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#684

December 15, 2016

Stuart M. Pape
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VIA FEDERAL EXPRESS

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

Re: GRAS Notice for Mung Bean Protein Isolate

To Whom It May Concern:

Accompanying this letter is a notice pursuant to regulations of the Food and Drug Administration found at 21 CFR Part 170 setting forth the basis for the conclusion reached by the submitter, Hampton Creek, Inc., that mung bean protein isolate is generally recognized as safe under the intended conditions of use described in the notice. The notice is contained in a binder. In addition, we include a USB flash drive that contains a complete copy of the notice. I hereby certify that the electronic files contained on the flash drive were scanned for viruses prior to submission, and thus certified as being virus-free using McAfee VirusScan 8.8.

Sincerely,

(b) (6)

Stuart M. Pape
Counsel to Hampton Creek, Inc.

SMP:vp
Enclosures

GRAS Notice for a Mung Bean Protein Isolate

684

Prepared for:

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

Submitted by:

Hampton Creek, Inc.
2000 Folsom Street
San Francisco, CA 94110

December 13, 2016

GRAS Notice for a Mung Bean Protein Isolate

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List of Figures and Tables

GRAS Notice for a Mung Bean Protein Isolate

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Hampton Creek, Inc.hereby informs the U.S. Food and Drug Administration (FDA) of the view that mung bean protein isolate, manufactured by Hampton Creek, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Hampton Creek's conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Part 1.3 below. In addition, as a responsible official of Hampton Creek, Jim Flatt, hereby certifies that all data and information presented in this notice constitutes a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Hampton Creek and pertinent to the evaluation of the safety and GRAS status of mung bean protein isolate as an ingredient for addition to food, as described herein.

Signed,

(b) (6)

12/13/16

Jim Flatt, Ph.D.
Chief of R&D
Hampton Creek
jflatt@hamptoncreek.com

Date

1.1 Name and Address of Notifier

James Flatt, Ph.D.
Chief of R&D
Hampton Creek, Inc.
2000 Folsom Street
San Francisco, CA
94110

Tel: +1 (858) 349-8338
Fax: +1 (415) 682-6990

1.2 Common Name of Notified Substance

Mung bean protein isolate

1.3 Conditions of Use

The mung bean protein isolate is intended for use as a direct protein replacement of animal- or vegetable-based protein in a variety of conventional food and beverage products across multiple categories at use levels ranging from 3 to 90% w/w of the final product. The intended food categories and use levels at which Hampton Creek's mung bean protein isolate will be added are summarized in Table 1.3-1. The mung bean protein isolate is also intended for use as a supplement to existing protein in food products.

Table 1.3-1 Summary of the Individual Proposed Food-Uses and Use Levels for Mung Bean Protein Isolate in Conventional Food and Beverage Products

Food Category	Proposed Food-Uses	Maximum Intended Use Level of Mung Bean Protein Isolate (%) in Final Product
Baked Goods and Baking Mixes	Cereal and granola bars	10
	Crackers	5
	Meal replacement/nutritional bars/energy bars	30
Beverages and Beverage Bases	Fermented beverages made from rice/barley/grains/legumes/tea[a]	8
	Non-milk based instant protein powders	90 (powder)
	Non-milk based nutritional beverages (RTD, and powdered types) including protein-enriched fruit smoothies[b]	20 (as consumed)
	Non-milk based weight control beverages, instant shakes, and protein drinks (RTD and powdered types)[c]	10 (as consumed)
Breakfast Cereals	Breakfast cereals (RTE)	3
Condiments and Relishes	Bean dips and spreads	5
	Seasoning sauces	3
Dairy Product Analogs	Non-dairy cheese	5
	Non-dairy cream cheese, spread, and dips[d]	5
	Non-dairy cream or sour cream (liquid and powder)	3
	Non-dairy ice cream and frozen desserts[d]	3
	Non-dairy milk	3
	Non-dairy coffee whiteners[e]	3
	Non-dairy yogurt and non-dairy drinkable yogurts[f]	8
	Whipped topping	3
Frozen Dairy Desserts and Mixes	Ice cream[g] and other frozen dairy desserts	3
Fruit and Water Ices	Ice pops and sorbets	3

Table 1.3-1 Summary of the Individual Proposed Food-Uses and Use Levels for Mung Bean Protein Isolate in Conventional Food and Beverage Products		
Food Category	**Proposed Food-Uses**	**Maximum Intended Use Level of Mung Bean Protein Isolate (%) in Final Product**
Gelatins, Puddings, and Fillings	Puddings and mousse	3
Grain Products and Pasta	Pasta	4
Milk Products	Milk-based instant protein powders	90 (powder)
	Milk-based nutritional beverages (RTD and powdered types)	5 (as consumed)
	Milk-based weight control beverages, instant milkshakes, protein drinks (RTD and powdered types), and milk-based smoothies	3 (as consumed)
Plant Protein Products	Egg product analogs (meringue)[h]	5
	Egg product analogs (quiche, frittata)[h]	8
	Egg product analogs (scrambled eggs, omelet, hard boiled, liquid)[i]	20
	Vegetarian food products and meat analogues	20
Snack Foods	Snack chips, popcorn, extruded snacks	5

NHANES = United States National Health and Nutrition Examination Survey; RTD = ready-to-drink; RTE = ready-to-eat.

[a] There were no food codes identified for 'fermented' versions of these beverage types in the 2011-2012 NHANES; as such, all beverages prepared from a base of barley, grains, legumes or tea (note: only iced and powdered teas) were selected as surrogates to represent typical consumption.

[b] Based on the food descriptors available in the Individual Foods files of 2011-2012 NHANES, it could not be discerned whether fruit smoothie food codes contained added protein or not; thus, *all* non-milk based fruit smoothie food codes were included in the intake assessment as a proxy for the consumption of protein-enriched fruit smoothies.

[c] There were no food codes identified to represent this food category in the 2011-2012 NHANES; however, it is expected that the intakes from this category are adequately represented in the "Milk-based weight control beverages, instant milkshakes, and protein drinks (RTD) and powdered types" included within the 'Milk Products' food category.

[d] These food-uses represent non-dairy products. However, as there were no food codes available for non-dairy versions of the product, food codes for the dairy-equivalent product were selected as surrogate food codes to represent typical consumption.

[e] Based on the food descriptors available in the Individual Foods files of 2011-2012 NHANES, it could not be discerned whether coffee whitener food codes were dairy-containing, or dairy-free; thus, *all* coffee whitener food codes were included in the intake assessment as a proxy for the consumption of dairy-free coffee whiteners.

[f] There were no food codes identified for non-dairy drinkable yogurts in the 2011-2012 NHANES; this is a specific type of yogurt product and it is expected that the intakes from this category are adequately represented by the 'non-dairy yogurts' category.

[g] Ice cream has a standard of identity under 21 CFR §135.110; however, mung bean protein isolate may be used as a non-milk-derived ingredient used in addition to milk products or ingredients.

[h] There were no food codes identified for 'egg product analogs' in the 2011-2012 NHANES; as such, the equivalent (traditional egg-containing) counterpart food codes were selected as surrogates to represent typical consumption.

[i] There were no food codes identified for 'egg product analogs' of hard boiled or liquid eggs, specifically; however, all food codes available for 'egg substitutes' consumed as part of the NHANES 2011-12 dataset (specifically for scrambled eggs and omelets) were included as a proxy for this entire food category.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR), mung bean protein isolate manufactured by Hampton Creek, has been concluded to have GRAS status for use as an ingredient for addition to specified conventional food and beverage products, as described in Part 1.3, on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the United States (U.S.) Food and Drug Administration (FDA) for review and copying upon request during business hours at the offices of:

Hampton Creek, Inc.
2000 Folsom Street
San Francisco, CA
94110

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency`s review of the notice, Hampton Creek will supply these data and information.

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

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

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

The mung bean protein isolate is derived from mung bean (*Vigna radiata*), a legume consumed by humans for around 4,500 years (Fuller and Harvey, 2006; Nip, 2007). The taxonomic identity of mung bean is shown in Table 2.1-1.

Table 2.1-1 Taxonomic Classification of Mung Bean	
Kingdom	Viridiplantae
Phylum	Streptophyta
Class	Pentapetalae
Subclass	Rosids
Order	Fabales
Family	Fabaceae
Subfamily	Papilionoideae
Tribe	Phaseoleae
Genus	*Vigna*
Species	*Vigna radiata*

2.2 Method of Manufacturing

2.2.1 Raw Materials and Processing Aids

The raw materials and processing aids used in the manufacture of the mung bean protein isolate are listed in Table 2.2.1-1. All raw materials used in the manufacture of the mung bean protein isolate are permitted for use as processing aids in the manufacture of human foods in the U.S.

Table 2.2.1-1 Processing Aids/Additives Used During Manufacturing

Material	Function	Regulatory Status
Water	Solvent	-
Sodium hydroxide (NaOH)	pH adjustment	21 CFR §184.1763
Citric acid	pH adjustment	21 CFR §184.1033
Ethylenediaminetetraacetic acid (EDTA)	Antioxidant	21 CFR §172.120 21 CFR §172.135
Sodium chloride (NaCl)	Ionic strength adjustment	21 CFR §182.1
Polyether polyol	De-foaming agent	Food-grade

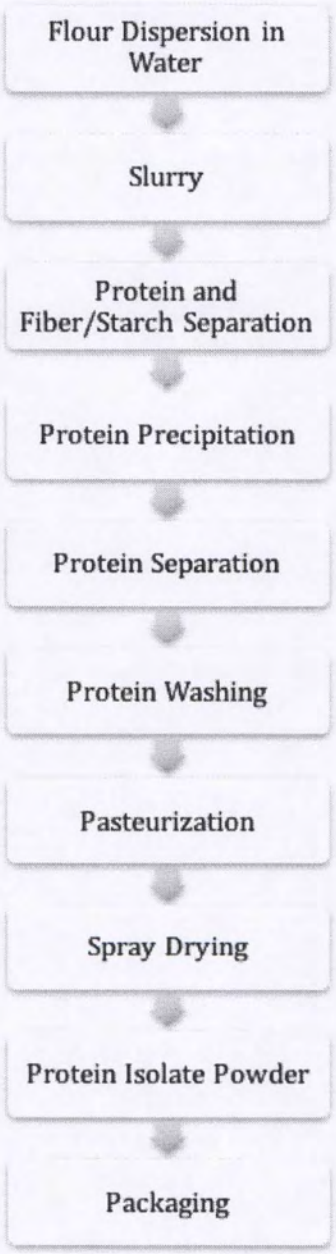
2.2.2 Manufacturing Process

The mung bean protein isolate is produced using a series of mechanical processes with the only chemicals used being pH adjusting agents, such as sodium hydroxide and citric acid; ethylenediaminetetraacetic acid (EDTA) to prevent lipid oxidation activities that may affect the flavor of the isolate; sodium chloride (NaCl) as an ionic strength adjuster, as well as a polyether polyol de-foaming agent.

