19 February 2020

RECEIVED
FEB 20 2020
OFFICE OF
FOOD ADDITIVE SAFETY

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

Re: GRAS Notice for Partially Defatted Almond Protein Flour

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Blue Diamond Growers, as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the company's conclusion that partially defatted almond protein flour, is GRAS on the basis of scientific procedures, for use conventional food and beverage products across multiple categories; these food uses of partially defatted almond protein flour are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Blue Diamond's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Kurt Waananen
Director, Research & Development
Blue Diamond Growers

Email: kwaananen@bdgrowers.com
Tel: 916-446-8309

GRAS NOTICE FOR PARTIALLY DEFATTED ALMOND PROTEIN FLOUR

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Blue Diamond Growers
1802 C Street
Sacramento, CA
95811 USA

DATE:
19 February 2020

GRAS Notice for Partially Defatted Almond Protein Flour

TABLE OF CONTENTS

List of Appendices

List of Figures and Tables

GRAS Notice for Partially Defatted Almond Protein Flour

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Blue Diamond Growers (Blue Diamond) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended use of partially defatted almond protein flour as an ingredient in conventional food and beverage products is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Blue Diamond's view that these notified food uses are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Blue Diamond, the undersigned hereby certifies that all data and information presented in this Notice represent a complete and balanced submission that is representative of the generally available literature. Blue Diamond considered all unfavorable, as well as favorable, information that is publicly available and/or known to Blue Diamond and that is pertinent to the evaluation of the safety and GRAS status of partially defatted almond protein flour as described herein.

Signed,

[redacted]

19 February, 2020

Kurt Waananen
Director, Research & Development
Blue Diamond Growers
kwaananen@bdgrowers.com

19 February 2020

1.1 Name and Address of Notifier

Blue Diamond Growers
1802 C Street
Sacramento, CA
95811 USA

1.2 Common Name of Notified Substance

The subject of this Notice is partially defatted almond protein flour obtained from natural and blanched almonds through a series of mechanical processes without the use of any processing aids or solvents.

1.3 Conditions of Use

Blue Diamond intends to market partially defatted almond protein flour in a variety of conventional food and beverage products as a source of plant protein to substitute for other protein sources in the diet. A summary of the proposed food categories and use levels for partially defatted almond protein flour is provided in Table 1.3-1 below. Food uses are organized according to 21 CFR §170.3.

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for Partially Defatted Almond Protein Flour in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019a)	Proposed Food Uses[a]	Partially Defatted Almond Protein Flour Use Level (%)
Baked Goods and Baking Mixes	Biscuits	5
	Cakes	10
	Cookies	5
	Cornbread, Corn Muffins, or Tortillas	5
	Crackers	5
	Doughnuts	5
	French Toast, Pancakes, Waffles	10
	Muffins	5
Beverages and Beverage Bases	Non-Milk-Based Nutritional Powders (plant based; incl. meal replacements)[b]	35
	Protein Powders	80
Coffee and Tea	Ready-to-Drink Coffee Drinks	5
Grain Products and Pastas	Cereal and Granola Bars	5
	Energy Bars or Protein Bars	25
	Meal Replacement Bars	10
Milk Products	Milk-based Smoothies	5
	Milk-based Nutritional Powders (incl. meal replacements)[b]	35
Processed Fruits and Fruit Juices	Fruit Smoothies (RTD)	5

CFR = Code of Federal Regulations; incl. = including; RTD = ready-to-drink; U.S. = United States.
[a] Partially defatted almond protein flour is intended for use in unstandardized products where standards of identity, as established under 21 CFR §130 to 169, do not permit its addition in standardized products.
[b] Includes ready-to-drink and powder forms.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2019b), Blue Diamond has concluded that the intended uses of partially defatted almond protein flour as described herein are GRAS 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 sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Blue Diamond Growers
1802 C Street
Sacramento, CA
95811 USA

Should the U.S. FDA have any questions or additional information requests regarding this Notification, Blue Diamond will supply these data and information upon request.

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

It is Blue Diamond'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 exempted from the Freedom of Information Act, 5 U.S.C. 552.

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

2.1 Identity

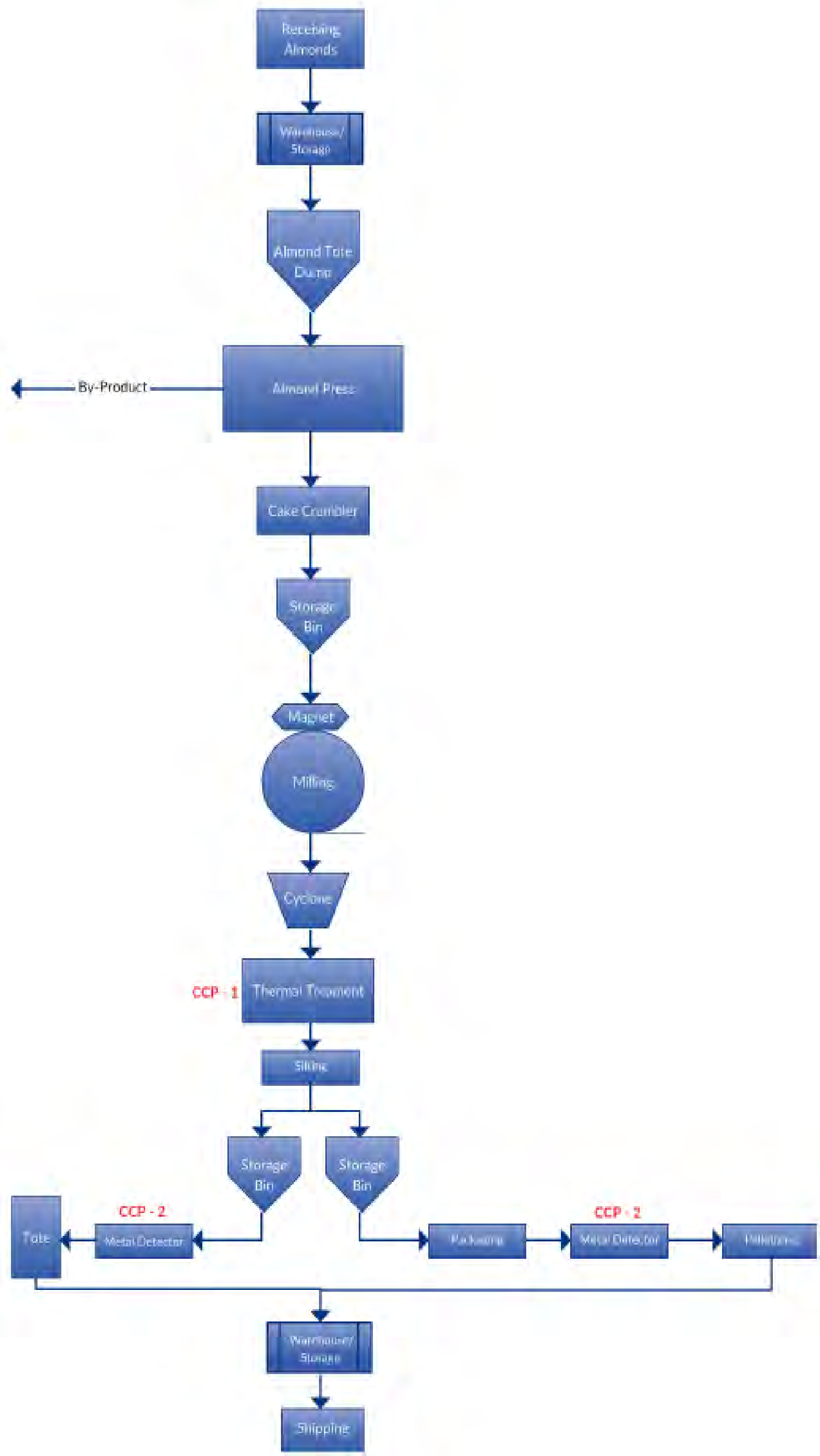
The partially defatted almond protein flour as manufactured by Blue Diamond is a fine flour that is tan or light cream in color when obtained from natural or blanched almonds, respectively. The almond protein flour has a clean, fresh odor and a flavor profile that is typical of sweet, fresh, natural almonds. As described in further detail in Section 2.2 below, the partially defatted almond protein flour is obtained through a series of mechanical processes without the use of any processing aids or solvents. Thus, the inherent protein quality of the ingredient is not changed and has been demonstrated to be comparable to the starting material.

2.2 Manufacturing Process

Partially defatted almond protein flour is produced from natural or blanched almonds using a series of mechanical processing steps, including crushing of pasteurized almonds (blanched and natural) with a mechanical press to remove the oil, crumbling the almond cake, and pneumatically conveying it to a mill where the crumbled almond cake is powdered into a flour. After milling, the almond flour is pneumatically conveyed through a thermal treatment and packaged and sealed into 55-lb multi-wall bags prior to storage and distribution.

The partially defatted almond protein flour is manufactured in accordance with the principles of current Good Manufacturing Practice (cGMP) and include critical control steps as part of the company's Hazards Analysis and Critical Control Point (HACCP) plan to limit the introduction of foreign materials and microbiological contaminants into the final product. A schematic overview of the manufacturing process of the partially defatted almond protein flour is provided in Figure 2.2-1.

Figure 2.2-1 Schematic Overview of the Manufacturing Process for Partially Defatted Almond Protein Flour from Blanched and Natural Almonds



2.3 Product Specifications

2.3.1 Chemical Specifications

The chemical specifications for partially defatted almond protein flour derived from both blanched and natural almonds are presented in Table 2.3.1-1. All methods of analysis are conducted using validated internationally recognized standard procedures.

