Pahm, et al. [55] reported DIAAS values of 51% for dehulled barley and 64% for polished white (Jasmine) rice.

IV. Allergenicity

Barley and rice are not listed as major allergens that are subject to allergenicity labeling under the Food Allergen Labeling and Consumer Protection Act (FALCPA). Furthermore, it is generally accepted that allergy to barley itself is rare although there have been case reports of allergic responses to barley exposure.[56] Likewise, the available information in the scientific literature indicates that allergy to rice is rare, and that its frequency, which can vary among populations, is lower in Western countries.[57] Therefore, despite the high levels of consumption of barley and rice globally, reported allergic reactions to both barley and rice protein are rare.

Products containing Barley Rice Protein will declare the presence of the barley and rice proteins in the ingredient list, thus allowing consumers to self-regulate. Therefore, while the potential exists for individuals with sensitivity to rice or barley protein to have an adverse response to consumption of products containing Barley Rice Protein, such reactions are expected to be very rare and pose an insignificant risk.

V. Summary Regarding the Safety and GRAS Status of Barley Rice Protein

Barley Rice Protein is a source of protein that is derived from brewers spent grains sourced from barley and rice, both of which has a long history of worldwide consumption. The manufacturing process is largely mechanical and results in a product that has a similar amino acid composition to the constituent grains (barley and rice) and literature reports for BSG. No adverse effects from the consumption of barley, rice, or brewers spent grains generally have been identified in the literature review. Barley Rice Protein is not expected to increase overall

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54 *Supra* note 34.

55 *Supra* note 36.

56 James JM et al., *Respiratory reactions provoked by double-blind food challenges in children*. 149 AM J RESPIR CRIT CARE MED 59-64 (1994); Räsänen L et al., *Allergy to ingested cereals in atopic children* 49 ALLERGY 871-876 (1994), *available at* https://doi.org/10.1164/ajrccm.149.1.8111598; Jones SM et al., *Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity,* 96 J ALLERGY CLIN IMMUNOL 341-351 (1995), *available at* https://doi.org/10.1016/s0091-6749(95)70053-6.

57 Jeon YH et al., *Identification of major rice allergen and their clinical significance in children*, 54 KOREAN J PEDIATR 414-421 (2011), *available at* https://dx.doi.org/10.3345%2Fkjp.2011.54.10.414.

exposure to protein and instead will serve as a replacement for other existing sources of plant protein. It therefore can be concluded that the intended use of Barley Rice Protein is as a source of protein is GRAS.

Part 7. References

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Appendix A

List of Screened Pesticides
(No Residues Detected)

PQA10 Pesticides - Spice, Herbs, Botanicals		
QA05Q Pesticides (Spice, Botanical, GC-MSMS)		
Acephate - (7300A146)	0.05 mg / kg	mg / kg
Acetochlor - (7300A479)	0.01 mg / kg	mg / kg
Aclonifen - (7300S041)	0.02 mg / kg	mg / kg
Acrinathrin - (7300A468)	0.01 mg / kg	mg / kg
Alachlor - (7300G008)	0.01 mg / kg	mg / kg
Aldrin - (7300S043)	0.01 mg / kg	mg / kg
Allethrin - (7300G006)	0.02 mg / kg	mg / kg
Ametryn - (7300A149)	0.02 mg / kg	mg / kg
Atrazine - (7300A152)	0.01 mg / kg	mg / kg
Azaconazole - (7300A663)	0.01 mg / kg	mg / kg
Azinphos-ethyl - (7300A153)	0.02 mg / kg	mg / kg
Azinphos-methyl - (7300S048)	0.02 mg / kg	mg / kg
Benalaxyl - (7300S050)	0.01 mg / kg	mg / kg
Benfluralin - (7300A156)	0.01 mg / kg	mg / kg
BHC, alpha- - (RC000006)	0.01 mg / kg	mg / kg
BHC, beta- - (RC000007)	0.01 mg / kg	mg / kg
BHC, delta- - (RC000009)	0.01 mg / kg	mg / kg
Bifenox - (7300A158)	0.01 mg / kg	mg / kg
Bifenthrin - (7300S054)	0.01 mg / kg	mg / kg
Bromacil - (7300S057)	0.02 mg / kg	mg / kg
Bromocyclen - (7300A162)	0.02 mg / kg	mg / kg
Bromophos - (7300A164)	0.01 mg / kg	mg / kg
Bromophos-ethyl - (7300A163)	0.01 mg / kg	mg / kg
Bromopropylate - (7300A165)	0.01 mg / kg	mg / kg
Butylate - (7300G014)	0.05 mg / kg	mg / kg
Captafol - (7300A168)	0.1 mg / kg	mg / kg
Captan - (7300A169)	0.1 mg / kg	mg / kg
Carbophenothion - (7300A373)	0.01 mg / kg	mg / kg
Carbophenothion-methyl - (7300G179)	0.01 mg / kg	mg / kg
Chlordane, cis- - (7300S002)	0.01 mg / kg	mg / kg
Chlordane, oxy- - (7300G168)	0.01 mg / kg	mg / kg
Chlordane, trans- - (7300S003)	0.01 mg / kg	mg / kg
Chlordene, gamma- - (SA000251)	0.01 mg / kg	mg / kg
Chlordimeform - (7300A174)	0.01 mg / kg	mg / kg
Chlorethoxyfos - (SF0001C5)	0.02 mg / kg	mg / kg
Chlorfenapyr - (7300A547)	0.05 mg / kg	mg / kg
Chlorfenson - (7300A175)	0.01 mg / kg	mg / kg
Chlorobenzilate - (7300A375)	0.01 mg / kg	mg / kg
Chloroneb - (7300A179)	0.01 mg / kg	mg / kg
Chloropropylate - (7300G099)	0.01 mg / kg	mg / kg
Chlorothalonil - (7300A180)	0.01 mg / kg	mg / kg
Chlorpropham (CIPC) - (7300A181)	0.01 mg / kg	mg / kg
Chlorpyrifos-ethyl - (7300S081)	0.01 mg / kg	mg / kg