In the first step of the process, raw mung beans are de-hulled. The de-hulled mung beans are then milled to produce flour. During extraction mung bean flour is then mixed with soft water and a food-grade de-foaming agent in a mix tank to form a slurry. The pH of the slurry is

adjusted with a food-grade sodium hydroxide solution for solubilization of the target protein into the aqueous solution. Next, the solubilized protein extract is separated from the slurry in a solid/liquid separation unit, typically consisting of a decanter centrifuge. Following this step, the clarified protein extract is acidified, which results in precipitation and separation of the target protein from the aqueous solution. In addition to the pH adjustment, food-grade EDTA is added to inhibit lipid oxidation that may produce off-flavor compounds. The precipitated protein slurry is then sent to a solid/liquid separation unit with the addition of food-grade sodium chloride to adjust the ionic strength, and a protein curd is recovered in the heavy phase. The protein curd is then washed to remove final residual impurities such as fibrous solids, salts, and carbohydrates, pasteurized in a high temperature/short time pasteurization step to kill any pathogenic bacteria that may be present in the solution, and finally passed through a spray dryer to remove any residual water content. The final dried protein isolate powder typically has less than 5% moisture content. A schematic overview of the manufacturing process of the mung bean protein isolate is provided in Figure 2.2.2-1.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for the Mung Bean Protein Isolate



2.2.3 Quality Control

The mung bean protein isolate is manufactured in compliance with current Good Manufacturing Practice (cGMP) as part of a preventative controls approach. In addition, Hampton Creek utilizes quality management systems based on the principles of Hazard Analysis Critical Control Point (HACCP) standards. Commercial production of the ingredient is in compliance with the preventive controls for human food regulation adopted by FDA as one of a series of critical regulations to implement the Food Safety Modernization Act (80 *Fed. Reg.* 55908 (September 17, 2015) (U.S. FDA, 2015a).

2.3 Product Specifications and Batch Analyses

2.3.1 Proposed Product Specifications

Appropriate food-grade product specifications have been established for Hampton Creek's mung bean protein isolate and are presented in Table 2.3.1-1 below. All methods of analysis are internationally recognized or are in-house methods that have been validated by Hampton Creek.

Table 2.3.1-1 Proposed Product Specifications of Hampton Creek's Mung Bean Protein Isolate

Parameter	Specification	Method of Analysis
Proximate analysis		
Moisture (%)	<7	AOAC 925.09 and 926.08
Protein (%)[a]	>80	AOAC 968.06 and 992.15
Fat (%)	3 to 5	AOAC 922.06, 954.02, 933.05, and 925.32
Ash (%)	<8	AOAC 923.03
Carbohydrate (%)	<10	SAM 07006 (calculation)
Microbiological		
Aerobic plate count (CFU/g)	<100,000	MFHPB-33
Listeria spp.	Negative	MFHPB-30
Salmonella spp.	Negative	MFHPB-20
Escherichia coli	Negative	MFHPB-34
Heavy metals		
Arsenic (ppm)	≤0.05	SAM 04001 (ICP-MS)
Cadmium (ppm)	≤0.05	SAM 04001 (ICP-MS)
Lead (ppm)	≤0.05	SAM 04001 (ICP-MS)
Mercury (ppm)	≤0.025	SAM 04007 (CVAA)

AOAC = Association of Analytical Chemists; CFU = colony-forming units; CVAA = cold vapor atomic absorption; ICP-MS = inductively-coupled plasma mass spectroscopy; MFHPB = Microbiology Food Health Protection Branch; SAM = Standard Addition Method.
[a] Protein values are measured on a dry weight basis.

2.3.2 Batch Analyses

Four (4) non-consecutive batches of the mung bean protein isolate were analyzed to verify that the manufacturing process produces a consistent product that meets the proposed product specifications. The results of the batch analyses are provided in Table 2.3.2-1.

Table 2.3.2-1 Results of Analyses of 4 Non-Consecutive Batches of Hampton Creek's Mung Bean Protein Isolate

Parameter	Specification	Manufacturing Lot No.			
		Batch 1 (122.1)	Batch 2 (123.1)	Batch 3 (124.1)	Batch 4 (133.1)
Proximate analysis					
Moisture (%)	<7%	4.2	3.4	4.3	3.1
Protein (%)[a]	>80%	82.3	83.9	85.2	82.8
Fat (%)	3 to 5	4.2	4.0	3.7	4.4
Ash (%)	<8%	6.8	6.1	6.0	6.8
Carbohydrate (%)	<10	7.0	5.4	4.5	5.4
Microbiological					
Aerobic plate count (CFU/g)	<100,000	24,000	31,000	42,000	55,000
Listeria spp.	Negative	Negative	Negative	Negative	Negative
Salmonella spp.	Negative	Negative	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative	Negative	Negative
Heavy metals					
Arsenic (ppm)	≤0.05	<0.05	<0.05	<0.05	<0.05
Cadmium (ppm)	≤0.05	<0.05	<0.05	<0.05	<0.05
Lead (ppm)	≤0.05	<0.05	<0.05	<0.05	<0.05
Mercury (ppm)	≤0.025	<0.025	<0.025	<0.025	<0.025

CFU = colony-forming units
[a] Protein values are measured on a dry weight basis.

2.3.3 Additional Analytical Information

2.3.3.1 Amino Acid Profile

The amino acid composition of 4 representative batches of the mung bean protein isolate was analyzed. The results of the analysis are provided in Table 2.3.3.1-1 below and show that the amino acid profile of the protein isolate is consistent from batch to batch, and the mung bean protein isolate contains a balanced amino acid profile.

Table 2.3.3.1-1 Amino Acid Composition of 4 Batches of the Mung Bean Protein Isolate				
Amino Acid	**Manufacturing Lot No. (% wt of total protein)**			
	Batch 1 (122.1)	**Batch 2 (123.1)**	**Batch 3 (124.1)**	**Batch 4 (133.1)**
Aspartic acid + asparagine	12.41	12.44	12.33	12.18
Threonine	2.82	2.77	2.89	2.75
Serine	5.35	5.30	5.32	5.24
Glutamic acid + glutamine	18.69	18.60	18.08	18.15
Glycine	3.39	3.34	3.43	3.30
Alanine	3.97	3.94	4.04	3.89
Valine	5.51	5.49	5.49	5.39
Methionine	1.33	1.25	1.32	1.26
Isoleucine	4.86	4.86	4.89	4.81
Leucine	8.60	8.59	8.65	8.49
Tyrosine	3.24	3.23	3.33	3.19
Phenylalanine	6.83	7.01	6.92	6.84
Lysine	7.03	7.09	7.09	7.07
Histidine	2.87	2.86	2.90	2.85
Arginine	7.39	7.51	7.43	8.85
Proline	4.43	4.44	4.49	4.39
Hydroxyproline	0.04	0.03	0.03	0.03
Cysteine	0.33	0.32	0.38	0.33
Tryptophan	0.94	0.91	0.99	0.96

2.3.3.2 Vitamins, Minerals, Carbohydrates, and Lipids

Three (3) non-sequential batches of the protein isolate were analyzed for vitamins, minerals, carbohydrates, and lipids, as presented in Table 2.3.3.2-1 below. Based on these analytical data and as expected, starch was absent from the final product confirming the efficiency of the manufacturing process to separate the solubilized protein extract from the slurry containing starch.

Table 2.3.3.2-1 Analyses for Vitamin, Mineral, Carbohydrate and Lipid Content of the Mung Bean Protein Isolate			
Parameter	**Manufacturing Lot No.**		
	Lot Numbers		
	IIIMNB75.3	**VMGB105.25**	**VIPS109.21**
Vitamins			
Vitamin A (IU/100g) Beta-carotene Retinol	 <200 <200	 <200 <200	 <200 <200
Vitamin C (mg/100g) Ascorbic acid	 <0.1	 <0.1	 <0.1
Vitamin D (IU/100g) D2 (ergocalciferol) D3 (cholcalciferol)	 N/A <200	 N/A <200	 N/A <200
Vitamin B5 (mg/100g) Calcium pantothenate	 0.62	 0.93	 0.34
Vitamin B6 (mg/100g) Pyridoxine HCl	 0.09	 0.07	 0.05
Vitamin B12 (µg/100 g) Cyanocobalamin	 10.10	 <2	 <2
Vitamin K1 (µg/100g) Phytonadione	 39.22	 37.13	 40.24
Vitamin K2 (µg/100g) MK-4 MK-7	 <20 <50	 <20 <50	 <20 <50
Tocopherols (mg/100g) *Beta-* D-*alpha-* *Delta-* *Gamma-*	 0.006 1.11 0.02 0.78	 0.01 2.4 0.04 2.0	 0.007 2.5 0.09 1.49
Thiamin (mg/100g)	0.13	0.12	0.10
Riboflavin (mg/100g)	0.13	0.09	0.06
Niacin (mg/100g)	0.90	0.13	0.47
Folic acid (µg/100 g)	3.55	4.78	7.59
Biotin (µg/100 g)	<2	<2	<2
Minerals			
Calcium (mg/100g)	116	44.27	22.20
Iron (mg/100g)	10.68	8.27	7.81
Sodium (mg/100g)	2,348	979	1,364
Potassium (mg/100g)	828	886	392
Magnesium (mg/100g)	108	143	114
Phosphorus (mg/100g)	570	545	494
Zinc (µg/100g)	3,210	1,584	897
Copper (mg/100g)	1.97	1.68	1.39
Molybdenum (µg/100g)	3.85	2.53	170
Selenium (µg/100g)	0.78	0.41	23.26

Table 2.3.3.2-1 Analyses for Vitamin, Mineral, Carbohydrate and Lipid Content of the Mung Bean Protein Isolate			
Parameter	**Manufacturing Lot No.**		
	Lot Numbers		
	IIIMNB75.3	**VMGB105.25**	**VIPS109.21**
Lipids			
Fat (%) Saturated Monounsaturated Polyunsaturated Trans	3.08 1.42 0.318 1.29 0.05	3.36 1.60 0.255 1.35 0.15	3.36 1.38 0.212 1.51 0.26
Carbohydrates			
Starch	Absent	Absent	Absent
Dietary fiber (g/100g)	<0.5	<0.5	<0.5

N/A = not available

2.3.3.3 Environmental Contaminants

2.3.3.3.1 Pesticide Residues

Due to the fact that the protein isolate is derived from a natural source, Hampton Creek conducted analyses for a number of chlorinated and organophosphate pesticide residues on 3 non-consecutive batches of the protein isolate. Chlorinated pesticides tested included alachlor, aldrin, *alpha*-BHC, *alpha*-chlordane, *beta*-BHC, DDD, DDE, DDT, *delta*-BHC, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, *gamma*-BHC, *gamma*-chlordane, heptachlor, heptachlor epoxide, methoxyclor, and permethrin. Organophosphate pesticides tested included azinophos methyl, carbophenothion, chlorfenvinphos, chlorpyrifos methyl, diazinon, dichlorvos, dursban, dyfonate, ethion, fenitrothion, malathion, methidathion, methyl parathion, parathion, phosalone, and pirimiphos methyl. The results of the batch analyses are provided in Table 2.3.3.3.1-1, and indicate that the level of chlorinated and organophosphate pesticide residues for the mung bean protein isolate complies with the set specifications and is below the level of detection of 0.1 ppm.

Table 2.3.3.3.1-1 Analyses for Residual Chlorinated and Organophosphate Pesticides in Representative Batches of the Mung Bean Protein Isolate				
Parameter	**Specification**	**Manufacturing Lot No.**		
		Lot Numbers		
		IIIMNB75.3	**VMGB105.25**	**VIPS109.21**
Chlorinated (ppm)	≤0.1	<0.1	<0.1	<0.1
Phosphates (ppm)	≤0.1	<0.1	<0.1	<0.1

2.3.3.3.2 Dioxins and Polychlorinated Biphenyls

In addition to pesticide residues, Hampton Creek also analyzed 3 non-consecutive batches of the mung bean protein isolate for residues of dioxins and polychlorinated biphenyls (PCBs). The results of the analyses are provided in Table 2.3.3.3.2-1. These compounds were determined to be either absent from the tested materials or present at levels that were of no toxicological significance.