Table 2.3.1-1 Chemical Specifications for Partially Defatted Almond Protein Flour from Blanched or Natural Almonds

Specification Parameter	Specification Limit		Method of Analysis
	Blanched Almonds	Natural Almonds	
Proximate Composition			
Moisture	≤6%	≤6%	AOAC 925.40 – Vacuum Oven
Fat (as is)	5.3 to 12%	5.3 to 12%	AOAC 933.05 – Mojo Acid Hydrolysis
Protein (as is)	41.5 to 48.7%	40.0 to 46.5.0%	AOAC 950.48/AOAC 991.20
Heavy Metals			
Lead	<0.5 ppm	<0.5 ppm	EPA 3050/6020, USP 730 – ICP-MS
Arsenic	<0.5 ppm	<0.5 ppm	EPA 3050/6020, USP 730 – ICP-MS
Cadmium	<0.5 ppm	<0.5 ppm	EPA 3050/6020, USP 730 – ICP-MS
Mercury	<0.5 ppm	<0.5 ppm	EPA 3050/6020, USP 730 – ICP-MS

AOAC = Association of Official Analytical Chemists; EPA = Environmental Protection Agency; ICP-MS = inductively coupled plasma-mass spectrometry; ppm = parts per million; USP = United States Pharmacopeia.

2.3.2 Microbiological Specifications

The microbiological specifications for partially defatted almond protein flour derived from both blanched and natural almonds are presented in Table 2.3.2-1. All methods of analysis are conducted using validated internationally recognized standard procedures.

Table 2.3.2-1 Microbiological Specifications for Partially Defatted Almond Protein Flour from Blanched or Natural Almonds

Specification Parameter	Specification Limit		Method of Analysis
	Blanched Almonds	Natural Almonds	
Standard plate count	<10,000 CFU/g	<10,000 CFU/g	AOAC 966.23
Yeast	<500 CFU/g	<500 CFU/g	FDA BAM
Mold	<500 CFU/g	<500 CFU/g	FDA BAM
Total coliforms	<100 CFU/g	<100 CFU/g	AOAC 991.14
Escherichia coli	<10 CFU/g	<10 CFU/g	AOAC 991.14
Salmonella	Negative in 2x375 g	Negative in 2x375 g	AOAC RI100201/AOAC 2003.09

AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; FDA BAM = Food and Drug Administration Bacteriological Analytical Manual.

2.4 Batch Analyses

2.4.1 Chemical Analysis of Partially Defatted Almond Protein Flour

Analysis of 5 non-consecutive lots of partially defatted almond protein flour derived from blanched almonds and 4 non-consecutive lots of partially defatted almond protein flour derived from natural almonds demonstrates that the manufacturing process, as described in Section 2.2, produces a consistent product that meets the defined product specifications. A summary of the chemical analyses for the partially defatted almond protein flour derived from both blanched and natural almonds is presented in Table 2.4.1-1.

Table 2.4.1-1 Summary of the Chemical Product Analysis for 5 Lots of Partially Defatted Almond Protein Flour from Blanched Almonds and 4 Lots of Partially Defatted Almond Protein Flour from Natural Almonds

Specification Parameter	Specification Limit	Partially Defatted Almond Protein Flour (Blanched)							Specification Parameter	Specification Limit	Partially Defatted Almond Protein Flour (Natural)					
		Manufacturing Lot					Mean	SD			Manufacturing Lot				Mean	SD
		18179NA BDB	18198NA BDB	18241NA BDB	18288NA BDB	18302NA BDB					18179N AWDB	18200N AWDB	18248N AWDB	18274N AWDB		
Proximate Composition																
Moisture (%)	≤6	3.42	6.63	4.56	4.91	4.30	4.76	1.18	Moisture (%)	≤6	5.70	5.10	3.25	4.54	4.65	1.05
Fat (as is) (%)	5.3 to 12	9.33	8.13	7.29	7.40	6.99	7.83	0.94	Fat (as is) (%)	5.3 to 12	8.85	8.60	7.94	6.60	8.00	1.01
Ash (as is)	-	6.18	6.09	6.18	6.35	6.44	6.25	0.14	Ash (as is)	-	6.25	6.33	6.55	6.57	6.43	0.16
Carbohydrate (as is)	-	36.19	34.70	35.94	35.10	34.57	35.30	0.73	Carbohydrate (as is)	-	37.32	37.95	38.90	41.74	38.98	1.95
Protein (as is) (%)	41.5 to 48.7	44.88	44.45	46.03	46.24	47.70	45.86	1.28	Protein (as is) (%)	40.0 to 46.5	41.88	42.02	43.36	40.55	41.95	1.15
Heavy Metals																
Lead (ppm)	<0.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.000	Lead (ppm)	<0.5	<0.01	<0.01	<0.01	<0.01	<0.01	0.000
Arsenic (ppm)	<0.5	0.02	0.02	0.02	0.03	0.02	0.017	0.001	Arsenic (ppm)	<0.5	0.03	0.03	0.03	0.02	0.03	0.005
Cadmium (ppm)	<0.5	0.017	0.016	0.017	0.017	0.016	<0.01	0.000	Cadmium (ppm)	<0.5	0.023	0.021	0.022	0.021	0.022	0.001
Mercury (ppm)	<0.5	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.000	Mercury (ppm)	<0.5	<0.005	<0.005	<0.005	<0.005	<0.005	0.00

CHO = carbohydrate; ppm = parts per million; SD = standard deviation.

2.4.2 Microbiological Analysis of Partially Defatted Almond Protein Flour

Analysis of 5 non-consecutive lots of partially defatted almond protein flour derived from blanched almonds and 4 non-consecutive lots of partially defatted almond protein flour derived from natural almonds demonstrates that the product meets the defined microbiological specifications. A summary of the microbiological analysis for the partially defatted almond protein flour derived from both blanched almonds and natural almonds is presented in Table 2.4.2-1.

Table 2.4.2-1 Summary of the Microbiological Product Analysis for Partially Defatted Almond Protein Flour from 5 Lots of Blanched Almonds and 4 Lots of Natural Almonds

Specification Parameter	Specification Limit	Partially Defatted Almond Protein Flour (Blanched)					Partially Defatted Almond Protein Flour (Natural)			
		Manufacturing Lot					Manufacturing Lot			
		18179NA BDB	18198NA BDB	18241NA BDB	18288NA BDB	18302NA BDB	18179NA WDB	18200NA WDB	18248NA WDB	18274NA WDB
Standard plate count (CFU/g)	<10,000	260	920	1,100	950	870	170	1,000	3,600	80
Yeast (CFU/g)	<500	<10[a]	<10	<10	<10	<10	<10	<10	10	<10
Mold (CFU/g)	<500	<10[a]	<10	<10	<10	10	<10	10	10	<10
Total coliforms (CFU/g)	<100	<10[a]	<10	<10	20	<10	<10	30	<10	<10
Escherichia coli (CFU/g)	<10	<10[a]	<10	<10	<10	<10	<10	<10	<10	<10
Salmonella	Negative in 2x375 g	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

CFU = colony-forming units.
[a] Limit of detection = 10 CFU/g

2.5 Additional Chemical Characterization

2.5.1 Pesticides

Blue Diamond has conducted analysis for residual pesticides and heavy metals on a variety of almonds sourced from California during 2017–2018, which are used as starting materials for partially defatted almond protein flour. The results of this analysis are presented in Table 2.5.1-1.

Table 2.5.1-1 Pesticides and Heavy Metal Analysis of Different Varieties of Almond Sourced from California

Variety	Pesticide Result	Heavy Metals			
		Arsenic	Cadmium	Lead	Mercury
Aldrich	Methoxyfenozide = ND to 0.02 ppm	0.05	ND	ND	ND
Avalon	Methoxyfenozide = ND to 0.02 ppm	0.05	ND	ND	ND
Butte	Methoxyfenozide = 0.02 ppm	0.06	ND	ND	ND
Butte/Padre	Piperonyl butoxide = ND to 0.02 ppm	0.05	ND	ND	ND
Carmel	Methoxyfenozide = ND to 0.02 ppm	0.05	ND	ND	ND
Fritz	Fluopyram = ND to 0.04 ppm Methoxyfenozide = ND to 0.02 ppm	0.04	ND	ND	ND
Independence	ND	0.04	0.02	ND	ND
Livingston	ND	0.03	ND	ND	ND
Mission	ND	0.03	0.03	ND	ND
Monterey	Methoxyfenozide = ND to 0.02 ppm	0.05	ND	ND	ND
Nonpareil	Methoxyfenozide = ND to 0.03 ppm Fluxapyroxad = ND to 0.04 ppm	0.02	ND	ND	ND
Price	ND	0.04	ND	ND	ND
Ruby	ND	0.03	0.02	ND	ND
Sonora	Methoxyfenozide = 0.02 to 0.03 ppm	0.06	0.01	ND	ND
Supareil	Piperonyl butoxide = 0.11 ppm	0.02	ND	ND	ND
Winters	Fluopyram = 0.03 ppm Methoxyfenozide = 0.02 ppm	0.02	0.01	ND	ND
Wood Colony	ND	0.04	0.01	ND	ND

ND = not detected; ppm = parts per million.

2.5.2 Amino Acid Profile

Analysis of 4 non-consecutive lots of partially defatted almond protein flour derived from blanched almonds and 6 batches of the partially defatted almond protein flour derived from natural almonds demonstrates that the amino acid profile is consistent and balanced across all lots. A summary of the amino acid profile for the partially defatted almond protein flour derived from both blanched almonds and natural almonds is presented in Table 2.5.2-1.