Chlorpyrifos-methyl - (7300A183)	0.01 mg / kg	mg / kg
Chlorthal-dimethyl - (7300J007)	0.01 mg / kg	mg / kg
Chlorthiofos - (7300A380)	0.01 mg / kg	mg / kg
Chlorthion - (7300A185)	0.02 mg / kg	mg / kg
cis-Chlordane - (7300G016)	0.01 mg / kg	mg / kg
Coumaphos - (7300A187)	0.01 mg / kg	mg / kg
Crotoxyphos - (7300G023)	0.01 mg / kg	mg / kg
Cyanazine - (7300A188)	0.01 mg / kg	mg / kg
Cyanofenphos - (7300A190)	0.01 mg / kg	mg / kg
Cyfluthrin - (7300S094)	0.01 mg / kg	mg / kg
Cyhalothrin lambda- - (7300A553)	0.01 mg / kg	mg / kg
Cyhalothrin, gamma- - (SA000197)	0.01 mg / kg	mg / kg
Cypermethrin - (7300A195)	0.01 mg / kg	mg / kg
Dacthal (DCPA) - (SA000258)	0.01 mg / kg	mg / kg
DDD, o,p- - (7300S099)	0.01 mg / kg	mg / kg
DDD, p,p'- - (7300S100)	0.01 mg / kg	mg / kg
DDE, o,p- - (7300S101)	0.01 mg / kg	mg / kg
DDE, p,p'- - (7300A406)	0.01 mg / kg	mg / kg
DDT, o,p'- - (7300A403)	0.01 mg / kg	mg / kg
DDT, p,p'- - (7300S103)	0.01 mg / kg	mg / kg
DEF - (SP000012)	0.01 mg / kg	mg / kg
Deltamethrin - (7300A199)	0.01 mg / kg	mg / kg
Demeton-S - (7300G029)	0.01 mg / kg	mg / kg
Demeton-S-methyl - (7300G028)	0.01 mg / kg	mg / kg
Dialifos - (7300A382)	0.01 mg / kg	mg / kg
Diallate - (7300G030)	0.02 mg / kg	mg / kg
Diazinon - (B001B097)	0.01 mg / kg	mg / kg
Dichlobenil - (7300A202)	0.01 mg / kg	mg / kg
Dichlofenthion - (7300A203)	0.01 mg / kg	mg / kg
Dichlofluanid - (7300A204)	0.01 mg / kg	mg / kg
Dichlone - (B001B287)	0.01 mg / kg	mg / kg
Dichlorvos - (7300A206)	0.01 mg / kg	mg / kg
Diclobutrazol - (7300A207)	0.01 mg / kg	mg / kg
Diclofop-methyl - (7300A385)	0.01 mg / kg	mg / kg
Dicloran - (7300A205)	0.01 mg / kg	mg / kg
Dicofol - (SA000160)	0.01 mg / kg	mg / kg
Dicrotophos - (7300G032)	0.05 mg / kg	mg / kg
Dieldrin - (B001B088)	0.01 mg / kg	mg / kg
Dimethachlor - (7300A386)	0.01 mg / kg	mg / kg
Dimethenamid - (7300A487)	0.01 mg / kg	mg / kg
Dioxabenzofos - (7300G189)	0.01 mg / kg	mg / kg
Dioxathion - (7300A387)	0.01 mg / kg	mg / kg
Diphenyl - (7300S006)	0.01 mg / kg	mg / kg
Diphenylamine - (7300A212)	0.01 mg / kg	mg / kg
Disulfoton - (7300A213)	0.01 mg / kg	mg / kg

Disulfoton-PS-sulfone - (7300G190)	0.01 mg / kg	mg / kg
Endosulfan I (alpha-endosulfan) - (7300J008)	0.01 mg / kg	mg / kg
Endosulfan II (beta-Endosulfan) - (7300J009)	0.01 mg / kg	mg / kg
Endosulfan sulphate - (7300A388)	0.01 mg / kg	mg / kg
Endrin - (7300S127)	0.01 mg / kg	mg / kg
Endrin ketone - (7300G116)	0.01 mg / kg	mg / kg
Endrin-aldehyde - (7300G115)	0.01 mg / kg	mg / kg
EPN - (7300S128)	0.01 mg / kg	mg / kg
EPTC - (7300G039)	0.02 mg / kg	mg / kg
Esfenvalerate - (7300A430)	0.01 mg / kg	mg / kg
Ethalfluralin - (7300G041)	0.01 mg / kg	mg / kg
Ethion - (B001B100)	0.01 mg / kg	mg / kg
Ethoprophos - (7300A220)	0.01 mg / kg	mg / kg
Etoxazole - (7300S134)	0.01 mg / kg	mg / kg
Etridiazole - (7300G043)	0.01 mg / kg	mg / kg
Etrimfos - (7300A222)	0.01 mg / kg	mg / kg
Famophos - (7300G182)	0.01 mg / kg	mg / kg
Fenamidone - (Z001AX04)	0.01 mg / kg	mg / kg
Fenchlorphos - (7300A224)	0.01 mg / kg	mg / kg
Fenfluthrin - (7300G175)	0.01 mg / kg	mg / kg
Fenitrothion - (7300A225)	0.01 mg / kg	mg / kg
Fenpropathrin - (7300S144)	0.01 mg / kg	mg / kg
Fenson - (7300A227)	0.01 mg / kg	mg / kg
Fensulfothion - (7300A228)	0.01 mg / kg	mg / kg
Fenthion - (7300A229)	0.01 mg / kg	mg / kg
Fenvalerate - (7300A230)	0.01 mg / kg	mg / kg
Flamprop-methyl - (7300A481)	0.01 mg / kg	mg / kg
Fluazifop-P-butyl - (7300S150)	0.01 mg / kg	mg / kg
Fluchloralin - (7300A232)	0.01 mg / kg	mg / kg
Fluchloralin (Basalin) - (SA000172)	0.01 mg / kg	mg / kg
Flucythrinate - (7300G176)	0.01 mg / kg	mg / kg
Fluotrimazole - (7300S154)	0.01 mg / kg	mg / kg
Fluquinconazole - (7300S155)	0.01 mg / kg	mg / kg
Flutriafol - (7300A460)	0.01 mg / kg	mg / kg
Folpet - (7300S158)	0.1 mg / kg	mg / kg
Fonofos - (7300A235)	0.01 mg / kg	mg / kg
Formothion - (7300A236)	0.01 mg / kg	mg / kg
Halfenprox - (7300S299)	0.01 mg / kg	mg / kg
Heptachlor - (7300H032)	0.01 mg / kg	mg / kg
Heptachlor epoxide - (B001B086)	0.01 mg / kg	mg / kg
Hexachlorobenzene (HCB) - (B001B081)	0.01 mg / kg	mg / kg
Hexazinone - (7300G047)	0.01 mg / kg	mg / kg
Hydroprene - (QA000027)	0.02 mg / kg	mg / kg
Iprobenfos - (7300G187)	0.01 mg / kg	mg / kg
Iprodione - (7300A241)	0.05 mg / kg	mg / kg