Table 2.3.3.3.2-1 Analyses for Residual Dioxin[a] and Polychlorinated Biphenyls[b] in Representative Batches of the Mung Bean Protein Isolate

Parameter	Level of Detection	Manufacturing Lot No.		
		Lot Numbers		
		IIIMNB75.3	VMGB105.25	VIPS109.21
Dioxins*	<1 ppt	2.2	2.4	0.55
Total PCB	<0.5 ppb	0.315	0.977	0.002
Monochloro	<0.5 ppb	ND	ND	ND
Dichloro	<0.5 ppb	0.311	0.967	ND
Trichloro	<0.5 ppb	ND	ND	ND
Tetrachloro	<0.5 ppb	ND	ND	ND
Pentachloro	<0.5 ppb	0.0037	0.0103	0.0021
Hexachloro	<0.5 ppb	ND	ND	ND
Heptachloro	<0.5 ppb	ND	ND	ND
Octachloro	<0.5 ppb	ND	ND	ND
Nonachloro	<0.5 ppb	ND	ND	ND
Decachloro	<0.5 ppb	ND	ND	ND

ND = not detected; PCB = polychlorinated biphenyls; ppb = parts per billion; ppt = parts per trillion.
[a] Environmental Protection Agency (EPA) Method 1613B [high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS)].
[b] Environmental Protection Agency (EPA) Method 1668A (HRGC/HRMS).

2.3.3.3.3 Mycotoxins

Three (3) non-sequential batches of the mung bean protein isolate were analyzed for the presence of mycotoxins, including aflatoxin B1, B2, G1, G2, and ochratoxin A, by liquid chromatography-mass spectrometry (LC-MS). The results of the analyses provided in Table 2.3.3.3.3-1 indicate that the protein isolate is devoid of any residual mycotoxins.

Table 2.3.3.3.3-1 Analyses for Residual Mycotoxins in Representative Batches of the Mung Bean Protein Isolate			
Parameter	**Manufacturing Lot No.**		
	Lot Numbers		
	IIIMNB75.3	**VMGB105.25**	**VIPS109.21**
Aflatoxin B1[a]	<5 ppb	<5 ppb	<5 ppb
Aflatoxin B2[a]	<5 ppb	<5 ppb	<5 ppb
Aflatoxin G1[a]	<5 ppb	<5 ppb	<5 ppb
Aflatoxin G2[a]	<5 ppb	<5 ppb	<5 ppb
Ochratoxin A[b]	<7 ppb	<7 ppb	<7 ppb

[a] Limit of detection = 5 to 10 ppb
[b] Limit of detection = 10 ppb

2.3.3.4 Anti-Nutritional Factors

Dietary anti-nutritional factors are chemical substances that can adversely impact the digestibility of protein, bioavailability of amino acids and protein quality of foods (Gilani *et al.*, 2012). The anti-nutritional factors reported in mung bean including tannins, phytic acid, hemagglutinins (lectins), polyphenols, trypsin inhibitors, α-amylase inhibitors, and protease inhibitors (Dahiya *et al.*, 2015), are partially or completely removed or degraded during certain processing steps such as dehulling, germination, soaking, and heating (Mubarak, 2005).

The presence of protein-based anti-nutritional factors in representative batches of the mung bean protein isolate and mung bean flour was analyzed using a 2-dimensional nano liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method combined with proteomic analysis. The results provided in Table 2.3.3.4-1 indicate that the protein isolation process resulted in a decrease in relative abundance of lectin and protease inhibitor proteins as compared to the mung bean flour samples. Following proteomic analyses, no matches to known α-amylase inhibitors were identified. In a separate analysis, the level of lectins in 3 representative batches of each protein isolate and mung bean flour showed low levels (<0.05 mg/g) in these samples.

Table 2.3.3.4-1 Relative Abundance of Protein-Based Anti-Nutritional Factors in Representative Batches of the Mung Bean Protein Isolate and the Mung Bean Flour

Anti-Nutritional Factor	Manufacturing Lot No.									
	Protein Isolate (%)					Flour (%)				
	15-686.0317-119.1	15-686-0319-120.1	15-686.0324-121.1	15686.0331-122.1	15-686.0402-123.1	15-686.0317-MNB-16-0001	15-686-0319-MNB-15-0012	15-686.0324-MNB-15-0020	15686.0331-MNB-16-0001	15-686.0402-MNB-16-001
Lectin[a]	ND	0.001	0.024	ND	ND	ND	0.001	0.032	ND	ND
Protease inhibitor[b]	0.0075	0.00775	0.0082	0.01	0.01	0.015	0.0228	0.03375	0.027	0.0172

ND = not detected
[a] Average of protein accession no. XP_014512565 and XP_014514843.
[b] Average of protein accession no. XP_014505181, XP_014501457, XP_014516943, XP_014517066, XP_014521704, and XP_014522196.

In addition to protein-based anti-nutritional factors (*i.e.*, protease inhibitors, α-amylase inhibitors, and lectins), levels of non-protein-based anti-nutritional factors (*i.e.*, polyphenols and phytic acid) were also measured in several representative batches of the mung bean protein and mung bean flour. Generally, low levels of total polyphenols were identified in the protein isolate (98 to 203 mg gallic acid equivalent (GAE)/100 g), as compared to the levels in the mung bean flour (117 to 344 mg GAE/100 g). Levels of phytic acid ranged from 759 to 918 mg/100 g in the protein isolate, as compared to a phytic acid range of 685 to 716 mg/100 g in the mung bean flour.

2.4 Analysis for Presence of Allergens

Hampton Creek conducted a comparative protein analysis of 5 putative protein allergens associated with mung bean according to the AllergenOnline database (http://www.allergenonline.org/) with a union set of 1,867 proteins identified across 5 batches of mung bean flour and their corresponding protein isolates (FARRP, 2016). In total, 18 sequences in the flours and protein isolates matched 4 of the putative mung bean allergens. The matches had >50% sequence identity calculated over full-length alignments, with E-values lower than 1e-7. The putative allergens were seed albumin (CAA50008.1, 4 hits), pathogenesis-related protein-10 (PR-10) (AAX19889.1, 2 hits), 8S globulin β-isoform precursor (ABG02262.1, 12 hits), and 8s globulin α-subunit (ABW23574.1, 12 hits). The relative abundance of putative allergen matches in representative batches of the protein isolate and the mung bean flour are shown in Table 2.4-1. The protein isolation process substantially removes or reduces the levels of the PR-10 protein allergen to those that are negligible to none. More specifically, PR-10 protein allergens were detected at levels of 0.002 to 0.003% in the mung bean flour, and when levels of these allergens were measured in the protein isolate, trace levels (0.001%) were detected in one batch (Lot No. 15-686-0319-120.1), while no PR-10 protein allergens were detected in the other 4 batches. The protein isolation process did not seem to change the relative abundance of the putative albumin and globulin allergens to a significant degree as compared to the mung bean flours, and the differences noted are likely within experimental error.

Table 2.4-1 Relative Abundance of Putative Allergen Matches in Representative Batches of the Mung Bean Protein Isolate and Mung Bean Flour										
Protein Type	**Manufacturing Lot No.**									
	Protein Isolate (%)					**Flour (%)**				
	15-686.0317-119.1	**15-686-0319-120.1**	**15-686.0324-121.1**	**15686.0331-122.1**	**15-686.0402-123.1**	**15-686.0317-MNB-16-0001**	**15-686-0319-MNB-15-0012**	**15-686.0324-MNB-15-0020**	**15686.0331-MNB-16-0001**	**15-686.0402-MNB-16-001**
Albumin[a]	0.153	0.159	0.242	0.071	0.166	0.262	0.367	0.258	0.437	0.263
8S globulin[b]	7.021	6.496	6.354	6.755	6.693	6.048	6.294	5.861	6.037	6.093
PR-10[c]	ND	0.001	ND	ND	ND	0.002	0.003	ND	0.002	0.002

ND = not detected; PR-10 = pathogenesis-related protein 10

[a] Average of protein accession no. XP_014524354, NP_001304229, XP_014523938, XP_014523928, XP_014523936, XP_014524353, XP_014515669, NP_001304202, XP_014523923, XP_014507363, XP_014492536, and NP_001304231.

[b] Average of protein accession no. XP_014513134, NP_001304082, XP_014511316, and XP_014512898.

[c] Average of protein accession no. XP_014506982 and XP_014508691.

A similar analysis was performed for the union set of 1,083 proteins identified in spray-dried protein isolate (finished product; Lot No. 123.1), and uncooked, and cooked samples prepared from the spray-dried protein isolate (Table 2.4-2). More specifically, the spray-dried sample was resuspended in 100 mM Hepes pH 8.6 and diluted in 10 mM sodium phosphate pH 8.0 buffer to 0.5 mg/mL. The spray-dried uncooked sample was then solubilized in water to make a 12% w/w protein solution and diluted in 10 mM sodium phosphate pH 8 buffer to 0.5 mg/mL. The cooked sample was prepared in a similar manner with an additional cooking step (250°F for 10 min) prior to addition of sodium phosphate buffer. No pathogenesis-related protein 10 (AAX19889.1) matches were detected. As shown in 2.4-2 below, the protein isolation process and cooking do not significantly alter the relative abundance of putative allergens (all changes were within 3% of the initial value for each sample). However, during the protein isolation process, levels of putative 8s globulin β-isoform precursor and 8s globulin α-subunit, both of which are major protein storage sources and function proteins in mung bean seeds, were slightly enriched or depleted.

Table 2.4-2 Relative Abundance of Putative Allergen Matches in Spray-Dried, Uncooked, and Cooked Mung Bean Protein Isolate Samples

Protein Type	Manufacturing Lot No. 123.1		
	Spray-Dried (%)	Uncooked (%)	Cooked (%)
8S globulin[a]	8.160	8.026	8.157
Albumin[b]	0.351	0.482	0.382

[a] Average of protein accession no. XP_014524354, XP_014523938, NP_001304202, XP_014523923, XP_014507363, NP_001304231, XP_014492536, XP_014523936, XP_014524353, and XP_014523928.
[b] Protein accession no. XP_014513134

2.5 Stability

2.5.1 Mung Bean Protein Isolate Stability

Hampton Creek is currently performing a 24-month stability study, wherein 4 non-consecutive batches of the mung bean protein isolate are stored at room temperature in airtight containers. The composition of the protein isolate (*i.e.,* moisture, protein, oil, ash, and carbohydrates) is to be measured at various time points throughout the study period (*i.e.,* 4, 6, 9, 12, 18, and 24 months). The interim results of the stability study are presented in Table 2.5.1-1 below. The moisture, protein content, oil content, ash, and carbohydrates of the mung bean protein isolate does not significantly change over time from the established product specifications, suggesting that the protein isolate is stable when stored up to 6 months. The values for the oil/lipid content of the protein isolate is presented below, and while no specification has been established for oil content, these values do not significantly change following storage up to 6 months.