Table 2.5.2-1 Amino Acid Profile of Partially Defatted Almond Protein Flour from Blanched and Natural Almonds

Specification Parameter (g/100 g) (as is)	Partially Defatted Almond Protein Flour (Blanched Almonds)						Partially Defatted Almond Protein Flour (Natural Almonds)							
	Manufacturing Lot				Mean	SD	Manufacturing Lot						Mean	SD
	RD18-011-026	RD18-011-025	RD18-011-021	RD18-011-040			RD18-011-022	RD18-011-023	RD18-011-020	RD18-011-041	RD18-011-039	RD18-011-038		
Moisture (%)	9.61	6.85	5.02	4.40	6.47	2.33	5.20	4.90	5.20	4.86	4.91	5.10	5.02	0.157
Aspartic acid	5.09	5.06	5.93	5.84	5.48	0.47	4.55	4.95	4.93	5.52	4.84	4.76	4.93	0.40
Threonine	1.3	1.23	1.43	1.58	1.39	0.15	1.2	1.23	1.27	1.51	1.26	1.23	1.28	0.14
Serine	1.84	1.63	2.03	2.17	1.92	0.23	1.7	1.76	1.78	2.07	1.81	1.79	1.82	0.17
Glutamic acid	12.36	11.47	14.81	15.32	13.49	1.87	11.95	12.35	12.72	14.33	12.22	11.99	12.59	1.04
Glycine	2.77	2.61	3.23	3.49	3.03	0.41	2.96	3.05	2.92	3.48	3.15	3.09	3.11	0.26
Alanine	2.05	1.89	2.26	2.36	2.14	0.21	1.85	1.93	1.94	2.23	2.02	1.98	1.99	0.17
Valine	2.12	1.85	2.25	2.27	2.12	0.19	1.87	1.96	1.95	2.11	1.96	1.92	1.96	0.10
Methionine	0.33	0.31	0.35	0.36	0.34	0.02	0.3	0.29	0.34	0.36	0.36	0.36	0.34	0.03
Isoleucine	1.79	1.65	1.96	2.01	1.85	0.16	1.64	1.7	1.7	1.89	1.73	1.7	1.73	0.11
Leucine	3.36	2.84	3.55	3.65	3.35	0.36	2.91	3.06	3.04	3.41	3.00	2.95	3.06	0.21
Tyrosine	1.33	1.12	1.4	1.45	1.33	0.15	1.17	1.24	1.22	1.42	1.22	1.21	1.25	0.11
Phenylalanine	2.44	2.33	2.78	2.85	2.60	0.25	2.27	2.36	2.38	2.68	2.41	2.36	2.41	0.18
Lysine	1.32	1.05	1.48	1.5	1.34	0.21	1.41	1.42	1.42	1.47	1.57	1.53	1.47	0.03
Histidine	1.12	0.94	1.27	1.37	1.18	0.19	1.08	1.12	1.12	1.31	1.12	1.11	1.14	0.10
Arginine	5.02	4.49	5.8	5.82	5.28	0.65	4.52	4.89	4.77	5.5	4.81	4.73	4.87	0.42
Proline	2.23	2	2.26	2.05	2.14	0.13	1.92	2.03	2.06	1.93	1.88	1.85	1.95	0.07
Hydroxyproline	<0.01	<0.01	<0.01	<0.01	<0.01	0.00	<0.01	<0.01	<0.01	<0.01	-	-	<0.01	0.00
Cysteine	0.65	0.59	0.66	0.7	0.65	0.05	0.6	0.61	0.62	0.69	0.5	0.49	0.59	0.04
Tryptophan	0.43	0.38	0.44	0.39	0.41	0.03	0.34	0.36	0.37	0.37	0.46	0.44	0.39	0.01

SD = standard deviation.

2.5.3 Minerals

Five non-consecutive lots of partially defatted almond protein flour derived from blanched almonds and 4 non-consecutive lots of the partially defatted almond protein flour derived from natural almonds were analyzed for their mineral content using inductively coupled plasma-mass spectrometry. A summary of the mineral profile for the partially defatted almond protein flour derived from both blanched and natural almonds is presented in Table 2.5.3-1.

Table 2.5.3-1 Mineral Profile of Partially Defatted Almond Protein Flour from Blanched and Natural Almonds

Specification Parameter	Partially Defatted Almond Protein Flour (Blanched Almonds)							Partially Defatted Almond Protein Flour (Natural Almonds)					
	Manufacturing Lot					Mean	SD	Manufacturing Lot				Mean	
	18179NAB DB	18198NAB DB	18241NAB DB	18288NAB DB	18302NAB DB			18179NA WDB	18200NA WDB	18248NA WDB	18274NA WDB		
Moisture (%)	3.42	6.63	4.56	4.91	4.30	4.76	1.18	5.70	5.10	3.25	4.54	4.65	1.05
Minerals (as is)													
Aluminum (mg/kg)	0.7	0.4	0.8	0.7	0.5	0.6	0.16	5.0	6.5	5.2	5.4	5.5	0.67
Antimony (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.00	<0.01	<0.1	<0.01	<0.01	<0.01	0.00
Barium (mg/kg)	6.58	6.41	6.25	7.17	7.26	6.73	0.46	12.0	11.2	11.1	11.5	11.5	0.40
Beryllium (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.00	<0.01	<0.01	<0.01	<0.01	<0.01	0.00
Bismuth (mg/kg)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.00	<0.02	<0.02	<0.02	<0.02	<0.02	0.00
Boron (mg/kg)	48.8	49.6	49.0	65.4	47.8	52.12	7.45	58.7	58.7	61.9	54.0	58.3	3.25
Calcium (mg/kg)	4,680	4,810	4,370	6,490	4,730	5,016	840	7,780	8,000	9,420	8,440	8,410	727
Chromium (mg/kg)	0.07	0.03	0.04	0.11	0.02	0.05	0.04	0.12	0.15	0.10	0.14	0.13	0.02
Cobalt (mg/kg)	0.09	0.09	0.09	0.09	0.12	0.10	0.01	0.11	0.14	0.10	0.14	0.12	0.02
Copper (mg/kg)	22.4	21.2	20.7	21.3	21.2	21.4	0.63	17.3	17.4	18.3	17.4	17.6	0.47
Iron (mg/kg)	70.7	68.0	70.1	70.8	67.5	69.4	1.56	71.1	72.9	75.7	68.4	72.0	3.07
Lithium (mg/kg)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.00	<0.1	<0.1	<0.1	<0.1	<0.1	0.00
Magnesium (mg/kg)	5,480	5,260	5,110	5,330	5,170	5,270	144	4,880	4,780	5,150	4,880	4,923	159
Manganese (mg/kg)	37.2	35.7	37.1	38.0	38.7	37.34	1.12	47.5	46.5	49.7	45.8	47.4	1.70
Mercury (mg/kg)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.00	<0.005	<0.005	<0.005	<0.005	<0.005	0.00
Molybdenum (mg/kg)	0.74	0.74	0.78	0.77	0.86	0.78	0.05	0.70	0.76	0.85	0.73	0.76	0.07
Nickel (mg/kg)	1.85	1.67	1.74	1.82	2.26	1.87	0.23	1.90	1.76	1.94	2.22	1.96	0.19
Phosphorus (mg/kg)	9,980	9,590	9,690	9,800	10,100	9,832	208	8,960	9,150	9,560	8,870	9,135	306
Potassium (mg/kg)	15,000	14,300	14,600	14,800	15,400	14,820	414	14,100	14,100	15,000	14,500	14,425	427
Selenium (mg/kg)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.00	<0.1	<0.1	<0.1	<0.1	<0.1	0.00
Silver (mg/kg)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.00	<0.02	<0.02	<0.02	<0.02	<0.02	0.00
Sodium (mg/kg)	569	460	526	600	684	568	83.5	19.9	18.2	18.7	13.7	17.6	2.71
Strontium (mg/kg)	21.7	21.2	25.9	24.8	24.4	23.6	2.05	31.7	32.2	34.4	34.5	33.2	1.46
Thallium (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.00	<0.01	<0.01	<0.01	<0.01	<0.01	0.00
Thorium (mg/kg)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.00	<0.1	<0.1	<0.1	<0.1	<0.1	0.00
Tin (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.00	<0.01	<0.01	0.02	<0.01	<0.01	0.005

Table 2.5.3-1 Mineral Profile of Partially Defatted Almond Protein Flour from Blanched and Natural Almonds

Specification Parameter	Partially Defatted Almond Protein Flour (Blanched Almonds)							Partially Defatted Almond Protein Flour (Natural Almonds)					
	Manufacturing Lot					Mean	SD	Manufacturing Lot				Mean	SD
	18179NABDB	18198NABDB	18241NABDB	18288NABDB	18302NABDB			18179NAWDB	18200NAWDB	18248NAWDB	18274NAWDB		
Titanium (mg/kg)	12.2	11.3	11.2	11.3	11.9	11.6	0.44	11.3	11.5	11.9	11.1	11.5	0.34
Uranium (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.00	<0.01	<0.01	<0.01	<0.01	<0.01	0.00
Vanadium (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00
Zinc (mg/kg)	57.3	54.6	56.4	55.4	54.4	55.6	1.23	53.9	54.8	57.7	52.5	54.7	2.20
Zirconium (mg/kg)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.00	<0.1	<0.1	<0.1	<0.1	<0.1	0.00

SD = standard deviation.