Isazophos - (7300S176)	0.01 mg / kg	mg / kg
Isocarbamid - (7300G057)	0.01 mg / kg	mg / kg
Isocarbofos - (7300S293)	0.01 mg / kg	mg / kg
Isodrin - (7300A242)	0.01 mg / kg	mg / kg
Isofenphos-methyl - (SP000002)	0.01 mg / kg	mg / kg
Isopropalin - (7300G075)	0.01 mg / kg	mg / kg
Isoprothiolane - (7300A664)	0.01 mg / kg	mg / kg
Isoxadifen-ethyl - (Z001AS8T)	0.01 mg / kg	mg / kg
Lenacil - (7300A250)	0.01 mg / kg	mg / kg
Leptophos - (7300A251)	0.01 mg / kg	mg / kg
Lindane (gamma-HCH) - (7300S186)	0.01 mg / kg	mg / kg
Malathion - (7300S187)	0.01 mg / kg	mg / kg
Mefenpyr-diethyl - (7300A617)	0.01 mg / kg	mg / kg
Mepronil - (Z001AS00)	0.01 mg / kg	mg / kg
Merphos (Tribufos) - (SA000208)	0.01 mg / kg	mg / kg
Metazachlor - (7300S190)	0.01 mg / kg	mg / kg
Methacriphos - (7300A257)	0.01 mg / kg	mg / kg
Methamidophos - (7300A256)	0.1 mg / kg	mg / kg
Methoprothryn - (7300S196)	0.01 mg / kg	mg / kg
Methoxychlor - (B001B093)	0.01 mg / kg	mg / kg
Mevinphos - (7300A264)	0.01 mg / kg	mg / kg
MGK-264 - (SA000363)	0.01 mg / kg	mg / kg
Mirex - (B001B092)	0.01 mg / kg	mg / kg
Monocrotophos - (7300G049)	0.01 mg / kg	mg / kg
Naled - (7300A399)	0.01 mg / kg	mg / kg
Napropamide - (7300G059)	0.01 mg / kg	mg / kg
Nitrapyrin - (7300G053)	0.01 mg / kg	mg / kg
Nitrofen - (7300A268)	0.01 mg / kg	mg / kg
Nonachlor, cis- - (SF0001DA)	0.01 mg / kg	mg / kg
Nonachlor, trans- - (7300G119)	0.01 mg / kg	mg / kg
Ofurace - (7300A603)	0.01 mg / kg	mg / kg
Omethoate - (7300A270)	0.01 mg / kg	mg / kg
o-phenyl phenol - (7300S007)	0.01 mg / kg	mg / kg
Other screened pesticides - (7300A583)	No unit / No unit	No unit / No unit
Oxyfluorfen - (7300A404)	0.01 mg / kg	mg / kg
Paclobutrazol - (7300S218)	0.02 mg / kg	mg / kg
Parathion - (B001B099)	0.01 mg / kg	mg / kg
Parathion oxygen analog - (7300A274)	0.01 mg / kg	mg / kg
Parathion-methyl - (B001B098)	0.01 mg / kg	mg / kg
Parathion-methyl oxygen analog - (7300A275)	0.01 mg / kg	mg / kg
PCB 101 - (7300A246)	0.01 mg / kg	mg / kg
PCB 138 - (7300A247)	0.01 mg / kg	mg / kg
PCB 153 - (7300A248)	0.01 mg / kg	mg / kg
PCB 180 - (7300A249)	0.01 mg / kg	mg / kg
PCB 28 - (7300A244)	0.01 mg / kg	mg / kg

PCB 52 - (7300A245)	0.01 mg / kg	mg / kg
Pentachloranisole - (7300A282)	0.02 mg / kg	mg / kg
Pentachloroaniline - (7300A284)	0.01 mg / kg	mg / kg
Pentachlorobenzene - (7300A283)	0.01 mg / kg	mg / kg
Pentachlorobenzonitrile - (SA000227)	0.02 mg / kg	mg / kg
Pentachlorothioanisole - (SP00000L)	0.02 mg / kg	mg / kg
Permethrin - (7300S227)	0.01 mg / kg	mg / kg
Perthane - (7300A408)	0.01 mg / kg	mg / kg
Phenkapton - (7300A409)	0.05 mg / kg	mg / kg
Phenothrin - (SF00000C)	0.01 mg / kg	mg / kg
Phenthoate - (7300G066)	0.01 mg / kg	mg / kg
Phorate - (7300A288)	0.01 mg / kg	mg / kg
Phorate-O-analogue - (LP000007)	0.01 mg / kg	mg / kg
Phorate-sulfone - (LP00001M)	0.01 mg / kg	mg / kg
Phosmet - (7300A290)	0.01 mg / kg	mg / kg
Phosphamidon - (7300G067)	0.01 mg / kg	mg / kg
Picolinafen - (7300S298)	0.01 mg / kg	mg / kg
Picoxystrobin - (7300A660)	0.01 mg / kg	mg / kg
Piperophos - (7300S037)	0.01 mg / kg	mg / kg
Prallethrin - (7300A555)	0.02 mg / kg	mg / kg
Procymidone (Sumilex) - (SA000238)	0.01 mg / kg	mg / kg
Profenofos - (7300A296)	0.01 mg / kg	mg / kg
Profluralin - (7300A297)	0.01 mg / kg	mg / kg
Prometryn - (7300A299)	0.01 mg / kg	mg / kg
Pronamide - (QD000187)	0.01 mg / kg	mg / kg
Propachlor - (7300A300)	0.01 mg / kg	mg / kg
Propanil - (7300H073)	0.02 mg / kg	mg / kg
Propazine - (7300A302)	0.01 mg / kg	mg / kg
Propetamphos - (7300A303)	0.01 mg / kg	mg / kg
Propyzamide - (7300A410)	0.01 mg / kg	mg / kg
Prothiofos - (7300A307)	0.01 mg / kg	mg / kg
Pyraclofos - (7300S038)	0.01 mg / kg	mg / kg
Pyrazophos - (7300A308)	0.01 mg / kg	mg / kg
Pyrifenox - (7300S255)	0.01 mg / kg	mg / kg
Quinalphos - (7300A310)	0.01 mg / kg	mg / kg
Quintozene - (7300A311)	0.01 mg / kg	mg / kg
Quizalofop-P-ethyl - (SP00001K)	0.01 mg / kg	mg / kg
Resmethrin - (7300A545)	0.02 mg / kg	mg / kg
S 421 (Octachlordipropylether) - (7300G114)	0.01 mg / kg	mg / kg
Screened pesticides - (PA0028L)	No unit / No unit	No unit / No unit
Simazine - (7300A312)	0.01 mg / kg	mg / kg
tau-Fluvalinate - (7300A551)	0.01 mg / kg	mg / kg
Tebupirimfos - (SF00000B)	0.01 mg / kg	mg / kg
Tecnazene - (7300A316)	0.01 mg / kg	mg / kg
Tefluthrin - (7300A432)	0.01 mg / kg	mg / kg

Terbacil - (7300S269)	0.01 mg / kg	mg / kg
Terbuthylazine - (7300A320)	0.02 mg / kg	mg / kg
Tetradifon - (7300A323)	0.01 mg / kg	mg / kg
Tetrahydrophthalimide (THPI) - (SP00001S)	0.01 mg / kg	mg / kg
Tetrasul - (7300A325)	0.01 mg / kg	mg / kg
Thiometon - (7300A327)	0.01 mg / kg	mg / kg
Thionazin - (7300A414)	0.01 mg / kg	mg / kg
Tolclofos-methyl - (7300A328)	0.01 mg / kg	mg / kg
Tolylfluanid - (7300A329)	0.01 mg / kg	mg / kg
Tralomethrin - (7300A469)	0.01 mg / kg	mg / kg
Triazophos - (7300A333)	0.01 mg / kg	mg / kg
Trichlorfon - (7300A334)	0.01 mg / kg	mg / kg
Trichloronat - (7300A335)	0.01 mg / kg	mg / kg
Trifluralin - (7300A336)	0.01 mg / kg	mg / kg
Triticonazole - (7300A643)	0.01 mg / kg	mg / kg
Vegedex (Sulfallate) - (SA000053)	0.01 mg / kg	mg / kg
Vinclozolin - (7300A339)	0.01 mg / kg	mg / kg
QA05R Pesticides (Quechers LC-MSMS)		
Abamectin - (7300A145)	0.01 mg / kg	mg / kg
Acetamiprid - (Z001AS01)	0.01 mg / kg	mg / kg
Aldicarb - (7300A147)	0.01 mg / kg	mg / kg
Aldicarb-sulfone - (7300A446)	0.01 mg / kg	mg / kg
Aldicarb-sulfoxide - (7300A447)	0.01 mg / kg	mg / kg
Aminocarb - (7300A150)	0.01 mg / kg	mg / kg
Amitraz - (7300A151)	0.1 mg / kg	mg / kg
Azadirachtin - (AS000004)	0.1 mg / kg	mg / kg
Azinphos-methyl oxon - (SA000242)	0.01 mg / kg	mg / kg
Azoxystrobin - (7300S049)	0.01 mg / kg	mg / kg
Bendiocarb - (7300A155)	0.01 mg / kg	mg / kg
Bensulide - (QD00018D)	0.01 mg / kg	mg / kg
Bitertanol - (7300G012)	0.01 mg / kg	mg / kg
Boscalid - (Z001AS8N)	0.01 mg / kg	mg / kg
Bromuconazole, cis- - (SP000019)	0.01 mg / kg	mg / kg
Bromuconazole, trans- - (SP00001A)	0.01 mg / kg	mg / kg
Bupirimate - (7300A166)	0.01 mg / kg	mg / kg
Buprofezin - (7300S064)	0.01 mg / kg	mg / kg
Butocarboxim-sulfoxide - (SF00019X)	0.05 mg / kg	mg / kg
Butralin - (7300G013)	0.01 mg / kg	mg / kg
Carbaryl - (7300A170)	0.01 mg / kg	mg / kg
Carbendazim - (Z001UD02)	0.01 mg / kg	mg / kg
Carbendazim/Benomyl (sum) - (7300A172)	0.01 mg / kg	mg / kg
Carbofuran - (7300A173)	0.01 mg / kg	mg / kg
Carbofuran, 3-OH - (7300A445)	0.01 mg / kg	mg / kg
Carbosulfan - (7300S071)	0.01 mg / kg	mg / kg
Carfentrazone-ethyl - (7300A673)	0.01 mg / kg	mg / kg