Table 2.5.1-1 Interim Results of Stability Testing of Mung Bean Protein Isolate when Stored at Room Temperature								
Parameter	**Manufacturing Lot No.**							
	Batch 1 (122.1)		**Batch 2 (123.1)**		**Batch 3 (124.1)**		**Batch 4 (133.1)**	
	Week 1	**Week 26**	**Week 1**	**Week 25**	**Week 1**	**Week 19**	**Week 1**	**Week 15**
Moisture (%)	4.78	5.39	4.66	5.40	5.14	5.34	4.76	5.16
Protein (%)	84.7	86.9	86.7	86.3	86.8	86.6	85.5	85.1
Oil (%)	0.84	0.64	0.65	0.62	0.70	0.34	0.70	0.55
Ash (%)	7.16	5.98	6.33	5.99	6.14	6.08	6.73	8.91
Carbohydrates (%)	6.52	5.79	5.66	6.39	5.72	6.24	6.71	4.68

2.5.2 Mung Bean Patty Stability

2.5.2.1 Moisture and pH

The stability of mung bean patties under frozen storage conditions (-20 and -80°C) was evaluated over 12 weeks. The mung bean patties were prepared using spray-dried mung bean protein isolate (Lot No. 122.1), water, salt, fat and minor food additives. The ingredients were blended and precooked at 121.1°C for 10 minutes, and bagged in polyethylene bags and stored in freezer over the course of the study. Changes in pH and moisture were measured at 0, 2, 4, 8, and 12 weeks. At each time point, frozen patties were thawed in a convection oven at 121.1°C for 20 to 24 minutes up to an internal temperature of 74°C. The pH was measured using a standard pH meter, and the moisture content of patties was measured gravimetrically using a loss-on-drying analyzer. Overall, the pH and moisture did not change significantly throughout the study period, suggesting that mung bean patties are stable over 12 weeks of storage at -20 and -80°C (Table 2.5.2.1-1).

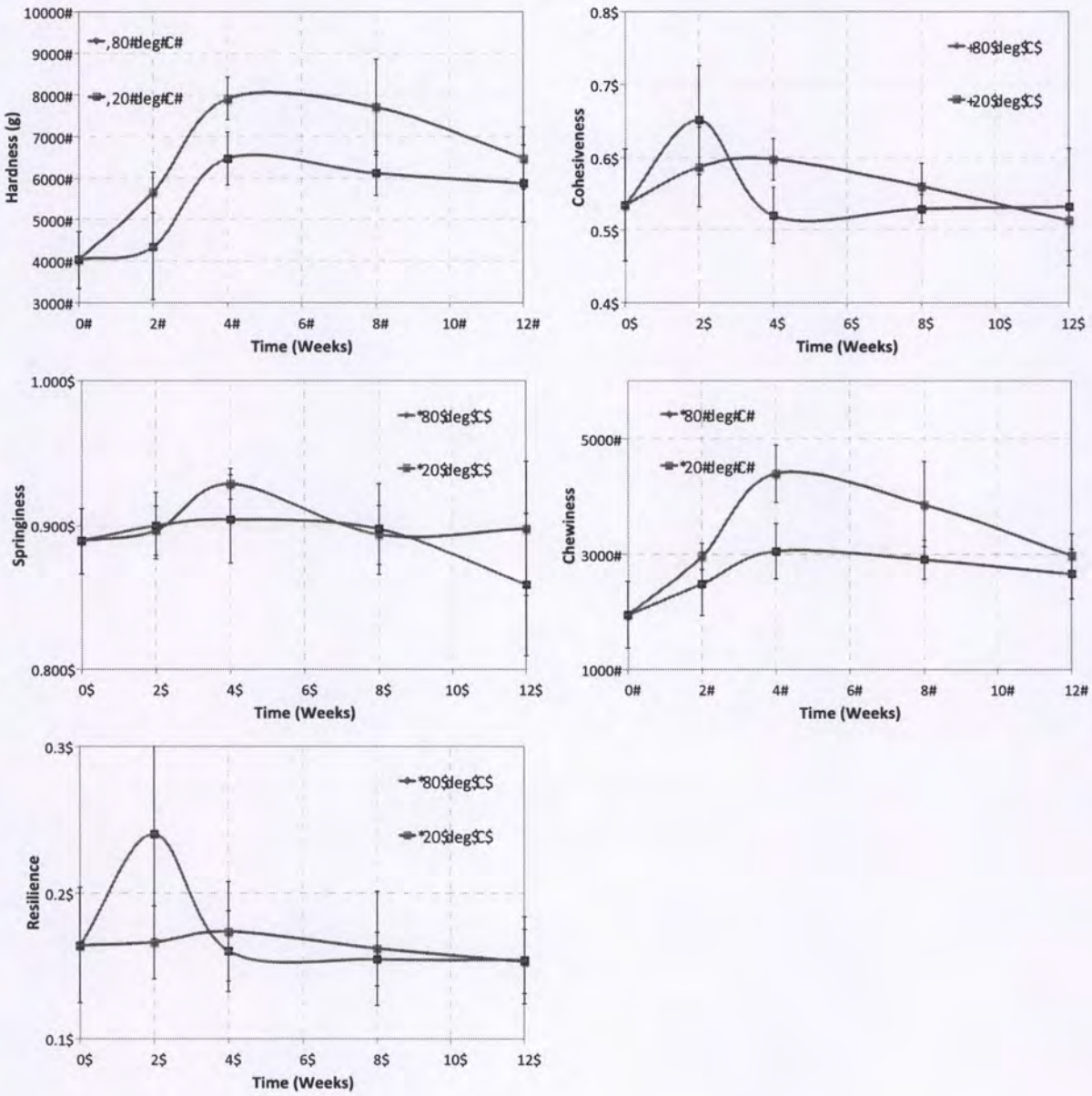
Table 2.5.2.1-1 Results of Stability Testing of Mung Bean Patties when Stored for 12 Weeks under Frozen Conditions (-20 and -80°C)				
Time Point	**pH**		**Moisture**	
	-80°C	**-20°C**	**-80°C**	**-20°C**
Day 0	8.07	8.07	68.43 ± 0.77	68.43 ± 0.77
Week 2	8.37	8.30	67.76 ± 1.66	64.09 ± 1.88
Week 4	8.13	8.18	62.95 ± 0.95	66.15 ± 0.78
Week 8	8.38	8.05	66.63 ± 0.33	67.39 ± 0.10
Week 12	8.23	8.10	68.02 ± 2.14	68.57 ± 1.32

2.5.2.2 Texture Profile

The texture profile (hardness, chewiness, springiness, resilience, and cohesiveness) of the mung bean patty prepared as described in Part 2.5.2.1 was evaluated at day 0, and weeks 2, 4,

8, and 12. The storage conditions (-20 and -80°C) were similar to what was previously indicated in Part 2.5.2.1. Texture profile analysis (TPA) was performed on a Brookfield CT3 analyzer using a cylindrical probe (38 mm diameter) set for 70% deformation, trigger load of 5.0 g, and test speed of 1 mm/s. The results of the analysis are provided in Figure 2.5.2.2-1. Overall, the hardness, chewiness, and cohesiveness of the mung bean patty increased in the first 4 weeks of the study, and were not significantly different between weeks 8 and 12. Although TPA measurements changed through first half of the study, sensory panel members perceived no changes on tasting and reported no significant differences in patty quality.

Figure 2.5.2.2-1 Texture Profile Analysis of Mung Bean Patties Stored at -20 and -80°C Over 12 Weeks



Part 3. §170.235 Dietary Exposure

3.1 Probable Consumption

Background Dietary Intakes of Mung Bean Protein Isolate

The protein content of mung bean has been reported to be in the range of 14.6 to 33.0 g/100 g dry matter or approximately 15 to 33% by weight (Dahiya *et al.,* 2015). In 2010, it was estimated that the U.S. consumption of mung beans was in the range of 15 to 20 million pounds (USDA-ERS, 2015). Using the 2010 U.S. population (*i.e.*, 308,745,538) and the highest consumption rate (20 million pounds), the annual *per capita* intake of mung bean is estimated to be 0.065 lbs or 30,000 mg, corresponding to a daily intake of approximately 82 mg. Assuming a mean protein content of 24% (ranging from 15 to 33%), daily dietary exposure to mung bean protein was calculated to be approximately 19 mg (USDA-ERS, 2015).

The annual *per capita* consumption of mung beans in China was reported to increase from 0.3 kg to 0.5 kg between 1986 to 2000 (Shanmugasundaram *et al.*, 2009; Global Pulse Confederation, 2015). This corresponds to a daily *per capita* intake ranging from approximately 822 to 1,400 mg for mung beans and approximately 197 to 336 mg for mung bean protein isolate (assuming a protein content of 24%).

In India, the annual *per capita* consumption of mung beans was reported to range from 0.8 to 2.1 kg (Vijayalakshmi *et al.*, 2003; Global Pulse Confederation, 2015), corresponding to a daily *per capita* intake ranging from approximately 2,192 to 5,753 mg. Based on the annual *per capita* estimate, and assuming a mean protein content of 24%, the daily dietary exposure to mung bean protein in India would be calculated as approximately 526 to 1,380 mg.

The *per capita* calculations presented herein provide estimates for the 'average' consumption across the population, assuming equal intake by every individual in the respective countries; in reality, intakes by consumers (or users) will be higher. In addition, these figures are not representative of intakes by 'heavy consumers' (WHO, 1985; U.S. FDA, 2006a). Empirical evidence illustrates that the 90th percentile estimate of intakes for commonly consumed foods is approximately 2 times the mean (equivalent to 2.76 g).

As summarized in Table 3.1-1, background dietary exposure to mung bean protein in the U.S. is approximately 72 times lower than the levels of safe consumption in India (19 *vs* 1,380 mg) that are considered as heavy consumers of mung beans.

Table 3.1-1 Global Consumption of Mung Bean Protein		
Country	**Daily *Per Capita* Intakes of Mung Bean* (mg)**	**Daily *Per Capita* Intakes of the Mung Bean Protein** (mg)**
United States	82	19
China	822 to 1,400	197 to 336
India	2,192 to 5,753	526 to 1,380

* Vijayalakshmi *et al.*, 2003; FPRI (Shanmugasundaram *et al.*, 2009); Global Pulse Confederation, 2015; USDA-ERS, 2015
** Calculated assuming a protein content of 24%.

Dietary Intake in General U.S. Population from all Proposed Food Uses

The estimates for the intake of mung bean protein isolate were generated using the maximum use level indicated for each intended food-use, as presented in Table 1.3-1, together with food consumption data available from the 2011-2012 NHANES dataset (USDA, 2014; CDC, 2015).

A summary of the estimated daily intake of mung bean protein isolate from proposed food-uses is provided in Table 3.1-2 on an absolute basis (g/person/day), and in Table 3.1-3 on a body weight basis (mg/kg body weight/day).

The percentage of users was high among all age groups evaluated in the current intake assessment; greater than 72.6% of the population groups consisted of users of those food products in which mung bean protein isolate is currently proposed for use (Table 3.1-2). Children had the greatest percentage of users at 97.6%. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed in detail.

Among the total population, the mean and 90th percentile all-user intakes of mung bean protein isolate was determined to be 10.1 and 22.5 g/person/day, respectively. Of the individual population groups, female teenagers were determined to have the greatest mean and 90th percentile all-user intakes of mung bean protein isolate on an absolute basis, at 14.8 and 34.3 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile all-user intakes of 4.9 and 10.7 g/person/day, respectively (Table 3.1-2).

Table 3.1-2 Summary of the Estimated Daily Intake of Mung Bean Protein Isolate from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants and Young Children	Up to 3	3.6	9.7	72.6	466	4.9	10.7
Children	3 to 11	6.7	14.1	97.6	1,472	6.9	14.5
Female Teenagers	12 to 19	13.6	34.3	91.8	494	14.8	34.3
Male Teenagers	12 to 19	9.7	24.6	90.4	467	10.8	25.9
Female Adults	20 and up	8.5	20.0	92.8	2,029	9.2	21.2
Male Adults	20 and up	10.3	24.1	87.4	1,802	11.8	26.6
Total Population	All Ages	9.1	21.2	90.6	6,730	10.1	22.5

NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, infants and young children were identified as having the highest mean and 90th percentile all-user intakes of any population group, of 403 and 919 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile all-user intakes of 129 and 280 mg/kg body weight/day, respectively (Table 3.1-3).