2.5.4 Antinutrients

Five non-consecutive lots of partially defatted almond protein flour derived from blanched almonds and 4 non-consecutive lots of the partially defatted almond protein flour derived from natural almonds were analyzed for their amygdalin content using liquid chromatography-tandem mass spectrometry. A summary of the amygdalin analysis for the partially defatted almond protein flour derived from blanched and natural almonds is presented in Table 2.5.4-1. A detailed description of the safety of amygdalin and the impact of amygdalin content on the overall safety of partially defatted almond protein flour is presented in Section 6.1.2.

Table 2.5.4-1 Summary of Amygdalin Analysis for Partially Defatted Almond Protein Flour from 5 Lots of Blanched Almonds and 4 Lots of Natural Almonds

Specification Parameter	Partially Defatted Almond Protein Flour (Blanched Almonds)							Partially Defatted Almond Protein Flour (Natural Almonds)					
	Manufacturing Lot					Mean	SD	Manufacturing Lot				Mean	SD
	18179NABDB	18198NABDB	18241NABDB	18288NABDB	18302NABDB			18179NAWDB	18200NAWDB	18248NAWDB	18274NAWDB		
Moisture (%)	3.42	6.63	4.56	4.91	4.30	4.76	1.18	5.70	5.10	3.25	4.54	4.65	1.05
Amygdalin (mg/kg) (as is)	45.2	53.0	83.6	88.0	80.7	70.1	19.5	101.2	144.2	139.2	188.7	143.3	35.8

SD = standard deviation.

2.5.5 Aflatoxins

Blue Diamond utilizes state-of-the-art technologies to identify and reject potentially contaminated or damaged almond kernels (from insects) to control potential aflatoxin contamination. The most common microorganisms that have been identified in almond crops are *Aspergillus flavus* and *Aspergillus parasiticus*. Insect activity can have a direct effect on the prevalence of aflatoxins, and therefore controlling and minimizing insect activity through good agricultural practices can reduce the potential for aflatoxin contamination. Blue Diamond removes insect damaged kernels by electronic or laser sorting, or manual sorting. The finished product is tested to ensure the absence of aflatoxins in an ISO 17025 accredited and United States Department of Agriculture (USDA) approved and certified laboratory. Blue Diamond also participates in the annual European Union FAPAS program and the quarterly American Oil Chemists' Society Laboratory Proficiency Program for aflatoxins. These risk management practices ensure that risk of aflatoxin contamination in the final product is significantly reduced.

2.6 Stability of Partially Defatted Almond Protein Flour

Production samples of partially defatted almond protein flour derived from both blanched and natural almonds were evaluated for organoleptic and analytical acceptability over an accelerated 12-month shelf-life study. Samples were placed in frozen (3°F/-16.1°C), ambient (68 to 72°F/20 to 22°C), and accelerated (104°F/40°C) storage conditions and analyzed at 0-, 2-, 4-, 6-, 8-, 10-, and 12-week intervals for moisture, water activity, hexanal, flavor, aroma, and overall difference from control. Accelerated shelf-life results indicate no variance from the product specification for moisture and no significant changes in other attributes compared to the control. The results demonstrate the product is stable and in compliance with the labeled shelf-life of 12 months in unopened bags when stored between 65 and 75°F (18 and 24°C) in a dry, odor-free area away from direct sunlight.

Part 3. §170.235 Dietary Exposure

3.1 Background Dietary Intakes of Almond Protein

An assessment of the estimated intake of almond protein from the background diet was conducted using data available in the 2015-2016 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (CDC, 2018a,b; USDA, 2018a), the same consumption survey used to assess the anticipated intakes from the proposed intended conditions of use of partially defatted almond protein flour (see Section 3.2 below). If necessary, product-specific adjustment factors were developed for composite foods/mixtures based on data provided in the Food and Nutrient Database for Dietary Studies (USDA ARS, 2019). The protein content of California almonds and almond-based food and beverage products were determined using the USDA Food Composition Databases (USDA, 2018b), summarized in Table 3.1-1 below. The average reported protein content, by type of almond food or beverage product, was applied in the background dietary intakes assessment of almond protein.

Table 3.1-1 Standard Protein Content of Whole California Almonds and Almond-Based Food and Beverage Products (USDA, 2018b)

USDA Food Composition Database	Type of Food or Beverage	Food Description	NDB Id	Reported Protein Content (g/100 g)	Average Protein Content (Value Applied in Assessment)
USDA Branded Food Products Database	Whole almonds	California Almonds	45285557	20.69	20.14
			45224058	21.43	
			45329710	21.05	
			45151257	20.69	
			45267069	21.43	
			45267066	17.86	
			45267064	17.86	
National Nutrient Database for Standard Reference Legacy Release	Almond milk	Beverages, almond milk, chocolate, ready-to-drink	14054	0.63	0.57
		Beverages, almond milk, unsweetened, shelf stable	14091	0.40	
		Beverages, almond milk, sweetened, vanilla flavor, ready-to-drink	14016	0.42	
		Beverages, chocolate almond milk, unsweetened, shelf-stable, fortified with vitamin D2 and E	14092	0.83	
	Almond oil	Oil, almond	04529	0.00	n/a
	Almond paste	Nuts, almond paste	12071	9.00	9.00
	Almond butter	Nuts, almond butter, plain, without salt added	12195	20.96	20.96
		Nuts, almond butter, plain, with salt added	12695	20.96	

NDB Id = nutrient database identification number; USDA = United States Department of Agriculture.

A summary of the estimated daily intake of almond protein from the background diet 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 consumers of almond protein from the background diet was low among all age groups in the U.S. NHANES, with between 5.2% (infants and young children) to 19.4% (female adults) of individuals identified to consume whole almonds or almond-based food and beverage products during the 2-day recording period (Table 3.1-2). Due to the low number of consumers, several of the mean and high-level estimates presented in Tables 3.1-2 and 3.1-3 are not statistically reliable (marked with an asterisk). Nonetheless, as consumer-only intakes are more likely to represent exposure in the target population, only consumer-only intake results will be discussed in detail.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of almond protein from the background diet were determined to be 2.51 and 6.82 g/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest statistically reliable mean and 90th percentile consumer-only intakes of almond protein on an absolute basis, at 3.13 and 6.89 g/person/day, respectively. While infants and young children had the lowest statistically reliable mean consumer-only intakes of 0.75 g/person/day, female adults had the lowest statistically reliable 90th percentile intake of 5.99 g/person/day (Table 3.1-2).

Table 3.1-2 Summary of the Estimated Daily Intake of Almond Protein from the Background Diet in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	0.04	na	5.2	33	0.75	1.97*
Children	3 to 11	0.08	na	7.0	76	1.13	2.55*
Female Teenagers	12 to 19	0.10	na	7.6	29	1.35*	3.43*
Male Teenagers	12 to 19	0.21	na	5.5	26	3.81*	4.72*
Female Adults	20 and up	0.44	0.86	19.4	361	2.27	5.99
Male Adults	20 and up	0.45	0.73	14.4	238	3.13	6.89
Total Population	All ages	0.36	0.52	14.3	763	2.51	6.82

n = sample size; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of almond protein from the background diet were determined to be 35 and 88 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean consumer-only intakes of any population group, of 63 mg/kg body weight/day, whereas male adults had the highest statistically reliable 90th percentile estimate of intake of 87 mg/kg body weight/day. Female adults had the lowest statistically reliable mean and 90th percentile consumer-only intakes of 33 and 82 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 Almond Protein from the Background Diet in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	3.3	na	5.1	32	63	163*

Table 3.1-3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Almond Protein from the Background Diet in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Children	3 to 11	3.3	na	6.9	75	47	92*
Female Teenagers	12 to 19	1.5	na	7.6	28	19*	54*
Male Teenagers	12 to 19	3.2	na	5.5	26	58*	99*
Female Adults	20 and up	6.3	12.2	19.5	361	33	82
Male Adults	20 and up	5.3	8.5	14.6	237	36	87
Total Population	All ages	5.1	7.0	14.4	759	35	88

bw = body weight; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

3.2 Intended Use of Partially Defatted Almond Protein Flour and Levels of Use in Foods

Blue Diamond intends to market the partially defatted almond protein flour in a variety of conventional food and beverages as a source of plant protein as defined under 21 CFR §170.3(n)(33) (U.S. FDA, 2019a). The ingredient is intended for use as a substitute to other protein sources in the diet, and will provide an alternative source of plant-based proteins to existing plant sources such as mung bean (GRN 684), pea (GRN 608), rice (GRN 609), and potato (GRN 447). A summary of the proposed food categories and use levels for partially defatted almond protein flour is provided in Table 3.2-1 below.

Table 3.2-1 Summary of the Individual Proposed Food Uses and Use Levels for Partially Defatted Almond Protein Flour in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019a)	Proposed Food Uses[a]	Partially Defatted Almond Protein Flour Use Level (%)
Baked Goods and Baking Mixes	Biscuits	5
	Cakes	10
	Cookies	5
	Cornbread, Corn Muffins, or Tortillas	5
	Crackers	5
	Doughnuts	5
	French Toast, Pancakes, Waffles	10
	Muffins	5
Beverages and Beverage Bases	Non-Milk-Based Nutritional Powders (plant based; incl. meal replacements)[b]	35
	Protein Powders	80
Coffee and Tea	Ready-to-Drink Coffee Drinks	5
Grain Products and Pastas	Cereal and Granola Bars	5
	Energy Bars or Protein Bars	25
	Meal Replacement Bars	10
Milk Products	Milk-based Smoothies	5
	Milk-based Nutritional Powders (incl. meal replacements)[b]	35
Processed Fruits and Fruit Juices	Fruit Smoothies (RTD)	5

CFR = Code of Federal Regulations; incl. = including; RTD = ready-to-drink; U.S. = United States.
[a] Partially Defatted almond protein flour is intended for use in unstandardized products where standards of identity, as established under 21 CFR §130 to 169, do not permit its addition in standardized products.
[b] Includes ready-to-drink and powder forms.