Chlorantraniliprole - (SF0001E3)	0.02 mg / kg	mg / kg
Chlorfenvinphos - (7300A176)	0.01 mg / kg	mg / kg
Chloridazone - (7300S076)	0.01 mg / kg	mg / kg
Chloroxuron - (7300G155)	0.01 mg / kg	mg / kg
Clethodim - (Z001AA0G)	0.01 mg / kg	mg / kg
Clodinafop-propargyl - (7300S019)	0.01 mg / kg	mg / kg
Clofentezine - (7300G003)	0.01 mg / kg	mg / kg
Clomazone - (7300G022)	0.01 mg / kg	mg / kg
Clothianidin - (Z001AS11)	0.01 mg / kg	mg / kg
Cyazofamid - (SP000005)	0.01 mg / kg	mg / kg
Cycloate - (7300S093)	0.01 mg / kg	mg / kg
Cycloxydim - (7300A340)	0.01 mg / kg	mg / kg
Cymoxanil - (7300A194)	0.01 mg / kg	mg / kg
Cyproconazole - (7300A196)	0.01 mg / kg	mg / kg
Cyprodinil - (7300A482)	0.01 mg / kg	mg / kg
Cyromazine - (7300A522)	0.1 mg / kg	mg / kg
Demeton-S-methyl-sulfone - (7300G038)	0.01 mg / kg	mg / kg
Desmedipham - (Z001AA0K)	0.01 mg / kg	mg / kg
Diafenthiuron - (Z001AA19)	0.01 mg / kg	mg / kg
Diazinon - (7300S106)	0.01 mg / kg	mg / kg
Diethofencarb - (7300S115)	0.01 mg / kg	mg / kg
Difenoconazole - (7300A209)	0.01 mg / kg	mg / kg
Diflubenzuron - (7300G085)	0.01 mg / kg	mg / kg
Dimethametryn - (Z001AA1B)	0.01 mg / kg	mg / kg
Dimethoate - (7300A210)	0.01 mg / kg	mg / kg
Dimethomorph - (7300A427)	0.01 mg / kg	mg / kg
Dimethylvinphos - (SP00000A)	0.01 mg / kg	mg / kg
Diniconazole - (7300A483)	0.01 mg / kg	mg / kg
Dinotefuran - (SF0001AX)	0.1 mg / kg	mg / kg
Dioxacarb - (7300A211)	0.01 mg / kg	mg / kg
Diuron - (7300A216)	0.01 mg / kg	mg / kg
Edifenphos - (7300G036)	0.01 mg / kg	mg / kg
Emamectin - (AS000003)	0.01 mg / kg	mg / kg
Epoxiconazole - (7300A484)	0.01 mg / kg	mg / kg
Etaconazole - (7300S131)	0.01 mg / kg	mg / kg
Ethiofencarb - (7300A219)	0.01 mg / kg	mg / kg
Ethiofencarb-sulfone - (AS00000L)	0.01 mg / kg	mg / kg
Ethiofencarb-sulfoxide - (AS00000M)	0.01 mg / kg	mg / kg
Ethofumesate - (7300G200)	0.01 mg / kg	mg / kg
Etobenzanid - (SA000190)	0.01 mg / kg	mg / kg
Etofenprox - (7300G202)	0.01 mg / kg	mg / kg
Fenamiphos - (7300G001)	0.01 mg / kg	mg / kg
Fenarimol - (7300A223)	0.01 mg / kg	mg / kg
Fenazaquin - (7300S138)	0.01 mg / kg	mg / kg
Fenbuconazole - (7300A415)	0.01 mg / kg	mg / kg

Fenhexamid - (Z001AA02)	0.1 mg / kg	mg / kg
Fenobucarb - (Z001JJ4A)	0.01 mg / kg	mg / kg
Fenoxycarb - (7300A416)	0.01 mg / kg	mg / kg
Fenpropimorph - (7300G004)	0.01 mg / kg	mg / kg
Fenpyroximate - (7300A539)	0.01 mg / kg	mg / kg
Fipronil - (Z001AS14)	0.01 mg / kg	mg / kg
Flonicamid - (AA000034)	0.01 mg / kg	mg / kg
Fludioxonil - (7300A486)	0.01 mg / kg	mg / kg
Flufenacet - (7300A615)	0.01 mg / kg	mg / kg
Flufenoxuron - (7300A575)	0.01 mg / kg	mg / kg
Fluopicolid - (AS000005)	0.01 mg / kg	mg / kg
Flutolanil - (7300A581)	0.01 mg / kg	mg / kg
Forchlorfenuron - (AS000007)	0.01 mg / kg	mg / kg
Fosthiazate - (7300A659)	0.01 mg / kg	mg / kg
Furalaxyl - (7300A342)	0.01 mg / kg	mg / kg
Furathiocarb - (7300S162)	0.01 mg / kg	mg / kg
Heptenophos - (7300A238)	0.01 mg / kg	mg / kg
Hexaconazole - (7300A239)	0.01 mg / kg	mg / kg
Hexaflumuron - (7300A574)	0.01 mg / kg	mg / kg
Hexythiazox - (7300A496)	0.01 mg / kg	mg / kg
Imazalil - (7002A019)	0.01 mg / kg	mg / kg
Imidacloprid - (7300A470)	0.01 mg / kg	mg / kg
Indoxacarb - (7300S034)	0.01 mg / kg	mg / kg
Iprovalicarb - (7300S175)	0.01 mg / kg	mg / kg
Isofenphos - (7300S178)	0.01 mg / kg	mg / kg
Isoprocarb - (7300S179)	0.01 mg / kg	mg / kg
Isoproturon - (7300A393)	0.01 mg / kg	mg / kg
Isoxaflutole - (7300A618)	0.01 mg / kg	mg / kg
Kresoxim-methyl - (7300A394)	0.01 mg / kg	mg / kg
Linuron - (7300A252)	0.01 mg / kg	mg / kg
Lufenuron - (7300A577)	0.01 mg / kg	mg / kg
Malaoxon - (7300A395)	0.01 mg / kg	mg / kg
Mecarbam - (7300A253)	0.01 mg / kg	mg / kg
Mepanipyrim - (7300A665)	0.01 mg / kg	mg / kg
Metalaxyl and Metalaxyl-M (sum) - (7300A255)	0.01 mg / kg	mg / kg
Metamitron - (7300A531)	0.01 mg / kg	mg / kg
Methabenztiazuron - (7300A254)	0.01 mg / kg	mg / kg
Methidathion - (7300A258)	0.01 mg / kg	mg / kg
Methiocarb - (7300A259)	0.01 mg / kg	mg / kg
Methiocarb sulfoxide - (7300A451)	0.01 mg / kg	mg / kg
Methiocarb-sulfone - (7300A450)	0.01 mg / kg	mg / kg
Methomyl - (7300A260)	0.1 mg / kg	mg / kg
Methoxyfenozide - (Z001AS02)	0.01 mg / kg	mg / kg
Metolachlor - (7300A262)	0.01 mg / kg	mg / kg
Metolcarb - (AS000008)	0.01 mg / kg	mg / kg