Table 3.1-3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Mung Bean Protein Isolate from Proposed Food-Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	Up to 3	293	748	72.6	463	403	919
Children	3 to 11	261	589	97.6	1,472	267	593
Female Teenagers	12 to 19	232	551	91.6	483	253	553
Male Teenagers	12 to 19	150	364	90.4	465	166	387
Female Adults	20 and up	120	278	92.8	2,008	129	280
Male Adults	20 and up	123	290	87.5	1,788	140	323
Total Population	All Ages	152	370	90.6	6,679	168	401

bw = body weight; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Consumption data from the 2011-2012 NHANES dataset and information pertaining to the individual proposed food-uses of mung bean protein isolate were used to estimate the all-person and all-user intakes for specific demographic groups and for the total U.S. population. Several conservative assumptions have been included in the present assessment, which means that resulting values should be considered 'worst case' estimates of exposure for the target

population. For example, it was assumed that all food products within a food category contain the ingredients at the maximum specified level of use. In reality, the levels of mung bean protein isolate added to specific foods will vary and are unlikely to have 100% market penetration. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently (Anderson, 1988).

In summary, on an all-user basis, the resulting mean and 90th percentile intakes of mung bean protein isolate by the total U.S. population from all proposed food-uses in the U.S., were estimated to be 10.1 g/person/day (168 mg/kg body weight/day) and 22.5 g/person/day (401 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of mung bean protein isolate were determined to be 14.8 g/person/day (253 mg/kg body weight/day) and 34.3 g/person/day (553 mg/kg body weight/day), respectively, as identified among female teenagers. When intakes were expressed on a body weight basis, infants and young children had the highest mean and 90th percentile all-user intakes of 403 and 919 mg/kg body weight/day, respectively. The mean calculated all-user intakes of the mung bean protein isolate by the total U.S. population from all proposed food-uses is approximately 7 times higher than the background intake of the mung bean protein in India (10.1g *versus* ~1.4g), as presented in Table 3.1-1.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with Hampton Creek's mung bean protein isolate.

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

Not applicable.

Part 6. §170.250 Narrative

The conclusion that mung bean protein isolate, as described herein, is GRAS under the conditions of its intended use in specified conventional food and beverage products is based on scientific procedures using generally available data and information characterizing the protein composition and quality of the mung bean protein isolate, and the substantial history of safe consumption of mung beans in the human diet. In the absence of product-specific safety data for mung bean protein isolate, the safety of the ingredient is addressed using an approach similar to a 2-tiered weight of evidence strategy that was previously proposed by the International Life Sciences Institute (ILSI) to assess the safety of newly expressed proteins in

agricultural products produced through biotechnology (Delaney *et al.*, 2008; Hammond *et al.*, 2013). This approach was developed in collaboration with experts on protein and food safety. In Tier I, no *in vivo* toxicology testing is considered necessary if the protein meets all of the following criteria: 1) there is a history of safe use of the protein or related proteins in foods; 2) the protein is not structurally or functionally related to proteins considered to be toxic (or allergenic) to humans or animals; 3) the protein has a molecular or biological function that raises no safety concerns; and (4) the protein is readily digested in validated *in vitro* digestive tests. If during Tier I assessment any safety issues are raised, or if assurance of safety is not feasible due to limited available information, then additional safety testing, such as repeat-dose animal toxicity studies, may be required for Tier II safety assessment (Delaney *et al.*, 2008; Hammond *et al.*, 2013). Using this approach, the safety of the protein isolate is assessed below based on the following criteria: Compositional analyses (Part 6.1); history of safe use (Part 6.2); nutritional information (Part 6.3); and allergenicity (Part 6.4). A discussion of the findings of an independent Expert Panel specially convened by Hampton Creek to evaluate the GRAS status of ingredient are discussed in Part 6.4 and a conclusion on GRAS status is presented in Part 6.5.

6.1 Compositional Analysis

As previously described in Part 2.2, considering that the manufacturing process of the mung bean protein isolate mainly involves physical processes, the protein is not chemically-modified and exogenous factors are not introduced into the manufacturing process that would alter the nutritional and functional properties of the protein isolate. Taking this into account, it can be deduced that the protein isolate is compositionally similar to the parent whole mung bean.

As presented in Part 2.3.3, analyses of several representative batches of the mung bean protein isolate indicated the absence of environmental contaminants, including pesticides, mycotoxins, PCBs, dioxins and heavy metals, lead, arsenic, cadmium, and mercury. In addition, the levels of anti-nutritional factors, including lectins, protease inhibitors, polyphenols, and phytic acid were shown to be reduced during the protein isolation process, as demonstrated by analytical data comparing the representative batches of the mung bean protein isolate with those of the mung bean flour, which was used as the starting material in the manufacturing process. Accordingly, the small presence of the anti-nutritional factors in the mung bean protein isolate is not expected to negatively impact the availability of other nutrients in foods to which the mung bean protein isolate is added. Overall, by means of analytical data it was demonstrated that the mung bean protein isolate is devoid of any toxicological, nutritional, or microbiological hazards that would arise from the production process.

6.2 History of Safe Use

Hampton Creek's mung bean protein isolate is comparable to the protein occurring as a normal constituent of mung beans, which are commonly consumed as part of the typical human diet

with a long history of safe use (Thirumaran and Seralathan, 1988; Shanmugasundaram *et al.*, 2009; Tang *et al.*, 2014). As discussed in Part 5.4.2, on an all-user basis, the resulting mean and 90th percentile intakes of the mung bean protein isolate by the total U.S. population from all proposed food-uses were estimated to be 10.1 g/person/day and 22.5 g/person/day, respectively. These intake levels are comparable with the estimated consumption from other plant-based proteins, such as pea protein concentrate (mean and 90th percentile intakes of 10.3 g/person and 17.3 g/person, respectively, GRN 608 – U.S. FDA, 2015b) and rice protein concentrate (mean and 90th percentile intakes of 10.3 g/person and 17.3 g/person, respectively, GRN 609 – U.S. FDA, 2015c), and are much lower than those occurring from use of cruciferin-rich canola/rapeseed protein isolate and napin-rich protein canola/rapeseed protein isolate (mean and 90th percentile intake of >20.9 and >57.9 g/day, respectively GRN 327 – U.S. FDA, 2010) and oat protein (46 g/day for women >19 years and 56 g/day for men >19 years, GRN 575 – U.S. FDA, 2015d), in various food products, which have already received a 'no questions letter' from the FDA, indicating that their intended uses are GRAS.

Furthermore, the FDA has established a Daily Reference Value (DRV) of 50 g for protein for adults and children 4 or more years of age. Although the 90th percentile all-user intakes of the mung bean protein isolate by the total U.S. population from all proposed food-uses (*i.e.*, 22.5 g/day) is almost half of the DRV, it should be noted that the mung bean protein isolate has been proposed as an alternative source of protein. As such, most of the population's protein intake is derived from, and will continue to be derived from, unprocessed foods, including meat, poultry, fish, and legumes. For those processed foods to which the mung bean protein isolate will be added, there are competitive products on the market. Thus, the addition of the mung bean protein isolate will simply serve as a replacement for these other competitive protein sources and is therefore unlikely to increase consumer exposure to protein.

6.3 Nutritional Information

6.3.1 *In Vitro* Protein Digestibility

The *in vitro protein* digestibility of mung beans was assessed in a study by Hira *et al.* (1988), who reported an *in vitro* protein digestibility value of 72.5% for raw mung beans after incubation with pepsin and pancreatin in an *in vitro* digestion model based on a methodology by Akeson and Stahmann (1964). The effects of domestic traditional processes, such as soaking, dehulling, boiling, autoclaving, microwave cooking, and germination on the *in vitro protein* digestibility of mung beans was evaluated by Mubarak (2005), using a two-digestive-enzyme system of trypsin and pancreatin based on a methodology by Salgó *et al.* (1985). In this study, the *in vitro protein* digestibility of raw mung beans was reported to be approximately 80%, and digestibility was shown to be improved following each aforementioned processing step (Mubarak, 2005). The improvement in digestibility was reported by the authors to be likely

attributed to denaturation of protein, destruction of the trypsin inhibitor, and reduction of anti-nutritional factors, including tannins and phytic acid (Mubarak, 2005).

In a similar study, Kataria *et al.* (1989) evaluated the *in vitro* protein digestibility of mung beans following domestic processing and cooking as determined using the method by Akeson and Stahmann (1964). Soaking of the seeds in water for 18 hours improved protein digestibility values from 56 to approximately 68%, as a result of a decrease in anti-nutrients such as phytic acid and polyphenols (Kataria *et al.*, 1989). Similarly, the soaking process was shown to improve protein digestibility compared to unsoaked seeds when ordinary-cooked (70 *versus* 63%) or pressure-cooked (83 *versus* 71%), which is likely due to the destruction of trypsin inhibitors and denaturation of the proteins (Kataria *et al.*, 1989). Similarly, Khatoon and Prakash (2006) found an improvement in protein digestibility when soaked mung beans were pressure-cooked, as determined by the Akeson and Stahmann (1964) methodology.

The *in vitro* protein digestibility of mung bean increased following germination with an increase in the protein digestibility from 70 to approximately 81%, as the period of germination increased from 24 to 60 hours (Kataria *et al.*, 1989). This is consistent with the results of a study by Khatoon and Prakash (2006) demonstrating that germination improved the *in vitro* protein digestibility of raw mung beans from 64.6 to 72.4%. This effect was reported to be likely attributed to the modification and degradation of storage proteins (Kataria *et al.*, 1989).

The *in vitro* protein digestibility of mung beans was reported to improve following irradiation under an ultraviolet lamp (Singh and Padmakar, 1991). When soaked mung beans were irradiated for 90 seconds, protein digestibility increased from 52 at time zero to 58% (Singh and Padmakar, 1991), as determined using the method by Akeson and Stahmann (1964).

Overall, the results of various *in vitro* protein digestibility studies reported values ranging from 52 to 83%, with processes such as soaking, cooking, irradiation and germination contributed to the improved protein digestibility. The variation noted in the protein digestibility is likely attributed to the difference in the actual protein content due to various locations where mung beans were sourced, differences in the methodologies, as well as the presence of anti-nutrients, which contribute to reduced protein digestibility.

Taken together, considering that the mung bean protein isolate is minimally processed, and thus, compositionally and nutritionally similar to the protein within the parent whole mung beans, it is expected that following consumption, biodisposition of the mung bean protein isolate will be the same as the parent mung beans or other commonly consumed dietary protein sources.

6.3.2 Protein Quality Evaluation

Protein quality evaluation aims to determine the capacity of food protein sources to meet the protein and essential amino acid requirements, and to satisfy the metabolic needs for amino

acids and nitrogen. Several methods are commonly used to assess the quality and nutritional value of a protein. These methods include the Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), Biological Value (BV), Protein Digestibility Corrected Amino Acid Score (PDCAAS), and Digestible Indispensable Amino Acid Score (DIAAS).

The 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 mung beans the PER value was reported to be range from 1.86 to 4.29, and was shown to be improved slightly by germination and cooking processes (Bhatty *et al.*, 2000; Mubarak, 2005; Dahiya *et al.*, 2015). Rat feeding studies demonstrated that a combination of 75% protein from rice and 25% protein from mung bean results in a PER value equivalent to 75% of casein protein (Tsou and Hsu, 1978). Although mung beans are adequate in most essential amino acids, as previously presented in Part 2.3.3.1, they have lower sulfur-containing amino acids methionine and cysteine.