3.3 Estimated Dietary Consumption of Partially Defatted Almond Protein Flour Based Upon Intended Food Uses

3.3.1 Methods

An assessment of the anticipated intake of partially defatted almond protein flour as an ingredient under the intended conditions of use was conducted using data available in the 2015-2016 cycle of the U.S. NHANES (CDC, 2018a,b; USDA, 2018a). The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2015-2016. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2018a,b; USDA, 2018a). The NHANES data were employed to assess the mean and 90th percentile intake of partially defatted almond protein flour for each of the following population groups:

- Infants and young children, up to and including 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;

- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of partially defatted almond protein flour by the U.S. population[1]. Estimates for the daily intake of partially defatted almond protein flour represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of partially defatted almond protein flour averaged over all individuals surveyed, regardless of whether they consumed food products in which partially defatted almond protein flour is proposed for use, and therefore includes individuals with "zero" intakes (*i.e*., those who reported no intake of food products containing partially defatted almond protein flour during the 2 survey days). "Consumer-only" intake refers to the estimated intake of partially defatted almond protein flour by those individuals who reported consuming food products in which the use of partially defatted almond protein flour is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which partially defatted almond protein flour is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of partially defatted almond protein flour was generated using the use level indicated for each intended food use, as presented in Table 3.2-1, together with food consumption data available from the 2015-2016 NHANES datasets. The results for this assessment are presented herein.

3.3.2 Intake Estimates for Partially Defatted Almond Protein Flour

A summary of the estimated daily intake of partially defatted almond protein flour from proposed food uses is provided in Table 3.3.2-1 on an absolute basis (g/person/day), and in Table 3.3.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was high among all age groups evaluated in the current intake assessment; more than 62.1% of the population groups consisted of consumers of food and beverage products in which partially defatted almond protein flour is currently proposed for use (Table 5.3-1). Children had the greatest proportion of consumers at 88.2%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of partially defatted almond protein flour were determined to be 15.2 and 22.0 g/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean consumer-only intakes of partially defatted almond protein flour on an absolute basis of 19.3 g/person/day, whereas female adults were determined to have the greatest 90th percentile consumer-only intakes of 29.5 g/person/day. Infants and young children had the lowest mean and 90th percentile consumer-only intakes of 3.0 and 7.4 g/person/day, respectively (Table 3.3.2-1).

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Table 3.3.2-1 Summary of the Estimated Daily Intake of Partially Defatted Almond Protein Flour from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	1.9	5.4	62.1	368	3.0	7.4
Children	3 to 11	5.2	11.8	88.2	998	5.9	12.3
Female Teenagers	12 to 19	5.6	13.5	75.3	356	7.5	16.1
Male Teenagers	12 to 19	7.7	13.6	73.1	353	10.5	16.2
Female Adults	20 and up	13.3	19.6	76.8	1,706	17.3	29.5
Male Adults	20 and up	14.3	19.9	74.2	1,432	19.3	29.2
Total Population	All ages	11.6	16.3	76.3	5,213	15.2	22.0

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

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of partially defatted almond protein flour were determined to be 221 and 391 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 249 and 555 mg/kg body weight/day, respectively. Female teenagers had the lowest mean and 90th percentile consumer-only intakes of 126 and 264 mg/kg body weight/day, respectively (Table 3.3.2-2).

Table 3.3.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Partially Defatted Almond Protein Flour from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	154	431	61.9	364	249	555
Children	3 to 11	209	471	88.3	995	236	495
Female Teenagers	12 to 19	95	240	75.3	349	126	264
Male Teenagers	12 to 19	119	227	73.1	352	163	268
Female Adults	20 and up	185	267	76.8	1,693	241	399
Male Adults	20 and up	157	226	73.9	1,410	212	296
Total Population	All ages	168	297	76.2	5,163	221	391

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

3.3.3 Summary and Conclusions

Consumption data and information pertaining to the intended food uses of partially defatted almond protein flour were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment, which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain partially defatted almond protein flour at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that partially defatted almond protein flour will have 100% market penetration in all identified food categories.

On consumer-only basis, the resulting mean and 90th percentile intakes of partially defatted almond protein flour by the total U.S. population from proposed food-uses in the U.S., were estimated to be 15.2 g/person/day (221 mg/kg body weight/day) and 22.0 g/person/day (391 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean intakes of partially defatted almond protein flour were determined to be 19.3 g/person/day (212 mg/kg body weight/day), as identified among male adults, and the highest 90th percentile intakes of partially defatted almond protein flour were determined to be 29.5 g/person/day (399 mg/kg body weight/day), as identified among female adults. While infants and young children had the lowest mean and 90th percentile consumer-only intakes on an absolute basis of 3.0 and 7.4 g/person/day, respectively, when expressed on a body weight basis, this age group had the highest daily intakes, of 249 and 555 mg/kg body weight/day at the mean and 90th percentile intake, respectively. The mean calculated consumer-only intakes of the partially defatted almond protein flour by the total U.S. population from all proposed food-uses is approximately 6 times higher than the background intake of the almond protein (15.2 g/person/day *versus* 2.51 g/person/day, respectively).

The U.S. FDA has established a Daily Reference Value (DRV) for protein of 50 g in adults and in children 4 years of age or older. The 90th percentile all-user intakes of partially defatted almond protein flour by the total U.S. population from all proposed food-uses (*i.e.*, 22.0 g/day), corresponding to protein intakes ranging between 8.8 and 10.2 g/person/day, based on protein specifications for this ingredient (40.0 and 46.5%), is approximately 5 to 6 times lower than the DRV for protein. It should also be noted that partially defatted almond protein flour is 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. Thus, the addition of partially defatted almond protein flour will simply serve as a replacement to other protein sources and is therefore unlikely to increase consumer exposure to protein.

Part 4. §170.240 Self-Limiting Levels of Use

The partially defatted almond protein flour is intended for use as a plant-based protein source in the diet. The intended uses of the ingredient are self-limiting in that the ingredient will be added to substitute protein in various food and beverage products; high levels of use of protein will adversely impact the organoleptic properties of the food or beverage product.

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

Not applicable.

Part 6. §170.250 Narrative and Safety Information

In order to identify scientific literature relevant to the safety of partially defatted almond protein flour, a comprehensive search of the scientific literature was conducted through February 2020 using the electronic search tool ProQuest Dialog™. The following databases were searched: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. The relevance and specificity of the literature search was increased through the implementation of search terms to reflect the compound of interest (*i.e.,* almond protein) in combination with metabolism and preclinical/clinical endpoints. The search results were retrieved and reviewed in 2 stages (titles and abstracts).

There was limited safety data available on almond and/or almond protein conducted in animal models or humans. Several studies conducted in rats evaluated efficacy-related endpoints, such as hepatoprotective effects of almond oil (Jia *et al.,* 2011), lipid profile, glucose, or antioxidant capacity of almonds (Groven *et al.,* 2017), and modulation of high-density lipoprotein (HDL) or low-density lipoprotein (LDL) cholesterol (Kim *et al.,* 2003). Song *et al.* (2010) and Arjariya *et al*. (2013) reported on safety-related endpoints in rats. However, these studies were conducted with partially defatted almond skins (Song *et al.,* 2010) or aqueous extracts of the Indian almond plant (Arjariya *et al.*, 2013), and therefore, are not relevant for the safety assessment of partially defatted almond protein flour. Clinical studies reported on the effects of almond consumption on risk factors of cardiovascular disease (HDL-cholesterol or LDL-cholesterol, lipid profile, blood pressure), vascular function, biomarkers of inflammation and lipid peroxidation, antioxidant effects, insulin resistance, or post-prandial metabolic response in healthy male and female subjects including pregnant women. There were no biological or nutritional effects reported in these studies, which would suggest that the use of partially defatted almond protein flour as a food ingredient would be unsafe.

Since partially defatted almond protein flour is derived from a whole food with a long-history of safe consumption, the safety assessment was evaluated using an adaptation to the 2-tiered weight of evidence approach described by the International Life Sciences Institute (ILSI) for the safety assessment of proteins produced in genetically engineered agricultural products (Delaney *et al.,* 2008). In the first step (Tier I), hazard identification of the ingredient, no traditional animal studies are necessary if the ingredient meets <u>all</u> of the following criteria:

1. There is a history of safe use of the ingredient in foods;

2. The ingredient is fully characterized with respect to exposure to natural toxins and anti-nutritional factors under the proposed conditions of use;

3. Nutritional implications of the ingredient are fully addressed, *e.g.*, protein quality, levels of nutrients and minerals; and

4. There are no biological adverse effects associated with the ingredient from clinical studies.

In the event that data gaps exist at the Tier I stage, additional information may be necessary for evaluation under Tier II. This information includes toxicological studies or other hypothesis-based testing strategies to address specific safety-related questions, clinical studies, and nutritional studies (*e.g.*, protein quality if uses in children and infants are intended). The approach described by ILSI has been used to evaluate the GRAS use of other plant-based proteins such as mung bean isolate described in GRN 684 (U.S. FDA, 2017). As described in detail in GRN 684, the notifier provided data on the compositional analyses of the protein isolate, digestibility, allergenicity potential, and information on the history of safe use.