Metoxuron - (7300A398)	0.01 mg / kg	mg / kg
Metribuzin - (7300S199)	0.01 mg / kg	mg / kg
Molinate - (7300G060)	0.01 mg / kg	mg / kg
Myclobutanil - (7300S203)	0.01 mg / kg	mg / kg
Neburon - (7300A400)	0.01 mg / kg	mg / kg
Nitenpyram - (SP000003)	0.01 mg / kg	mg / kg
Novaluron - (SP000006)	0.01 mg / kg	mg / kg
Nuarimol - (7300A478)	0.01 mg / kg	mg / kg
Other screened pesticides - (7300A583)	mg / kg	mg / kg
Oxadixyl - (7300A272)	0.01 mg / kg	mg / kg
Oxamyl - (7300A452)	0.01 mg / kg	mg / kg
Oxydemeton-methyl - (7300G196)	0.01 mg / kg	mg / kg
Penconazole - (7300A280)	0.01 mg / kg	mg / kg
Pencycuron - (E001DI01)	0.01 mg / kg	mg / kg
Pendimethalin - (7300A281)	0.01 mg / kg	mg / kg
Phenkapton - (7300A409)	0.01 mg / kg	mg / kg
Phenmedipham - (7300G061)	0.01 mg / kg	mg / kg
Phorate-sulfoxide - (LP000010)	0.01 mg / kg	mg / kg
Phosalone - (7300A289)	0.01 mg / kg	mg / kg
Piperonyl butoxide (PBO) - (7300A291)	0.01 mg / kg	mg / kg
Pirimicarb - (7300A292)	0.01 mg / kg	mg / kg
Pirimicarb, desmethyl- - (7300G201)	0.01 mg / kg	mg / kg
Pirimicarb, desmethyl-formamido- - (SP00000F)	0.01 mg / kg	mg / kg
Pirimifos-ethyl - (7300S235)	0.01 mg / kg	mg / kg
Pirimiphos-methyl - (7300S234)	0.01 mg / kg	mg / kg
Prochloraz - (7300G068)	0.01 mg / kg	mg / kg
Profoxydim - (Z001AA0H)	0.01 mg / kg	mg / kg
Promecarb - (7300A298)	0.01 mg / kg	mg / kg
Propamocarb - (7300A426)	0.01 mg / kg	mg / kg
Propanil - (7300H073)	0.01 mg / kg	mg / kg
Propaquizafop - (Z001AA0P)	0.01 mg / kg	mg / kg
Propargite - (7300A418)	0.01 mg / kg	mg / kg
Propham - (7300A304)	0.01 mg / kg	mg / kg
Propiconazole - (7300A305)	0.01 mg / kg	mg / kg
Propoxur - (7300A306)	0.01 mg / kg	mg / kg
Prosulfocarb - (7300S251)	0.01 mg / kg	mg / kg
Pymetrozine - (7300A630)	0.01 mg / kg	mg / kg
Pyraclostrobin - (Z001AA14)	0.01 mg / kg	mg / kg
Pyridaben - (7300S254)	0.01 mg / kg	mg / kg
Pyridaphenthion - (7300G193)	0.01 mg / kg	mg / kg
Pyrimethanil - (7300S256)	0.01 mg / kg	mg / kg
Pyriproxyfen - (7300S028)	0.01 mg / kg	mg / kg
Quinoxyfen - (7300S029)	0.01 mg / kg	mg / kg
Rotenone - (7300A534)	0.01 mg / kg	mg / kg
Screened pesticides - (PA0028L)	No unit / No unit	No unit / No unit

Sethoxydim - (7300A135)	0.01 mg / kg	mg / kg
Spinetoram - (SA000008)	0.02 mg / kg	mg / kg
Spinosad - (Z001AX02)	0.01 mg / kg	mg / kg
Spirodiclofen - (AS00000A)	0.01 mg / kg	mg / kg
Spiromesifen - (Z001AS8Z)	0.01 mg / kg	mg / kg
Spiroxamine - (7300A657)	0.01 mg / kg	mg / kg
Sulfentrazone - (AS00000B)	0.01 mg / kg	mg / kg
Sulfotep - (7300A313)	0.01 mg / kg	mg / kg
Sulprofos - (7300A314)	0.01 mg / kg	mg / kg
Tebuconazole - (7300A318)	0.01 mg / kg	mg / kg
Tebufenpyrad - (7300S266)	0.01 mg / kg	mg / kg
Tepraloxydim - (Z001AS03)	0.01 mg / kg	mg / kg
Terbufos - (7300A413)	0.01 mg / kg	mg / kg
Terbutryn - (7300A321)	0.01 mg / kg	mg / kg
Tetrachlorvinphos - (7300J010)	0.01 mg / kg	mg / kg
Tetraconazole - (7300A499)	0.01 mg / kg	mg / kg
Thiabendazole - (7300S278)	0.01 mg / kg	mg / kg
Thiacloprid - (7300A661)	0.01 mg / kg	mg / kg
Thiamethoxam - (Z001AX03)	0.01 mg / kg	mg / kg
Thiobencarb - (7300A579)	0.01 mg / kg	mg / kg
Thiodicarb - (7300A453)	0.01 mg / kg	mg / kg
Thiofanox-sulfoxide - (Z001AS05)	0.01 mg / kg	mg / kg
Thiophanate-methyl - (7300H058)	0.01 mg / kg	mg / kg
Tralkoxydim - (AS00000C)	0.01 mg / kg	mg / kg
Triadimefon - (7300A330)	0.01 mg / kg	mg / kg
Triadimenol - (7300A331)	0.01 mg / kg	mg / kg
Triallate - (7300A332)	0.01 mg / kg	mg / kg
Tricyclazole - (Z001AS90)	0.01 mg / kg	mg / kg
Tridemorph - (7300A444)	0.01 mg / kg	mg / kg
Trifloxystrobin - (7300A631)	0.01 mg / kg	mg / kg
Triflumizole - (7300S288)	0.01 mg / kg	mg / kg
Triflumuron - (7300A515)	0.01 mg / kg	mg / kg
Triforine - (7300A337)	0.01 mg / kg	mg / kg
Trimethycarb, 3,4,5- - (7300A650)	0.01 mg / kg	mg / kg

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April 29, 2022

Via Electronic Mail

Marissa Santos, M.S.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: Response to FDA's Additional Questions for GRAS Notice No. 001031

Dear Ms. Santos:

We are writing in response to FDA's additional questions regarding GRAS Notice No. 001031 for Barley Rice Protein. As discussed during our April 20, 2022 call, we are initially responding to questions 1-3 and 5-7 and our response to question 4 will follow. For ease of reference, we reproduce each question below, followed by our response.