Another factor in determining the quality of protein is the NPU value, as a measure of the percentage of amino acids consumed that are eventually converted into proteins and utilized by the body. The NPU values for mung beans were reported to range from 53 to approximately 60 (Dahiya *et al.*, 2015), indicating that on average about 56% of the mung bean protein is absorbed and retained (and in theory utilized) within the body. This is comparable with the average BV value of 64% reported for mung beans (Mubarak, 2005), which measures how efficiently the human body utilizes dietary protein.

6.3.2.1 Protein Digestibility Corrected Amino Acid Score for the Mung Bean Protein Isolate

The PDCAAS rating, which was proposed by the Food and Agriculture Organization of the United Nations (FAO) in 1989, was adopted by the FDA in 1993 as "the preferred best" method to evaluate protein quality (FAO/WHO, 1991; U.S. FDA, 1993). This method is based on the principle that the nutritive value of a protein depends upon its capacity to provide nitrogen and amino acids in adequate amounts to meet human (essential) amino acid requirements. While the quality of some proteins can be assessed directly using amino acid score values, others cannot because of poor digestibility and/or bioavailability. Consequently, both amino acid composition and digestibility measurements are considered necessary to accurately predict the protein quality of foods for human diets (FAO/WHO, 1991). In practice, the PDCAAS relates the content of the first limiting essential amino acid of the test protein to the content of the same amino acid in a reference pattern of essential amino acids (*i.e.*, amino acid score), correcting for fecal digestibility, which is often measured using a rat balance assay (FAO/WHO, 1991).

The PDCAAS for the mung bean protein isolate is calculated using the following formula, where the reference pattern of essential amino acids is based on amino acid requirements of 2- to

5-year-old pre-school aged children, which was determined by the FAO/WHO/UNU in 1985 (see Table 6.3.2.1-1).

$$\text{PDCAAS (\%)} = \frac{\text{mg of limiting amino acid in 1 g of test protein}}{\text{mg of same amino acid in 1 g of reference protein}} \times \text{fecal digestibility} \times 100\%$$

Table 6.3.2.1-1 Calculation of Amino Acid Scores for the Mung Bean Protein Isolate

Essential Amino Acid	Total Amino Acid Content* (mg/g protein)	FAO Reference Requirements for Amino Acids** (mg/g crude protein)	Calculated Amino Acid Scores Using FAO Reference Requirements
Histidine	28.7	19	1.51
Isoleucine	48.6	28	1.73
Leucine	85.8	66	1.30
Lysine	70.7	58	1.22
Methionine	16.3	25	**0.65**
Cysteine			
Tyrosine	101.5	63	1.61
Phenylalanine			
Threonine	28.1	34	0.83
Tryptophan	9.5	11	0.86
Valine	54.7	35	1.56

FAO = Food and Agriculture Organization of the United Nations
* The values for each amino acid is the mean of 4 batch data presented in Table II.C.3.1-1.
** Reference requirements for amino acids as determined by the FAO for 2- to 5-year-old pre-school aged children (FAO/WHO/UNU, 1985).

As presented in Table 6.3.2.1-1, the limiting amino acids in the protein isolate are the sulfur-containing amino acids, methionine and cysteine having the lowest amino acid score of 0.65. Taking the amino acid score of 0.65 into account and based on a true fecal digestibility of 84% reported for mung beans (Khan *et al.*, 1979), the % PDCAAS score for the mung bean protein isolate is calculated as 55% (*i.e.*, 0.65 x 84%).

Additionally, Hampton Creek has conducted an *in vivo* fecal digestibility study to determine the true digestibility (TD) of their protein isolate (2 batches) and to calculate the PDCASS score. In this study, groups of albino male Sprague-Dawley rats (4/group) were fed 15 g/day test diets containing 10% protein (casein, uncooked protein isolate or cooked protein isolate prepared as described in Part 2.5.2.1) and other nutrients including vitamins, minerals and calories necessary for rat survival for 9 consecutive days. The control group received a protein-free diet formulated to contain the same level of nutrients as the test diets except for protein that was replaced with cornstarch. Feces were collected from day 5 through 9, composited, dried and analyzed for nitrogen content using Kjeldahl method. As presented in Table 6.3.2.1-2, the TD of the mung bean protein isolate ranged from 94 to 97% and the % PDCAAS score was calculated

ranging from 50 to 56%, which is in agreement with the score of 55% calculated using the true fecal digestibility values reported by Khan *et al.*, 1979, as described in the previous paragraph. True digestibility was not affected by the cooking process (see Table 6.3.2.1-2).

Table 6.3.2.1-2 Fecal Digestibility of Cooked and Uncooked Mung Bead Protein Isolate in Rats

Parameter	Manufacturing Lot No.			
	Batch 1 (124.1)		Batch 2 (143.1)	
	Uncooked	Cooked (ML_16)	Uncooked	Cooked (ML_14)
True Digestibility (%)	95.696	97.321	97.004	94.754
Amino Acid Score	0.592	0.561	0.580	0.532
PDCAAS (%)	56.65	54.60	56.26	50.41

PDCAAS = Protein Digestibility Corrected Amino Acid Score

6.3.2.2 Digestible Indispensable Amino Acid Score for the Mung Bean Protein Isolate

Recently, FAO has recommended a new and advanced method for assessing the quality of dietary proteins called the DIAAS to replace PDCAAS as the preferred method of measuring protein quality, 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 (FAO, 2013).

Accordingly, the DIAAS method was used to estimate the protein quality of mung bean protein isolate. 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 mung bean protein isolate would be:

$$\text{DIAAS (\%)} = 100\% \times \frac{mg\ of\ digesible\ IAA\ in\ 1\ g\ of\ mung\ bean\ protein\ isolate}{mg\ of\ the\ same\ IAA\ in\ 1\ g\ of\ the\ reference\ protein}$$

where digestible IAA is the amount of the amino acid in mung bean protein isolate (in mg per 1 g of protein) adjusted by the true ileal IAA digestibility. The calculation of digestible IAA in mung bean protein isolate (*i.e.,* the numerator of the formula) is provided in Table 6.3.2.2-1.

Table 6.3.2.2-1 Calculation of Digestible Indispensable Amino Acid in Mung Bean Protein Isolate			
IAA	**IAA Content in Mung Bean Protein Isolate (mg AA/g protein)[a]**	**True Ileal Digestibility of IAA in Cooked Mung Beans (%)[b]**	**Digestible IAA in Mung Bean Protein Isolate (%)[c]**
Histidine	28.7	82	23.5
Isoleucine	48.6	75	36.4
Leucine	85.8	80	68.7
Lysine	70.7	92	65.0
Methionine	12.9	77	9.9
Cysteine	3.4	70	2.4
Tyrosine	32.5	87	28.3
Phenylalanine	69.0	84	58.0
Threonine	28.1	84	23.6
Tryptophan	9.5	77	7.3
Valine	54.7	80	43.8

IAA = indispensable amino acid.
[a] Based on the average of the amino acid compositions provided in Table 2.3.3.1-1.
[b] Based on the true ileal digestibility of IAA in cooked mung beans provided in Moughan *et al.* (2012). Two sets of true ileal digestibility of IAA in cooked mung beans were provided in the publication, differing in how the endogenous amino acid losses were determined (*i.e.,* either based on the protein-free diet method *versus* based on the enzyme hydrolyzed ultrafiltration method). The values based on the protein-free diet are provided in this table. The DIAAS of mung bean protein based on values derived by the enzyme hydrolyzed ultrafiltration method will be provided below.
[c] Digestible Indispensable AA in mung bean protein isolate was calculated by multiplying indispensable AA content in mung bean protein isolate with true ileal digestibility of indispensable AA in cooked mung beans (*i.e.,* column 2 multiplied by column 3).

The amount of IAA in the reference protein (*i.e.,* the denominator of the DIAAS formula) is also known as the amino acid pattern of the reference protein and is based on the amino acid requirements of different age groups. The calculation of DIAAS of mung bean protein isolate based on the FAO recommended amino acid scoring patterns of different age groups is provided Table 6.3.2.2-2.

Table 6.3.2.2-2 Calculation of Digestible Indispensable Amino Acid Score of Mung Bean Protein Isolate							
Indispensable AA	**Digestible IAA in Mung Bean Protein Isolate (%)[a]**	**IAA in the Reference Protein (mg IAA per g of protein)[b]**			**DIAAS (%)[c]**		
		Infant	**Child**	**Older Children**	**Infant**	**Child**	**Older Children**
Histidine	23.5	21	20	16	112.1	117.7	147.1
Isoleucine	36.4	55	32	30	66.2	113.8	121.4
Leucine	68.7	96	66	61	71.5	104	112.6
Lysine	65.0	69	57	48	94.3	114.1	135.5
Methionine + Cysteine	12.3	33	27	23	**37.3[d]**	**45.6[d]**	**53.5[d]**
Tyrosine + Phenylalanine	86.3	94	52	41	91.7	165.8	210.3
Threonine	23.6	44	31	25	53.6	76.1	94.3
Tryptophan	7.3	17	8.5	6.6	43.0	86.1	110.8
Valine	43.8	55	43	40	79.6	101.8	109.4

IAA = indispensable amino acid.
[a] Based on the digestible IAA in mung bean protein isolate as calculated in Table 6.4.2-1.
[b] Based on the FAO recommended amino acid scoring pattern. Amino acid scoring patterns for infants, 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. The amino acid scoring pattern for older children is used for calculating the DIAAS of adolescents and adults.
[c] Based on dividing "Digestible IAA in mung bean protein isolate" by "IAA in the Reference Protein" (*i.e.,* dividing column 2 by columns 3 through 5).
[d] Methionine and cysteine (*i.e.,* sulphur-containing IAA) are the limiting IAA for mung bean protein isolates.

As presented in Table 6.3.2.2-2, and consistent with the PDCAAS analysis, methionine and cysteine are the only limiting digestible indispensable amino acids for the mung bean protein isolate. The DIAAS of the mung protein isolate is calculated as 37.3, 45.6, and 53.5% for infants, children, and older children, adolescents, or adults, respectively[1].

6.4 Allergenicity Potential

In comparison with other legumes, reported allergenic reactions to mung bean are not common. Allergenicity associated with consumption of legumes in decreasing order has been shown to be peanut, soybean, lentil, chickpea, pea, mung bean, and red gram (Verma *et al.,* 2013). Rare cases of allergic reactions to mung beans have been mainly associated with consumption of sprouted mung beans (Mittag *et al.*, 2005), rather than the whole bean or the protein isolate. Mung beans are reported to contain 4 proteins that exhibited pepsin resistance and immunoglobulin E (IgE) binding capability with sensitized human and mice sera (Misra *et al.*, 2011). These proteins named Vig r1, Vig r2, Vig r3, and Vig r4 showed significant sequence similarity with known allergens of soybean, lentil, pea and lupin (Misra *et al.*, 2011; Verma *et al.*, 2013). The majority of protein allergens in mung bean belong to the seed storage family of

[1] Using the true ileal digestibility of indispensable amino acids in cooked mung beans when endogenous amino acid was based on the enzyme hydrolyzed ultrafiltration method, the DIAAS of mung protein isolates are 38.3, 46.8, and 54.9% for infants, children, and older children, adolescents, or adults, respectively.

proteins and cupin superfamily, namely, Vig r2 (8S globulin β-isoform precursor), Vig r3 (8S globulin α-subunit), and Vig r4 (seed albumin) (Misra *et al.*, 2011; Verma *et al.*, 2013). Conversely, the protein allergen, Vig r1, falls under the family of pathogenesis-related 10 (PR-10) proteins that are characterized by their small size, stability in acidic conditions, as well as resistance to proteolytic degradation (Verma *et al.*, 2013).