Almonds have a long history of use in the diet. Considering the history of safe use of almonds and given the fact that partially defatted almond protein flour derived from almonds is minimally processed, it can be concluded that partially defatted almond protein flour is unlikely to contain constituents of toxicological concern. Specific safety concerns related to the use of partially defatted almond flour in the diet arise from the manufacturing process to potentially concentrate natural toxins, minerals, or anti-nutrients factors, the allergenicity potential, nutritional considerations for food applications where protein quality may impact the growth and development of the consumer (*e.g*., foods intended for use by infants or growing children), and data relevant to the digestibility of the protein and how it would be handled by the body following ingestion. These specific safety concerns of partially defatted almond protein flour are addressed in the following sections.

6.1 History of Use

Almonds have a long history of consumption in the U.S. and globally (O'Neil *et al.,* 2016). The cultivation of almonds has been reported to occur in the Eastern Mediterranean around the second millennium BC, with evidence of extensive trade in the 4^{th} century BC (Gradziel, 2011). There exists documentation supporting the long history of almonds in culinary and medical uses throughout human history and in different global regions, suggesting that the spread of cultivation of almonds was through well established and intercontinental trade routes. Almonds were one of the first tree nuts to be domesticated, and, currently, remains to be one of the most important commercial crops in the U.S. It is estimated that California produces over 80% of the world's supply of almonds, and almost 100% of the almonds consumed in the U.S. According to the USDA, approximately 2.2 billion pounds of almonds were produced in 2017 (USDA, 2018c). Almonds may be consumed raw, toasted, as part of a meal, incorporated into certain dishes or processed to produce a butter, oil, or milk (O'Neil *et al.,* 2016). Based on a search of the Food Commodity Intake Database (2005-2010) for "almonds", it is estimated that the *per capita* intake of almonds is in the region of 1.15 g/day or 0.02 g/day body weight/day over a 2-day average (U.S. EPA, 2019). It is concluded that there is an established history of safe consumption of almonds in the U.S.

6.2 Compositional Analysis

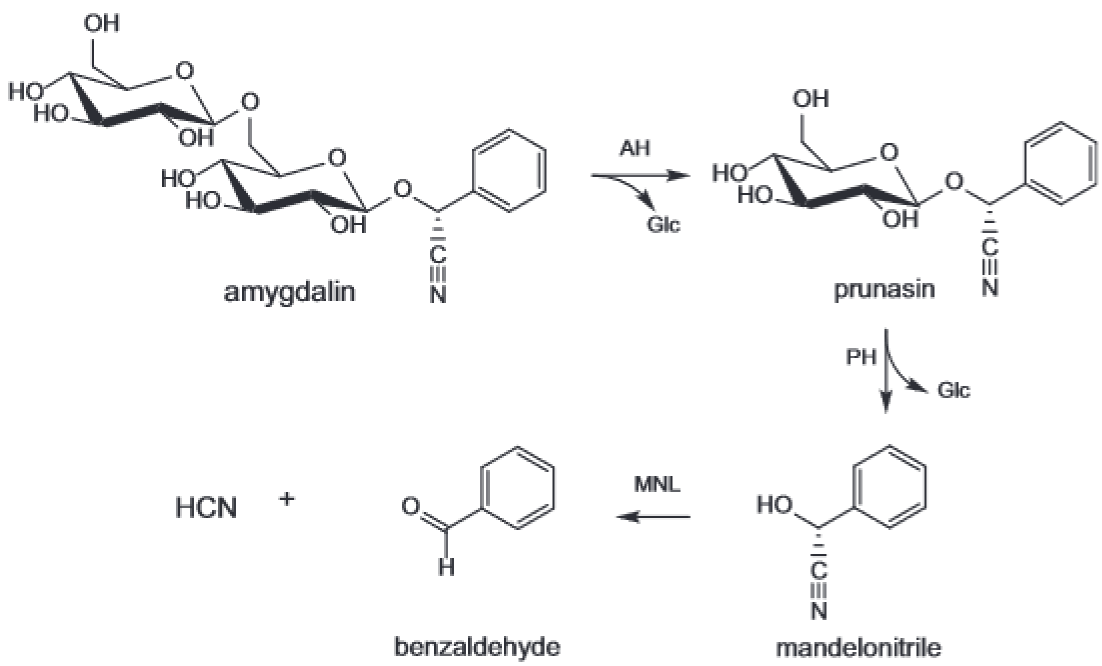
6.2.1 Proximate Analysis

The manufacturing process of partially defatted almond protein flour utilizes a series of mechanical processing steps that do not involve the use of any solvents or processing aids. Therefore, there is no selective isolation or concentration step that would potentially concentrate any constituent of toxicological concern (*e.g.,* amygdalin) that may be naturally present in natural or blanched almonds used as starting materials. The partially defatted almond protein flour is fully characterized. The final ingredient is comprised of protein (41.95 to 45.86%), carbohydrates (35.30 to 38.98%), moisture (4.65 to 4.76%), fat (7.83 to 8.00%), and ash (6.25 to 6.43%). The results of additional chemical characterization of the partially defatted almond protein flour demonstrated the absence of heavy metals, residual pesticides, or any anti-nutritional factors (*i.e.,* amygdalin) that may be of toxicological concern.

6.2.2 Natural Toxins/Anti-Nutritional Factors

Dietary anti-nutritional factors or antinutrients are natural or synthetic compounds found in a variety of foods, more specifically, grains, beans, legumes, and nuts that can adversely impact the absorption of vitamins, minerals and other nutrients. Therefore, the presence of any natural anti-nutritional factors or toxins that are characteristic of almonds were considered during the GRAS evaluation. Some antinutrients reported in almonds include phytic acid (Duong *et al.*, 2017), oxalic acid (Chai and Liebman, 2004), cyanogenic glycosides (Chaouali *et al*., 2013), and various polyphenols (Bolling *et al.*, 2010). Among these, the major safety concern is related to cyanogenic glycosides, which can release hydrogen cyanide upon hydrolysis, resulting in acute cyanide poisoning (Rietjens *et al*., 2005). Amygdalin is the major cyanogenic glycoside present in almonds (JECFA, 1993; Chaouali *et al.,* 2013; EFSA, 2016). The toxicity of amygdalin and hydrogen cyanide resulting from the hydrolysis of amygdalin was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA). In their evaluation, these scientific bodies noted the potential toxicity of amygdalin to be dependent on 2 mechanisms. First, maceration of the almond results in the release of β-glucosidase, which hydrolyzes the cyanogenic glycoside to produce hydrogen cyanide and glucose, ketones, or benzaldehyde. Secondly, the β-glucosidase released may enter the stomach, where it is deactivated in the low pH environment, and then reactivated in the alkaline conditions of the gut. The mechanism of degradation of amygdalin is shown in Figure 6.2.2-1. The complete hydrolysis of 1 g of amygdalin yields 59 mg of hydrogen cyanide (JECFA, 1993; JECFA, 2012; EFSA, 2016). The resulting cyanide is detoxified by rhodanese, forming thiocyanate, which is ultimately eliminated in the urine (JECFA, 2012). Based on the available information, the EFSA Panel on Contaminants in the Food Chain (CONTAM) noted that cyanide is of high acute toxicity in humans, with a reported lethal dose of 0.5 to 3.5 mg/kg body weight (EFSA, 2016). The EFSA CONTAM Panel derived an acute reference dose of 20 µg/kg body weight for cyanide toxicity. JECFA derived an acute reference dose of 0.09 mg/kg body weight as cyanide equivalents and a provisional maximum tolerable daily intake (PMTDI) of 20 µg/kg body weight/day as cyanide equivalents.

Figure 6.2.2-1 Mechanism of Formation of Hydrogen Cyanide and Other Metabolites from Amygdalin (Adapted from EFSA, 2016)



The mean level of amygdalin detected in the partially defatted almond protein flour produced from blanched and natural almonds was 70.1±19.5 and 143.3±35.8 mg/kg, respectively. Considering that complete hydrolysis of amygdalin results in the release of approximately 59 mg of hydrogen cyanide, these levels of amygdalin could, theoretically, result in the release of 2.99 to 10.57 mg of hydrogen cyanide per kg of the partially defatted almond protein flour. Dietary exposure to hydrogen cyanide as a result of hydrolysis of amygdalin have been determined based on the estimated dietary intakes of the partially defatted almond protein flour. The estimated dietary exposure to hydrogen cyanide due to consumption of the partially defatted almond protein flour (90^{th} percentile) in children, female adults (highest exposure group), and the total population were in the range of 1.3 to 4.7, 0.79 to 2.8, and 0.88 to 3.1 µg/kg body weight/day, respectively. These dietary exposure estimates are well below the acute reference dose of 90 µg/kg body weight and PMTDI of 20 µg/kg body weight/day, as established by EFSA (2016) and JECFA (2011), respectively. Therefore, the levels of amygdalin present in the partially defatted almond protein flour do not pose a safety concern to the consumer under the proposed conditions of use.

6.3 Allergenicity of the Partially Defatted Almond Protein Flour

Almond is considered a major food allergen under "tree nuts" and is therefore, subject to labelling under the Food Allergen Labeling and Consumer Protection Act (FALCPA), as regulated by the U.S. FDA (U.S. FDA, 2004). In the U.S., almond allergy is the third most commonly reported tree nut allergy (behind cashew and walnut) impacting 15% of patients (Costa *et al.,* 2012). Eight groups of proteins have been identified and characterized as allergenic in almonds, including pathogenesis related (PR)-10 (Pru du 1), thaumatin-like proteins (TLP) (Pru du 2), prolamins (Pru du 2S albumin, Pru du 3), profilins (Pru du 4), 60sRP (Pru du 5), and cupin (Pru du 6, Pru du γ-conglutin) (Costa *et al.,* 2012; Mandalari and Mackie, 2018; Che *et al.,* 2019). Of these groups, Pru du 3, Pru du 4, Pru du 5, and Pru du 6 are listed as putative allergens in the World Health Organization/International Union of Immunological Societies list (WHO/IUIS, 2019). Costa *et al.* (2012) reviewed the allergenic protein groups present in almonds and provided an overview of the protein, biological function, and clinical relevance. These findings are summarized in Table 6.3-1 below.