1. *On page 5 of the notice, you state that the ingredient is derived from brewers spent grains and consists of a mixture of barley and rice protein hydrolysates at levels of 40 to 100% and 0 to 60% respectively. On Page 8 of your notice, you indicate that the ingredient is derived from a 'grain mixture', consisting of non-wheat containing brewer's grains from the mash step of beer production. Further on the same page you mention 'incoming grains' at the start of the manufacturing process. Please clarify if this refers to the mash from rice grains and mash of barley grains being introduced separately to the extraction vessel, or if the starting material consists of one mash from a brewer's spent grains which consists of both rice and barley.*

The starting material (*i.e.*, incoming grains) which is introduced into the extraction vessel consists of a single mash from brewer's spent grains always consisting of both rice and barley.

Washington, DC ♦ Brussels ♦ San Francisco ♦ Shanghai ♦ Boulder

2. *On Page 11 of your notice, you state that the tested batches of the ingredient consist of 60 to 70% barley protein hydrolysate and 30 to 40% rice protein hydrolysate. We note that on page 5 of the notice you described the ingredient as a mixture of barley and rice protein hydrolysates at levels of 40 to 100% and 0 to 60%, respectively, and that a grain mixture containing 100% barley protein hydrolysate (i.e., 0% rice protein hydrolysate) would not be considered rice and barley protein hydrolysate. Please clarify the identity of the ingredient that is the subject of the notice.*

   Please note that we may have additional safety questions based on your response regarding the identity of the ingredient and if it is deemed not to be compositionally or nutritionally similar to native rice and barley.

The mash from brewer's spent grains that enters the extraction vessel will consist of barley and rice at ratios of 40 to 99% barley and 1 to 60% rice. EverGrain wishes to clarify that there will always be some combination of barley and rice and that there will never be mash from brewer's spent grains that only consists of barley. In other words, there will never be a product that will be produced from 100% barley brewer's spent grains and thus, there will never be an ingredient that is 100% barley protein.

The amino acid composition of Barley Rice Protein remains very similar to native barley and rice protein across all batches of Barley Rice Protein that have been tested, regardless of the starting ratios of barley and rice. The table below reproduces the data from Table 7 of the GRAS Notice and includes data from four additional lots of Barley Rice Protein with varying barley and rice compositions. The additional data from these four lots is in red text. The consistency of the amino acid profiles of these batches demonstrates that the variability in the ratio of barley to rice does not significantly impact protein quality.

Table 7: Amino Acid Profile of Four Non-Consecutive Batches of Barley Rice Protein											
Amino Acids	**Rice (g/100g protein)**	**Barley (g/100g protein)**	**BSG (g/100g protein)**	**Barley Rice Protein (g/100g protein)**							
				Lot No. 101121 BR-NWK-VF19	**Lot No. 101121 BR-NWK-VF20**	**Lot No. 121121 BR-NWK-VF19**	**Lot No. MU finished powder (=Everpro 55% rice inclusion)**	**Lot No. 080318 BRSP01**	**Lot No. 122018 SP01**	**Lot No. 012919 EV01**	**Lot No. 020419 SP01**
Barley / Rice Ratio	-	-	-	60/40	60/40	70/30	45/55	60-70/ 30-40*	60-70/ 30-40*	60-70/ 30-40*	60-70/ 30-40*
Aspartic acid	9.85	6.24	6.9	9.61	9.37	9.44	9.9	9.12	9.06	8.84	9.48
Threonine	3.86	3.4	1.9-3.4	4.04	3.87	3.79	4.04	3.61	3.65	3.74	3.8
Serine	5.45	4.22	2.8-4.4	4.61	4.44	4.48	5	4.13	4.52	4.39	4.58
Glutamic acid	21.46	26.13	20.1-22.3	26.56	26.06	23.83	22.03	25.64	22.98	23.70	23.38
Glycine	5.19	3.62	3.3-4.7	4.56	4.67	4.54	4.62	4.20	4.12	4.25	4.42
Alanine	6.14	3.89	4.3-7.4	5.15	5.24	5.03	5.34	4.39	4.82	4.58	4.69
Valine	6.18	4.9	4.2-6.0	6.35	6.28	5.98	6.11	5.47	5.77	5.65	5.65
Methionine	2.37	1.92	1.1-2.3	2.28	2.19	2.3	2.16	1.82	1.88	1.95	1.93
Isoleucine	4.46	3.65	1.5-4.2	4.62	4.42	4.32	4.36	4.00	4.40	4.05	3.98
Leucine	8.71	6.79	5.8-10.8	8.24	7.94	7.8	8.07	6.97	8.10	7.27	7.38
Tyrosine	3.95	2.87	3.2-3.7	4.41	4.24	4.27	4.71	3.99	4.15	3.97	4.17
Phenylalanine	5.44	5.61	5.8-6.2	6.38	6.23	5.95	5.79	5.88	6.61	5.68	5.64
Lysine	4.02	3.65	2.1-3.2	4.17	4	4.05	3.71	3.43	3.23	3.33	3.39

Histidine	2.68	2.25	2.2-3.6	2.3	2.37	2.36	2.45	2.03	2.12	2.21	2.28
Arginine	7.98	5.01	1.0-6.0	5.45	5.26	5.49	5.99	4.41	4.69	4.68	4.96
Proline	4.93	11.89	4.0-12.1	12.37	11.79	10.27	8.23	11.49	10.45	10.25	9.69
Cysteine	1.27	2.21	0.4-1.6	1.61	1.57	1.41	1.29	1.60	1.51	1.48	1.48
Tryptophan	1.34	1.67	2.6-5.5	1.56	1.6	1.62	1.54	1.47	1.51	1.52	1.53

* As stated in the GRAS Notice, these four lots consist of 60-70% barley protein and 30-40% rice protein.

3. *On page 10, in Table 1 of the notice, you list the specifications for rice and barley protein hydrolysate and list the chemical parameters as protein (≥ 85%) and moisture (<8%). You also note that the ingredient consists of fat, carbohydrates, fiber and ash, and refer to these parameters as 'typical parameters' in Table 2. It is our understanding that the Total Carbohydrates were calculated using the equation: %carbohydrates (by calculation) = 100 - %moisture - %protein - %lipid - %ash. If so, Table 2 appears incomplete in that it is missing moisture and protein levels. Please clarify. Additionally, on Page 11, in Table 3 of the notice, you list specification/typical parameters for your ingredient and provide data from 5 lots. We note that the sum of the percentages of the listed parameters and lot data are above 100% for all 5 lots. Please provide an explanation regarding why the listed parameters do not equate to 100% of the ingredient.*

The "typical parameters" in Table 2 are not specifications and they simply reflect the typical values observed. The specifications are included in Table 1. Thus, it would be inaccurate to include protein and moisture in Table 2. In response to your comment, we have added a footnote to Table 2 referring to the protein and moisture values from Table 1, so it is clear how the total carbohydrate level is calculated.