While data on the cross-reactivity potential of mung bean with other legumes or major allergens are limited, there has been one report indicating co-reactivity between peanut and mung bean seeds and sprouted mung beans (Jensen *et al.*, 2008). In this study, protein extracts of mung bean seeds and mung bean hypocotyls (parts of the embryo located below the cotyledon attachment) induced histamine release from cells sensitized with 10 peanut-allergic individuals' sera, with hypocotyl extracts of mung bean being more potent than the corresponding seed extract (median:10 *versus* 80 µg/mL) (Jensen *et al.*, 2008). These effects, however, were much weaker than those observed with seeds and hypocotyls of soybean (median: 8 *versus* 9 µg/mL) and lupine (median: 5 *versus* 1 µg/mL), and seed and epicotyl of pea (median: 20 *versus* 6 µg/mL) (Jensen *et al.*, 2008). These findings suggest that ingestion of pea, soybean, and lupine are more likely to induce an allergic reaction in peanut allergic-individuals than mung beans. Furthermore, while the prevalence of allergic reactions to pea and its cross-reactivity potential to other legumes has been reported to be greater than mung bean with several studies demonstrating cross-reactivity between pea proteins and peanut (Wensing *et al.*, 2003; Sanchez-Monge *et al.* 2004; Jensen *et al.*, 2008; Szymkiewicz and Chudzik-Kozlowska, 2013), various forms of pea protein, including pea protein isolate (GRN 182), un-hydrolyzed and hydrolyzed pea protein (GRN 581), and pea protein concentrate (GRN 608) have been determined to be GRAS for use as food ingredients and have subsequently received "good day" letters from the FDA thereby supporting the safety of their use in the food supply (U.S. FDA, 2006b, 2016a,b).

There have been reports of cross-reactivity between mung bean seedlings and birch pollen as a result of common antigenic determinants (Mittag *et al.*, 2005). The food allergy to mung bean seedlings was reported to be caused by primary sensitization to birch pollen and mediated by Vig r1 (Mittag *et al.*, 2005). Vig r1 in mung bean is homologous to Bet v1, the major birch pollen allergen belonging to the PR-10 family of proteins (Mittag *et al.*, 2005). PR-10 proteins are heat labile and their allergenic potential was reported to be destroyed by heating (Vieths *et al.*, 2002), as such, cooking mung beans will reduce its allergenic potential mediated by Vig r1.

As discussed in Section 2.4, no or trace levels (<0.003%) of PR-10 protein were found following proteomic analyses of 5 representative batches of the mung bean protein isolate. This finding is in agreement with the understanding that the major allergen in mung bean, Vig r1, is only detected in seedlings (Mittag *et al.*, 2005), while the mung bean protein isolate is derived from the de-hulled mung bean seeds that were not germinated. In addition, the analytical data presented in Section 3.4 indicated that the level of other putative allergens belonging to seed

storage family in the mung bean protein isolate was low and comparable to that in the mung bean flour, which was used as the starting material.

In summary, mung bean is not one of the major 8 food allergens that include milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans, and the available information suggests that the use of the mung bean protein isolate in foods is expected to have a very low potential to induce allergic reactions. Cross-reactivity in birch pollen allergic patients is not expected to occur due to the absence or negligible level of PR-10 proteins, such as Vig r1 in the mung bean protein isolate. As previously indicated, the prevalence of allergic reactions to mung beans and its cross-reactivity potential to other legumes is much lower than various forms of pea protein, including pea protein isolate (GRN 182), un-hydrolyzed and hydrolyzed pea protein (GRN 581), and pea protein concentrate (GRN 608) that have been considered GRAS by the FDA for their use in food and beverage products, despite several reports demonstrating cross-reactivity between pea proteins and peanut. Taken together, the use of the mung bean protein isolate as a food ingredient is not expected to raise a safety concern due to allergenic potential. (U.S. FDA, 2006b, 2016a,b)

6.5 Expert Panel Evaluation

Hampton Creek has concluded that the protein product (mung bean protein isolate), isolated from mung beans in its original form through a series of mechanical processing steps, manufactured consistent with cGMP and meeting appropriate food grade specifications, is GRAS for use as an ingredient in specified conventional food and beverage products, as described in Part 1.3, on the basis of scientific procedures using protein composition and quality measures, corroborated by a substantial history of safe consumption.

Hampton Creek's conclusion on the GRAS status of mung bean protein isolate under the conditions of its intended use is based on data generally available in the public domain using an approach similar to a 2-tiered weight of evidence strategy proposed by the ILSI (Delaney *et al.*, 2008; Hammond *et al.*, 2013) to assess the safety of the protein isolate. Based upon the outcome of the Tier 1 evaluation, as discussed in Parts 6.1 through 6.4, it was determined that *in vivo* toxicology testing was not necessary. More specifically, utilizing publically available data, the safety of the mung bean protein isolate was established on the basis of 1) product-specific compositional data; 2) a history of safe use (Thirumaran and Seralathan, 1988; Shanmugasundaram *et al.,* 2009; Tang *et al.,* 2014) and consumption estimates; 3) *in vitro* digestibility data (Hira *et al.*, 1988; Kataria *et al.*, 1989; Singh and Padmakar, 1991; Mubarak, 2005; Khatoon and Prakash, 2006); and 4) the non-allergenicity potential (Mittag *et al.*, 2005; Misra *et al.*, 2011; Verma *et al.*, 2013).

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of the mung bean protein isolate under conditions of its intended use. The Expert Panel consisted of the

following qualified scientific experts: Professor Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Professor Emeritus George C. Fahey, Jr. (University of Illinois), and Professor Stephen L. Taylor (University of Nebraska).[2]

The Expert Panel, convened by Hampton Creek, independently and critically evaluated all data and information presented herein and concluded that the mung bean protein isolate, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable for use as an ingredient in specified conventional food and beverage products, as described in Part 1.3, and is GRAS based on scientific procedures using protein composition and quality measures, corroborated by a substantial history of safe consumption. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the mung bean protein isolate is presented in Appendix A.

6.6 Conclusion

Based on the above data and information presented herein, Hampton Creek has concluded that the intended uses of the mung bean protein isolate in specified conventional food and beverage products, as described in Part 1.3, are GRAS based on scientific procedures using protein composition and quality measures, and corroborated by a substantial history of safe consumption. The GRAS status of mung bean protein isolate is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of mung bean protein isolate in conventional food and beverage products, as described herein, is GRAS.

The mung bean protein isolate, therefore. may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

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[2] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

Part 7. §170.255 List of Supporting Data and Information

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APPENDIX A

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Mung Bean Protein Isolate for Uses in Conventional Food and Beverage Products

November 1, 2016

INTRODUCTION

Hampton Creek convened a panel of independent scientists (the "Expert Panel"), qualified by their scientific training and relevant national and international experience in the safety evaluation of food ingredients, to conduct a critical and comprehensive assessment of the available pertinent data and information related to protein, isolated from mung beans in its original form through a series of mechanical processing steps, and to determine whether the intended uses of mung bean protein isolate in conventional food and beverage products (as described in Table A-1), would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of the below-signed qualified scientific experts: Professor Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Professor Emeritus George C. Fahey, Jr. (University of Illinois), and Professor Stephen L. Taylor (University of Nebraska).

The Expert Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data compiled from the literature. This information was presented in a dossier provided by Hampton Creek [Documentation Supporting the Evaluation of a Mung Bean Protein Isolate as Generally Recognized as Safe (GRAS) for Use in Conventional Food and Beverage Products in the United States, October 17, 2016, revised November 3, 2016], which included an evaluation of all available scientific data and information relevant to the safety of the intended food uses of Hampton Creek's ingredient. This information was prepared in part from a comprehensive search of the scientific literature and also included information characterizing the identity and purity of the ingredient, manufacture of the ingredient, product specifications, supporting analytical data, stability data, intended conditions of use, estimated exposure under the intended uses, information on the history of consumption, and studies investigating the allergenicity potential of mung beans. In addition, the Expert Panel evaluated other information deemed appropriate or necessary.

Following its independent critical evaluation, the Expert Panel unanimously concluded that mung bean protein isolate, produced using current Good Manufacturing Practices (cGMP) is Generally Recognized as Safe (GRAS), based on scientific procedures, corroborated by a substantial history of safe consumption, under the conditions of intended use in foods and beverage products as described in Table A-1. A summary of the information reviewed by the Expert Panel is provided below.

SUMMARY AND BASIS FOR GRAS

Hampton Creek intends to market mung bean protein isolate for use in a variety of food and beverage products in the U.S. The manufacturing process for the mung bean protein isolate is consistent with cGMP standards, and involves a series of mechanical processes using mung bean flour as the starting material. The mung bean flour is prepared by milling raw mung beans which have been de-hulled through a 3-step process of pitting, soaking, and drying. Considering that no processing agents (*e.g.,* solvents) are used during manufacturing that would chemically modify the protein structure, the mung bean protein isolate has similar nutritional and functional properties of the parent mung beans. The total protein content of the mung bean protein isolate is greater than 80% (on a dry weight basis) with the remainder being composed of carbohydrates, lipids, and mineral salts. All the materials used in the manufacture of the mung bean protein isolate are food-grade and permitted for use in food. The results of the analysis of 4 non-sequential batches of the mung bean protein isolate demonstrate that the manufacturing process produces a consistent product that meets product specifications. The interim results of an ongoing 24 month stability study indicate that the dry protein ingredient remains compositionally stable for a minimum of 6 months when stored at room temperature. In addition, preliminary results of an ongoing stability test of a representative finished food product formulated with the mung bean protein isolate as a major ingredient (mung bean patties), indicate that the protein within the product remains stable for a minimum of 12 weeks, when stored as recommended (frozen).

In the absence of product-specific safety data for mung bean protein isolate, the safety of the protein product was addressed using an approach based upon a 2-tiered weight of evidence strategy proposed by the International Life Sciences Institute (ILSI) to assess the safety of newly expressed proteins in agricultural products developed through biotechnology (Delaney *et al.*, 2008; Hammond *et al.*, 2013). This approach was developed in collaboration with experts in protein and food safety. Using this safety paradigm, the following criteria supported 1) there was a history of safe use of the protein or related proteins in foods; 2) the protein was not structurally or functionally related to proteins considered to be toxic (or allergenic) to humans or animals; 3) the protein has a molecular or biological function that raises no safety concerns; and (4) the protein is readily digested in validated *in vitro* digestive tests. Based upon the outcome of these Tier 1 evaluations, it was determined that *in vivo* toxicology testing was not necessary. More specifically, utilizing publically available data, the safety of the mung bean protein isolate was established on the basis of 1) product-specific compositional data; 2) a history of safe use (Thirumara and Seralathan, 1988; Shanmugasundaram *et al.,* 2009; Tang *et al.,* 2014) and consumption estimates; 3) *in vitro* digestibility data (Hira *et al.*, 1988; Kataria *et al.*, 1989; Singh and Padmakar, 1991; Mubarak, 2005; Khatoon and Prakash, 2006); and 4) the non-allergenicity potential (Mittag *et al.*, 2005; Misra *et al.*, 2011; Verma *et al.*, 2013). Chemical analysis of the mung bean protein isolate demonstrated that it is devoid of any toxicological, nutritional, or microbiological hazards either from the mung bean itself following agricultural practices and

cultivation or that may arise through the production process. These analyses include any potentially toxic inherent constituents (*e.g.*, anti-nutritional factors), or external contaminants (*e.g.*, pesticides, heavy metals, PCBs, dioxins, mycotoxins). The results of various *in vitro* protein digestibility studies reported values ranging from 52 to 83% for mung beans, with processes such as soaking, cooking, irradiation and germination contributing to the improved protein digestibility (Hira *et al.*, 1988; Kataria *et al.*, 1989; Singh and Padmakar, 1991; Mubarak, 2005; Khatoon and Prakash, 2006). The variation noted in the protein digestibility is likely attributed to the difference in the actual protein content due to various locations where mung beans were sourced from, differences in the methodologies, as well as the presence of anti-nutrients, which contribute to the reduced protein digestibility.