Table 6.3-1 Allergenic Proteins in Almonds and Their Biological Function and Clinical Relevance (Adapted from Costa *et al*., 2012)

Allergen	Biochemical Designation	Protein Families	Molecular Weight (kDa)	Biological Function	Clinical Relevance
Pru du 1	Bet v 1-homologous	PR-10 family	17 (~160 aa)	Protection against pathogenic constraints and adaptation to stressful environment	• Mild immune reactions and related to OAS • Severe allergic reactions reported in some patients with birch pollen allergy • Cross-reactivity with Bet v 1 and other PR-10
Pru du 2	TLP	PR-5 family	23 to 27 (246 to 330 aa)	Thaumatins	• Recognized as potential allergens, but the clinical relevance is yet subject of study
Pru du 2S albumin	2S albumin	Prolamin superfamily	12 (28 aa)	Seed storage proteins for seed development	• Specific allergic symptoms not yet defined
Pru du 3	nsLTP	Prolamin superfamily	9 (116 to 123 aa)	Lipid transfer protein	• Systemic and life-threatening symptoms • Cross-reactivity among Rosaceae fruits
Pru du 4	Profilin	Profilin-specific IgE usually cross-reacts with homologues from virtually every plant source	14 (131 aa)	Actin-binding proteins	• Symptoms are mild and limited to oral cavity
Pru du 5	R60sRP	Autoimmune reactions to human P2	10 (113 aa)	Intervenes in the elongation step of protein synthesis	• Specific allergic symptoms not yet defined
Pru du 6	Amandin, 11S globulin, or AMP	Cupin superfamily	360 (~1,055 aa)	Legumin-like protein (major storage protein)	• Reported to induce severe allergic reactions
Pru du γ-conglutin	γ-conglutin	Cupin superfamily	45 (25 aa)	7S vicillins	• Specific allergic symptoms not yet defined

aa = amino acids; AMP = almond major protein; IgE = immunoglobulin E; nsLTP = non-specific lipid transfer protein; OAS = oral allergy syndrome; PR = pathogenesis-related; TLP = thaumatin-like protein.

Pru du 6, an 11S globulin, is also known as amandin. This storage protein was one of the first allergenic proteins identified in almonds. Pru du 6 accounts for *ca.* 65% of total almond protein content and has been associated with severe allergenic reactions upon consumption of almonds in sensitive individuals (Mandalari and Mackie, 2018). Pru du 6 and its various isoforms have been demonstrated to be highly resistant to heat treatments during food processing (Costa *et al.,* 2012). Using a simulated *in vitro* model of gastric digestion, Pru du 6 was found to be readily digested by pepsin; however, incorporation of almond flour into a food matrix was shown to decrease its digestibility by pepsin (Mandalari and Mackie, 2018). As discussed in Section 6.5.1 below, following *in vitro* digestion of raw almond flour, peptide fragments sharing similarity to Pru du 6 were identified (De Angelis *et al.,* 2018), suggesting that the epitope associated with allergenicity of this protein is likely not digested and remains intact.

A search of the list of known and putative allergens in the AllergenOnline database (Version 19; dated 10 February 2019) and Comprehensive Protein Allergen Resource (COMPARE) database for "*Prunus dulcis*" identified 7 known allergen proteins present in *P. dulcis*, most of which were the Pru du 6 proteins (Table 6.3-2) (FARRP, 2019). Among proteins identified in almonds, Pru du 6 has shown immunoglobulin E (IgE)-binding activity *via* Western blot or enzyme-linked immunosorbent assay (ELISA) analyses that is characteristic of allergenic proteins. Furthermore, 2 proteins, Pru 4 Profilin and Pru p 2 have been demonstrated experimentally to contain IgE-binding activity *via* Western blot or ELISA analysis and skin prick test, in addition to biological activity as measured by basophil activation (Chen *et al.*, 2008; Palacín *et al.*, 2010; Das *et al.*, 2011; Costa *et al.*, 2012).

Table 6.3-2 Known Allergens from *Prunus dulcis* According to the AllergenOnline Database (Version 19) and COMPARE Database

Allergen	Allergenicity Rating	Amino Acid Length	Accession No.
Prunus persica Pru p 2 IUIS	IgE plus basophil+ or SPT+	241	ACE80974.1
Prunus Pru 4 Profilin peach cherry almond	IgE plus basophil+ or SPT+	131	AAL91662.1
Prunus Pru du 6 Amandin	IgE but no biological test	531	3EHK_A
Prunus Pru du 6 Amandin	IgE but no biological test	178	AGR27935.1
Prunus Pru du 6 Amandin	IgE but no biological test	551	ADN39440.1
Prunus Pru du 6 Amandin	IgE but no biological test	504	ADN39441.1
Prunus Seed allergenic protein 2 (Conglutin gamma)	IgE but no biological test	25	P82952.1

COMPARE = Comprehensive Protein Allergen Resource; IgE = immunoglobulin E.

The totality of available evidence demonstrates that almonds may pose allergenic risk to the consumers, despite the high digestibility of the protein (see Section 6.4.1). Since almonds are a type of tree nut, a major food allergen, it is subject to labelling under FALCPA. Therefore, foods containing the partially defatted almond protein flour will be clearly labeled as containing almonds.

6.4 Nutritional Considerations

6.4.1 *In Vitro* Protein Digestibility

The structure and biological activity of most proteins is lost during the digestion process that occurs along the gastrointestinal tract (Delaney *et al.,* 2008). This is likely due to the denaturation and degradation processes that occur. Proteins that are readily digested by these processes are more likely to not pose a safety concern compared to those that are resistant to digestion. Proteins that are resistant to proteolytic digestion can reach the intestinal mucosa, where they are absorbed into the systemic circulation, and may elicit an immune (allergenic) response (Toomer *et al.,* 2013). A number of *in vitro* and *in vivo* digestibility studies on almonds, processed almonds (*i.e.,* defatted or roasted), or ground almonds were identified in the literature, and these are briefly discussed below.

The *in vitro* digestion of raw almonds and almond flour subjected to an autoclave step was investigated as part of an assessment of the allergenic potential of almonds (De Angelis *et al.,* 2018). Simulated salivary fluid (SSF), gastric fluid (SGF), and intestinal fluid (SIF) were employed to mimic the physiological conditions of gastric and duodenal digestion. During the duodenal phase, bile salts and digestive enzymes trypsin, chymotrypsin, pancreatic lipase and/or pancreatic α-amylase were added. Untreated almonds subject to SSF, SGF, or SIF without enzymes were treated as the control. The results suggest that raw and treated almond flour are readily digested in the gastrointestinal tract into small peptide chains, specifically in the region of >50, 37 to 50, 20 to 25, and ≤16 kDa. The authors analyzed each band on the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) *via* high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The peptide fragments after digestion of the control sample without digestive enzymes were attributed to the following almond proteins: (R)-mandelonitrile lyase 2, abscisic acid response protein, prunin 1 and 2, and putative lipid-transfer protein. Of these proteins, only prunin 1 and 2 have been associated with allergenicity as they are key components of the allergen amandin (Jin *et al.,* 2009; see Section 6.4 for further details). Analysis of the control samples subject to simulated gastrointestinal digestion resulted in peptide fragments sharing identity with (R)-mandelonitrile lyase 2 and other digestive enzymes. Upon analysis of the treated almond flour, only the digestive enzymes were identified, suggesting that almond proteins are completely digested by the simulated gastric and duodenal system. The authors performed a subsequent experiment in which raw and treated almond samples, collected at the end of the duodenal phase, were subject to size exclusion chromatography with a 6 kDa cut-off and analyzed using HPLC-MS/MS. The results indicate that the small peptide fragments (≤6 kDa) identified in the samples shared similarity to known allergens in almonds (*e.g.,* Pru du 3, Pru du 6, Pru du 4, Pru du 5, Pru du 2S). The results of this study suggest that almond flour, either raw or heat-treated, is readily digestible in an *in vitro* model of gastrointestinal digestion, and the resulting protein fragments were shown to share similarity to known almond allergens, indicating that these allergens are not digested and may be available for absorption.

The protein digestibility of composite samples of raw almonds (varieties Butte, Independence, Monterey, and Nonpareil) that were grounded by an electric mill was investigated in an *in vitro* digestion model and *in vivo* in rats (House *et al.,* 2019). The *in vitro* protein digestion was measured using the pH drop method as described by Tinus *et al.* (2012). The *in vivo* protein digestibility of raw almonds was measured according to the test protocol described in AOAC 991.29. The authors reported that the percent true fecal protein digestibility was greatest for the Monterey variety (80.6%) and lowest for Butte varieties (78.3%). The true fecal protein digestibility for Independence and Nonpareil varieties were 78.9% and 78.6%, respectively. In comparison, the authors reported a true fecal protein digestibility of 96% for casein (House *et al.,* 2019).