Table 2: Typical Parameters for Barley Rice Protein		
Fat	<2%	AOAC 954.02
Total carbohydrates	<10%	Calculation*
Total fiber	<5%	AOAC 991.43
Ash	<10%	AOAC 942.05

* %carbohydrates (by calculation) = 100 - %moisture - %protein - %lipid (fat) - %ash. See the specifications above in Table 1 above for the values of moisture, <8%, and protein (dry basis), ≥85%.

The values in Table 3 do not add to 100% because the protein values are reported on a dry basis. Table 3 in the GRAS Notice is reproduced below with protein reported on both a dry basis and on an "as is" basis (added in red). The sum of the protein (as is), moisture, fat, total carbohydrate, and ash content equals 100%.

Table 3: Results of Chemical Analysis of Five Non-Consecutive Batches of Barley Rice Protein

Parameter	Specification/ Typical Parameter	Manufacturing Lot Numbers				
		080318SP01	122018BRSP01	0129191EV01	060319BRSP01	060319BRSP01
Protein (dry basis)	≥85%	84.6	89.1	86.9	89.7	88.5
Protein (as is)	-	80.8	86.5	83.1	85.8	84.0
Moisture	<8%	4.5	2.9	4.4	4.3	5.1
Fat	<2%	0.26	0.84	1.26	1.0	0.45
Total carbohydrates	<10%	8.92	6.79	6.36	5.83	7.25
Total fiber	<5%	2.8	2.8	3.6	3.2	3.2
Soluble	-	2.0	2.0	3.1	2.3	2.8
Insoluble	-	0.8	0.8	0.5	0.9	0.4
Ash	<10%	5.51	2.97	4.85	3.06	3.37

4. *On Page 17 of the notice, you state that the intended use of rice and barley protein hydrolysate is as a protein substitute. You also state that there is no expected increase to dietary exposure to protein for consumers, that it will remain below IOM established Daily Reference Intakes (DRI) for adults (mean: 56-104 g/p/d and 90TH percentile: 76¬142 g/p/d), and below the Daily Reference Value (DRV) for protein for adults and children 4 or more years of age (50 g/p/d). We consider that while this DRV-based approach has been accepted, we find that it is limited and has been used without consideration of the actual uses of the*

> *ingredient. We further note that DRVs are values used for food labeling and that citing DRVs for a nutrient may not be an accurate estimate of the amount of a nutrient consumed by the U.S. population or subpopulations of interest. We have estimated the dietary exposure to your ingredient based on the maximum intended use levels and food categories that you provided, to be 96 g/p/d and 193 g/p/d at the mean and 90t^{h} percentile, respectively for the U.S. population aged 4 years or older. Given that the ingredient contains approximately 88% protein, we estimated the mean and 90t^{h} percentile dietary exposure to protein to be 84.8 g/p/d and 169 g/p/d, respectively. Please provide a mean and a 90th percentile dietary exposure estimate for the intended uses of the ingredient for the US population aged 2 years and older to support your narrative that there will be no expected increase to the dietary exposure to protein. This estimate may be based on National Health and Nutrition Examination Survey (NHANES) food consumption survey data and uses proposed in the notice, or you may assume partial or total replacement of dietary protein with your ingredient with citation to a published reference reporting consumption of protein or citation of a source such as the USDA, Agricultural Research Service (USDA/ARS) Usual Nutrient Intakes from Food and Beverage by Gender and Age, based on What We Eat in America, (WWEIA, NHANES) 2013-2016 data (https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/usual/Usual_Intake_gender_WWEIA_2013_2016.pdf).*

As noted above, once we have received information regarding how FDA estimated exposure to Barley Rice Protein, we will prepare a response.

5. *The protein digestibility-corrected amino acid score (PDCAAS) rating draws conclusions using unpublished digestibility data for barley rice protein. Additionally, the reported PDCAAS rating for barley rice protein is lower than for native barley protein; suggesting a compositional difference, which is in direct conflict with your safety narrative. Please reconcile this suggestion.*

As discussed in the GRAS Notice on page 26, the digestibility of native barley protein has been well documented in published sources. Specifically, the true digestibility of native barley protein was found to be 86.5±2.6% in one study (Nitrayová, et al. (2018)), although as discussed in the submitted Notice, the values reported in the literature vary from a high of 93% to a low as 75% (Cervantes-Pahm *et al.* (2014)). As evident by the manufacturing process, Barley Rice Protein is a minimally processed ingredient, and therefore we would expect that its digestibility would be similar to that of its constituent proteins (native barley and rice). While Barley Rice Protein does contain rice protein (at a level of up to 60%), rice has a higher digestibility and so estimating the digestibility of Barley Rice Protein with that of native barley protein is a conservative assumption (and one ultimately supported by the unpublished study). Thus, even without reference to the unpublished Gallaher study, we would expect that the digestibility of Barley Rice Protein could be conservatively estimated with the digestibility of native barley protein. The unpublished Gallaher study's reported digestibility of Barley Rice Protein of 86% is consistent with the noted published studies on native barley protein digestibility. Therefore, in our view, the unpublished Gallaher study is not pivotal to the GRAS conclusion and merely corroborates a conclusion (*i.e.*, the digestibility of barley rice protein is similar to that of native barley protein) that can be drawn from published sources and the manufacturing process for Barley Rice Protein. Reference to the unpublished study is therefore appropriate. *See* 21 CFR 170.30(b) (stating that GRAS conclusions "may be corroborated by the application of unpublished scientific data, information, or methods").

We disagree that the reported PDCAAS score of 60% for Barley Rice Protein is lower than that of native barley protein. First, the reported PDCAAS score for Barley Rice Protein was based on a digestibility correction of 86% which, as just noted, is approximately that same as the digestibility reported for native barley protein. Similarly, as noted on page 30, the PDCAAS for barley has been reported as 61% (Nitrayová, et al. (2018)), which is virtually identical to the reported PDCAAS for Barley Rice Protein. Finally, we also note that PDCAAS values for native barley protein have also been reported to be *lower* than 60%, which further supports the conclusion that there is no decrease in digestibility of barley rice protein when compared to native barley. *See* Bai et al., *Effect of tempering moisture and infrared heating temperature on the nutritional properties of desi chickpea and hull-less barley flours, and their blends,* 108 FOOD RESEARCH INT'L 430-39 (2018), *available at* https://doi.org/10.1016/j.foodres.2018.02.061 (reporting PDCAAS score of barley between 44% and 52%).

6. *Please discuss any potential allergenicity of the source material, brewer's spent grain.*

A literature search was conducted on April 26, 2022 on Google Scholar and Pubmed Central for reports of allergencity to brewer's spent grain. No reports of allergenicity were found.

7. *An updated literature search should be performed, noting the date it was performed. Any new findings that would impact the current GRAS conclusion should be discussed.*

We conducted an updated literature search on April 26, 2022. The following databases were searched: Google Scholar, Pubmed Central, Toxline, and ChemIDplus Advanced. The following search terms were used: "barley protein", "rice protein", "barley and rice protein", "brewer's spent grains", "BSG", "BSG animal studies", "BSG human studies", "BSG food." No additional safety studies were identified.