Considering that the mung bean protein isolate is minimally processed, and thus, compositionally and nutritionally similar to the protein within the parent whole mung beans, it is expected that following consumption, biodisposition of the mung bean protein isolate will be the same as the parent mung beans or other commonly consumed dietary protein sources. From a nutritional perspective, the mung bean protein isolate was also found to contain a balanced amino acid profile that compares favorably with the FAO reference protein (FAO, 2013).

The mung bean protein isolate is a comminuted product of a whole food (mung bean) that has a history of widespread consumption as a staple food commodity in countries outside the U.S. (*e.g.*, India and China). In 2010, it was estimated that the U.S. consumption of mung beans was in the range of 15 to 20 million pounds (USDA-ERS, 2015). Using the 2010 U.S. population (*i.e.*, 308,745,538) and the highest consumption rate (20 million pounds), the annual *per capita* intake of mung bean was estimated to be 0.065 lbs or 30,000 mg, corresponding to a daily intake of approximately 82 mg. Assuming a mean protein content of 24% (ranging from 15 to 33%) (Dahiya *et al.*, 2015), the daily dietary exposure to mung bean protein was calculated to be approximately 19 mg (USDA-ERS, 2015). In comparison, the annual *per capita* consumption of mung beans in China was reported to increase from 0.3 kg to 0.5 kg between 1986 to 2000 (Shanmugasundaram *et al.*, 2009; Global Pulse Confederation, 2015). This corresponds to a daily *per capita* intake ranging from approximately 822 mg to 1,400 mg for mung beans and approximately 197 mg to 336 mg for mung bean protein isolate (assuming a protein content of 24%). In India, the annual *per capita* consumption of mung beans was reported to range from 0.8 kg to 2.1 kg (Vijayalakshmi *et al.*, 2003; Global Pulse Confederation, 2015), corresponding to a daily *per capita* intake ranging from approximately 2,192 mg to 5,753 mg. Based on the annual *per capita* estimate, and assuming a mean protein content of 24%, the daily dietary exposure to mung bean protein in India was calculated to be approximately 526 mg to 1,380 mg. The *per capita* calculations presented herein provide estimates for the 'average' consumption across the population, assuming equal intake by every individual in the respective countries; in reality, intakes by consumers (or users) will be higher. In addition, these figures are not representative of intakes by 'heavy consumers'. Empirical evidence illustrates that the 90th percentile estimate of intakes for commonly consumed foods is approximately 2 times the

mean (WHO, 1985; U.S. FDA, 2006a). Hampton Creek intends to use the mung bean protein isolate as a direct protein replacement of animal- or vegetable-based protein currently used in food and beverages in the U.S., and as a supplement to the protein occurring in existing food products at use levels ranging from 3 to 90%, w/w of the final product. From all proposed food-uses of the mung bean protein isolate in the U.S., on an all-user basis, the resulting mean and 90th percentile intakes of mung bean protein isolate by the total U.S. population were estimated to be 10.1 g/person/day (168 mg/kg body weight/day) and 22.5 g/person/day (401 mg/kg body weight/day), respectively. These daily consumption levels are comparable with the estimated intakes of other GRAS determined plant-based proteins, such as pea protein concentrate and rice protein concentrate, which have received a 'no questions letter' from the FDA, following notification (U.S. FDA, 2015a,b). In addition, the mean calculated all-user intakes of the mung bean protein isolate by the total U.S. population from all proposed food-uses will be approximately 7 times higher than the background *per capita* intake of the mung bean protein in India, as the heaviest consumers of mung beans (10.1 g *versus* ~1.4 g). The estimated mean and 90th percentile figures for mung bean protein isolate by the U.S. population illustrates good agreement with the statement above regarding the typically observed difference between these two percentiles, *i.e.*, a 2.2-fold difference is noted. This indicates that a similar difference would be expected between 90th percentile consumers of mung bean protein isolate and high consumers of mung bean protein in India. While the mung bean protein isolate is considered an alternative source of dietary protein, it is still envisioned that most of the population's protein intake will continue to be derived from unprocessed foods, including meat, poultry, fish, and legumes. For those processed foods to which the mung bean protein isolate will be added, it is likely that this product will substitute for other competitive products in the marketplace, and therefore, will not increase the overall consumer exposure to protein.

Mung bean is a widely consumed legume throughout the world and is not a major food allergen, and available information suggests that the use of the mung bean protein isolate in foods is expected to have a very low potential to induce allergic reactions. Rare cases of allergic reactions to mung beans have been mainly associated with consumption of sprouted mung beans, rather than the whole bean or the protein isolate. Cross-reactivity in birch pollen allergic patients is not expected to occur due to the absence or trace levels of PR-10 proteins, such as Vig r1 in the mung bean protein isolate. There is limited evidence on the cross-reactivity potential of mung beans with other legumes or any of the major food allergens. While there is one report showing possibility of co-reactivity between peanut and mung bean, the study suggests potentially higher likelihood of an induced allergic reaction from pea, soybean and lupine than mung bean (Jensen *et al.,* 2008). Various forms of pea protein have received a "good day" letter from the FDA (GRN 182, GRN 581, GRN 608), despite several reports of the cross-reactivity of pea proteins with peanut, one of the 8 major food allergens (U.S. FDA, 2006b, 2016a,b). The weight of the available evidence suggests the use of the mung bean protein isolate as a food ingredient is not expected to raise a safety concern due to potential allergenicity.

CONCLUSION

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that the mung bean protein isolate, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable for use as an ingredient in specified conventional food and beverage products described in Table A-1.

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that the mung bean protein isolate, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice is Generally Recognized as Safe (GRAS), based on scientific procedures using protein composition and quality measures, corroborated by a substantial history of safe consumption, for use in specified conventional food and beverage products as described in Table A-1.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

11 November 2016

Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

Date

(b) (6)

14 November 16

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Date

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Date

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Attachment A1

Intended Food and Beverage Uses and Use Levels for the Mung Bean Protein Isolate in the United States

Table A-1 Summary of the Individual Proposed Food-Uses and Use Levels for Mung Bean Protein Isolate in the United States		
Food Category	**Proposed Food-Uses**	**Maximum Intended Use Level of Mung Bean Protein Isolate (%) in Final Product**
Baked Goods and Baking Mixes	Cereal and granola bars	10
	Crackers	5
	Meal replacement/nutritional bars/energy bars	30
Beverages and Beverage Bases	Fermented beverages made from rice/barley/grains/legumes/tea[a]	8
	Non-milk based instant protein powders	90 (powder)
	Non-milk based nutritional beverages (RTD, and powdered types) including protein-enriched fruit smoothies[b]	20 (as consumed)
	Non-milk based weight control beverages, instant shakes, and protein drinks (RTD and powdered types)[c]	10 (as consumed)
Breakfast Cereals	Breakfast cereals (RTE)	3
Condiments and Relishes	Bean dips and spreads	5
	Seasoning sauces	3
Dairy Product Analogs	Non-dairy cheese	5
	Non-dairy cream cheese, spread, and dips[d]	5
	Non-dairy cream or sour cream (liquid and powder)	3
	Non-dairy ice cream and frozen desserts[d]	3
	Non-dairy milk	3
	Non-dairy coffee whiteners[e]	3
	Non-dairy yogurt and non-dairy drinkable yogurts[f]	8
	Whipped topping	3
Frozen Dairy Desserts and Mixes	Ice cream[g] and other frozen dairy desserts	3
Fruit and Water Ices	Ice pops and sorbets	3
Gelatins, Puddings, and Fillings	Puddings and mousse	3
Grain Products and Pasta	Pasta	4
Milk Products	Milk-based instant protein powders	90 (powder)
	Milk-based nutritional beverages (RTD and powdered types)	5 (as consumed)
	Milk-based weight control beverages, instant milkshakes, protein drinks (RTD and powdered types), and milk-based smoothies	3 (as consumed)

Table A-1 Summary of the Individual Proposed Food-Uses and Use Levels for Mung Bean Protein Isolate in the United States		
Food Category	**Proposed Food-Uses**	**Maximum Intended Use Level of Mung Bean Protein Isolate (%) in Final Product**
Plant Protein Products	Egg product analogs (meringue)[h]	5
	Egg product analogs (quiche, frittata)[h]	8
	Egg product analogs (scrambled eggs, omelet, hard boiled, liquid)[i]	20
	Vegetarian food products and meat analogues	20
Snack Foods	Snack chips, popcorn, extruded snacks	5

NHANES = United States National Health and Nutrition Examination Survey; RTD = ready-to-drink; RTE = ready-to-eat.

[a] There were no food codes identified for 'fermented' versions of these beverage types in the 2011-2012 NHANES; as such, all beverages prepared from a base of barley, grains, legumes or tea (note: only iced and powdered teas) were selected as surrogates to represent typical consumption.

[b] Based on the food descriptors available in the Individual Foods files of 2011-2012 NHANES, it could not be discerned whether fruit smoothie food codes contained added protein or not; thus, *all* non-milk based fruit smoothie food codes were included in the intake assessment as a proxy for the consumption of protein-enriched fruit smoothies.

[c] There were no food codes identified to represent this food category in the 2011-2012 NHANES; however, it is expected that the intakes from this category are adequately represented in the "Milk-based weight control beverages, instant milkshakes, and protein drinks (RTD) and powdered types" included within the 'Milk Products' food category.

[d] These food-uses represent non-dairy products. However, as there were no food codes available for non-dairy versions of the product, food codes for the dairy-equivalent product were selected as surrogate food codes to represent typical consumption.

[e] Based on the food descriptors available in the Individual Foods files of 2011-2012 NHANES, it could not be discerned whether coffee whitener food codes were dairy-containing, or dairy-free; thus, *all* coffee whitener food codes were included in the intake assessment as a proxy for the consumption of dairy-free coffee whiteners.

[f] There were no food codes identified for non-dairy drinkable yogurts in the 2011-2012 NHANES; this is a specific type of yogurt product and it is expected that the intakes from this category are adequately represented by the 'non-dairy yogurts' category.

[g] Ice cream has a standard of identity under 21 CFR §135.110; however, mung bean protein isolate may be used as a non-milk-derived ingredient used in addition to milk products or ingredients.

[h] There were no food codes identified for 'egg product analogs' in the 2011-2012 NHANES; as such, the equivalent (traditional egg-containing) counterpart food codes were selected as surrogates to represent typical consumption.

[i] There were no food codes identified for 'egg product analogs' of hard boiled or liquid eggs, specifically; however, all food codes available for 'egg substitutes' consumed as part of the NHANES 2011-12 dataset (specifically for scrambled eggs and omelets) were included as a proxy for this entire food category.

SUBMISSION END