Additional studies which describe customer acceptance of food products made using different brewer's spent grain ingredients were identified. *See* Cuomo F. et al., *Sustainable re-use of brewer's spent grain for the production of high protein and fibre pasta*, 11 FOODS 642 (2022), *available at* https://dx.doi.org/10.3390%2Ffoods11050642 and Combest S. and Warren C., *The effect of upcycles brewers' spent grain on consumer acceptance and predictors of overall liking in muffins*, JOURNAL OF

FOOD QUALITY (2022), *available at* https://doi.org/10.1155/2022/6641904. The studies describe pasta and bakery products which exhibited satisfactory nutritional and sensory qualities. No adverse events were reported.

* * *

We appreciate the Agency's review of GRAS Notice 1031. Please let us know if you have any other questions or if you need any additional information.

Cordially yours,

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June 3, 2022

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Re: Response to FDA Additional Question Number 4 for GRAS Notice No. 001031

Dear Ms. Santos:

We are now providing our response to FDA's additional question number 4 regarding GRAS Notice No. 001031 for Barley Rice Protein. For convenience, we have repeated question number 4 below.

4. On Page 17 of the notice, you state that the intended use of rice and barley protein hydrolysate is as a protein substitute. You also state that there is no expected increase to dietary exposure to protein for consumers, that it will remain below IOM established Daily Reference Intakes (DRI) for adults (mean: 56-104 g/p/d and 90TH percentile: 76¬142 g/p/d), and below the Daily Reference Value (DRV) for protein for adults and children 4 or more years of age (50 g/p/d). We consider that while this DRV-based approach has been accepted, we find that it is limited and has been used without consideration of the actual uses of the ingredient. We further note that DRVs are values used for food labeling and that citing DRVs for a nutrient may not be an accurate estimate of the amount of a nutrient consumed by the U.S. population or subpopulations of interest. We have estimated the dietary exposure to your ingredient based on the maximum intended use levels and food categories that you provided, to be 96 g/p/d and 193 g/p/d at the mean and 90t^{h} percentile, respectively for the U.S. population aged 4 years or older. Given that the ingredient contains approximately 88% protein, we estimated the mean and 90t^{h} percentile dietary exposure to protein to be 84.8 g/p/d and 169 g/p/d, respectively. Please provide a mean and a 90th percentile dietary exposure estimate for the intended uses of the ingredient for the US population aged 2 years and older to support your narrative that there will *be no expected increase to the dietary exposure to protein. This estimate may be based on National Health and Nutrition Examination Survey (NHANES) food consumption survey data and uses proposed in the notice, or you may assume partial or total replacement of dietary protein with your ingredient with citation to a published reference reporting consumption of protein or citation of a source such as the USDA, Agricultural Research Service (USDA/ARS) Usual Nutrient Intakes from Food and Beverage by Gender and Age, based on What We Eat in America, (WWEIA, NHANES) 2013-2016 data (https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/usual/Usual_Intake_gender_WWEIA_2013_2016.pdf).*

In your May 13, 2022 email, FDA responded as follows:

FDA estimated the dietary exposure to your ingredient based on the maximum intended use levels for specific food categories listed in Table 11 of the notice, and NHANES food consumption survey data. The dietary exposure to protein was estimated by considering that your ingredient contains approximately 88% protein. Please note that though FDA provided exposure estimates, the notifier should estimate their own dietary exposures to the ingredient based on the intended use to support their narrative that there will be no expected increase in the exposure to protein. We noted in our request that the dietary exposure may be estimated using WWEIA/NHANES 2013-2018 data, by assuming the partial or total replacement of dietary protein in the diet with the ingredient. In response to our request, you proposed using mean protein exposure from WWEIA and using twice the mean value to estimate the 90th percentile dietary exposure. FDA believes that this would be an appropriate method to calculate the dietary exposure to your ingredient.

We had a call with FDA on May 23, 2022 to further clarify FDA's approach regarding exposure estimates for protein ingredients. Our response to number 4 is provided below.

As an initial matter, EverGrain LLC proposes to modify the intended use levels of Barley Rice Protein as indicated in Table 8 below. Table 8 from the GRAS Notice is copied below, with an additional column added demonstrating the changes from the initial proposed use levels. The food categories of "Fats and Oils" and "Jams and Jellies" have been removed entirely, while the maximum use levels in several other food categories have been reduced; no new uses have been added and the maximum use level has not increased for any category of food.

Table 8: Examples of Food Applications and Use Levels for Barley Rice Protein		
Food Category (21 C.F.R. § 170.3)	**Barley Rice Protein Use Levels (%) [As proposed in initial GRAS Notice submission]**	**Revised Barley Rice Protein Use Levels (%)**
Baked Goods and Baking Mixes (e.g., bread, quick breads, brownies, cookies, crackers, etc.)	0.5 to 15	0.5 to 5
Beverages and Beverage Bases [e.g., ready-to-drink energy drinks, ready-to-drink (liquid) nutritional, meal replacement, and protein beverages (non-milk-based), etc]	1 to 90	1 to 90 (No change)
Breakfast cereals (e.g., hot breakfast cereals, ready-to eat breakfast cereals, etc.)	1 to 30	1 to 10
Dairy Product Analogs (e.g., non-dairy milk, non-dairy milk shakes and smoothies, non-dairy cream, etc)	1 to 50	1 to 40
Fats and Oils (e.g., margarine/butter-type spreads, salad dressings, etc.)	1 to 10	EverGrain no longer wishes to include this intended use in the GRAS Notice.
Grain Products and Pastas (e.g., protein bars, nutrition bars, dry rice sides, etc.)	1 to 30	1 to 30 (No change)
Gravies and Sauces (e.g., specialty types and tomato-, milk-, and buttery-based sauces)	1 to 10	1 to 10 (No change)

Jams and Jellies	1 to 10	EverGrain no longer wishes to include this intended use in the GRAS Notice.
Nuts and Nut Products (e.g., nut-based spreads and butters)	1 to 20	1 to 20 (No change)
Plant Protein Products (e.g., meat analogues, plant-based spreads)	1 to 30	1 to 20
Snack Foods	1 to 10	1 to 10 (No change)
Soft Candy	1 to 15	1 to 15 (No change)
Soups and Soup Mixes	1 to 15	1 to 15 (No change)
Sweet Sauces, Toppings, and Syrups	1 to 20	1 to 10

Using protein intake as reported in WWEIA (*See* Table A2) to estimate a conservative upper limit on intake of Barley Rice Protein, we estimate that mean and 90th percentile exposure to Barley Rice Protein for all persons 1 year and older will not exceed 79.2 g/person/day and 158.4 g/person/day, respectively. 90th percentile exposure was estimated by doubling the mean intake of protein reported by WWEIA.

These estimates are based on the fact that Barley Rice Protein will replace existing protein in the food supply and will not increase a consumer's overall exposure to protein. We note that these estimates are very conservative as Barley Rice Protein will not be used in every category of food that contains protein, we would not expect Barley Rice Protein to penetrate the market completely, it is highly unlikely if not impossible that a person would consume all the listed food categories containing Barley Rice Protein in a day, and many sources of animal protein in the diet are unlikely to be completely replaced by plant proteins such as Barley Rice Protein.

Cordially yours,

Evangelia C. Pelonis