Mandalari *et al.* (2008) investigated the release of protein from almond seeds during digestion using *in vitro* models of gastric and duodenal digestion. Protein digestion was measured from whole blanched and natural almonds, finely ground almond, and defatted finely ground almonds. The protein loss of the ground almonds (*ca.* 38% and 41%) was significantly greater compared to the whole almonds (*ca.* 12% and 14%) in the *in vitro* gastric and duodenal digestion models. In another study evaluating the effect of simulated gastric (pepsin) and intestinal (pancreatin) digestion on the temporal stability and immunoreactivity of defatted almond protein extract, it was reported that the allergenic almond proteins are completely hydrolyzed in the gastric lumen (Toomer *et al.,* 2013). The authors reported the proteins must remain under denaturing conditions for up to 80 minutes for complete hydrolysis and for loss of immunoreactivity to occur. Sze-Tao and Sathe (2000) reported complete and rapid hydrolysis of a defatted almond protein isolate (Nonpareil) by pepsin within 2 minutes in an *in vitro* digestibility model with porcine pepsin. The protein digestibility of raw and roasted almonds was investigated in male growing pigs (Bornhorst *et al.,* 2016). Animals were provided a single meal of raw or roasted almonds at a level of 5% of metabolic body weight (*i.e.,* half of the daily food portion), and were euthanized at 20, 60, 180, 300, 480, or 720 minutes (n=6 at each timepoint). The stomach was removed, and chyme samples were taken from the proximal and distal stomach regions. The results of this study provide further evidence that almonds are completely digested by proteases of the gastrointestinal tract; the gastric hydrolysis of almond proteins increases over time, and greater protein hydrolysis leads to increases in gastric emptying of protein into the small intestine.

The true protein digestibility of raw, whole almond flour (*P. dulcis* var. Carmel, Mission, and Nonpareil) containing protein levels of 20.6 to 23.3% was reported to range from 82.6 to 92.3% (Boye *et al.,* 2012). The true protein digestibility was determined *in vivo*, in which male weanling Sprague-Dawley rats (n=7/group) were provided a protein-free diet (control, casein) or test diet containing the almond flour at levels of 14% for 10 days.

Based on the available *in vitro* and *in vivo* digestibility studies, it is anticipated that the partially defatted almond protein flour would be readily digested in the gastrointestinal tract into the amino acid and small peptide components and would therefore provide a nutritional source of dietary protein.

6.4.2 Nutritive Value and Protein Quality Evaluation

Evaluation of the protein quality aims to determine the capacity of food protein sources to meet the protein and essential amino acid requirements and to satisfy the metabolic requirements for amino acids and nitrogen. Several methods are commonly used to assess the quality and nutritional value of a protein, specifically 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 that are 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. A search of the scientific literature indicates that almonds have a PER of 0.4 (CFIA, 2018). Information on the NPU, BV, and DIAAS of partially defatted almond protein flour or other related almond flour were not identified in the scientific literature. A detailed discussion on the PDCAAS rating of the partially defatted almond protein flour is provided in Section 6.4.3 below.

The PER and net protein ratio (NPR) were determined in weanling male Sprague-Dawley rats (10 animals/group) consuming almond protein sourced from defatted almond powder (*Prunus amygdalus*) containing 45 to 50% protein as the sole protein source (Cowan *et al.,* 1963). The test groups were provided 10% almond protein or casein. The reported PER for the almond protein group was 0.32, while the PER for the casein control group was 2.5. The authors attributed the significant different in PER to the limiting amino acids, methionine, lysine, tryptophan, and threonine, and, when the diets were supplemented with these limiting amino acids, the PER of the almond protein group was determined to be 2.44. The NPR of 5.80 and 2.73 were reported for the casein control and almond protein groups, respectively. When the diets of the almond protein group were supplemented with the limiting amino acids, the NPR was reported to be 5.22. The results of this study suggest that almond protein is not sufficient for adequate growth when used as the sole source of protein in the diet due to the limitations in amino acid quality.

6.4.3 Protein Digestibility Corrected Amino Acid Score (PDCAAS) for Partially Defatted Almond Protein Flour

The PDCAAS rating proposed by the Food and Agriculture Organization of the United Nations (FAO) in 1989 (FAO/WHO, 1991) was adopted by the U.S. FDA as "the preferred best" method to evaluate protein quality (U.S. FDA, 1993). The PDCAAS approach to evaluating protein quality is based on the principle that the nutritive value of a protein is dependent on its capacity to provide nitrogen and amino acids in sufficient amounts to meet the essential amino acid requirements of humans. The quality of some proteins can be assessed directly using amino acid score values, while others cannot due to poor digestibility and/or bioavailability. The PDCAAS approach utilizes both the amino acid composition of a protein and its digestibility profile to accurately predict the protein quality of foods for human diets (FAO/WHO, 1991). The PDCAAS rating relates the content of the first limiting essential amino acid of the protein of interest (*i.e.,* partially defatted almond protein flour) to the content of the same amino acid in a reference pattern of essential amino acids (*i.e.,* amino acid score), corrected for fecal digestibility, which is often measured using a rat balance assay.

As presented in Table 6.4.3-1 below, the partially defatted almond protein flour contains a balanced amino acid composition, for the most part, but is deficient in lysine, tryptophan, methionine, and cysteine. The lack of methionine in almonds was reported by Sze-Tao and Sathe (2000) and methionine and lysine and threonine have been reported to be limiting amino acids in almond-based diets (Ahrens *et al.,* 2005). The addition of methionine, tryptophan, lysine, and threonine improved the NPR and PER of rats consuming almonds as the sole protein source (Cowan *et al.,* 1963). Note that the partially defatted almond protein flour is not intended for use as a sole source of protein in foods targeting growing children, and therefore, nutritional insufficiencies of these amino acids are not anticipated to be a concern.

Table 6.4.3-1 Calculation of Amino Acid Scores for Partially Defatted Almond Protein Flour (Natural and Blanched Almonds)

Essential Amino Acids (mg/g protein)	Partially Defatted Almond Protein Flour		FAO Reference Requirements for Amino Acids (mg/g protein)[b]	Calculated Amino Acid Scores Using FAO Reference Requirements	
	Blanched Almonds [a]	Natural Almonds [a]		Blanched Almonds	Natural Almonds
Histidine	26	27	19	1.3	1.4
Isoleucine	40	41	28	1.4	1.5
Leucine	73	73	66	1.1	1.1
Lysine	29	35	58	**0.5**	**0.6**
Methionine + Cysteine	22	22	25	0.9	0.9
Phenylalanine + Tyrosine	86	87	63	1.4	1.4
Threonine	30	31	34	0.9	0.9
Tryptophan	9	9	11	0.8	0.8
Valine	46	47	35	1.3	1.3

FAO = Food and Agriculture Organization of the United Nations.
[a] The values were obtained using the essential amino acid content as listed in Table 3.4.2-1 in g/100 g and taking into account a mean protein content of 45.86 and 41.95% for blanched and natural almonds, respectively (refer to Table 2.5.2-1).
[b] Reference requirements for amino acids as determined by the FAO for 2 to 5 years old preschool aged children (FAO/WHO/UNU, 1985).

Blue Diamond evaluated the true fecal protein digestibility of partially defatted almond protein flour from both blanched and natural almonds in rats and reported values of 93.78% and 90.87%, respectively, indicating that the protein is readily digestible *in vivo*. These values are consistent with those reported by Ahrens *et al.* (2005) for 3 varieties of almonds (Carmel, Mission, and Nonpareil) ranging from ~83 to 92%. Considering the amino acid score of lysine (*i.e.,* limiting amino acid as per Table 6.4.3-1) and the fecal digestibility values of 93.78% and 90.87%, as reported for blanched and natural almonds, respectively, the PDCAAS rating for the partially defatted almond protein flour from blanched and natural almonds can be calculated using the following formula[2]:

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

Using the above formula, PDCAAS values of 46.9% and 54.5% were calculated for partially defatted almond protein flour from blanched and natural almonds, respectively. These PDCAAS values are higher than the reported PDCAAS values for 3 varieties of almonds (Carmel, Mission, and Nonpareil), which ranged from 0.22 (22%) to 0.24 (24%) for children (2 to 5 years) and 0. 32 to 0.36 for adults ≥18 years (Ahrens *et al.,* 2005), suggesting that the protein extraction process likely resulted in a better digestibility and increased protein quality when compared to whole almonds. In addition, PDCAAS values ranging from 44.3 to 47.8 were reported for Butte, Independence, Monterey, and Nonpareil varieties of raw almonds based on the amino acid requirements of children 2 to 5 years of age (House *et al.,* 2019). The PDCAAS for casein, the gold standard protein, is 1.0 (Pacheco *et al.,* 1997).

6.4.4 Estimated Exposure to Minerals

The exposure to various minerals present in partially defatted almond protein flour (see Table 2.5.3-1) has been assessed for children as a sensitive population and female adults, which have the highest 90th percentile intakes of partially defatted almond protein flour on an absolute basis (see Table 3.3.2-1). The mineral intakes for the aforementioned population groups were well below the established tolerable upper limits proposed by EFSA and Institute of Medicine; therefore, intakes of minerals resulting from the use of partially defatted almond protein flour as a food ingredient do not pose a safety concern.

Table 6.4.4-1 Estimated Exposure to Minerals from the Partially Defatted Almond Protein Flour in the Total Population (90th Percentile)

Mineral	Partially Defatted Almond Protein Flour (Blanched Almonds)		Partially Defatted Almond Protein Flour (Natural Almonds)		Tolerable Upper Limit (EFSA, 2018)	Tolerable Upper Limit (IOM, 2006)
	Children (mg/day)	Female Adults (mg/day)	Children (mg/day)	Female Adults (mg/day)		
Boron	0.58	1.01	0.65	1.13	10 mg/day	20 mg/day
Calcium	56.18	97.31	94.19	163.15	2,500 mg/day	2,500 mg/day (19 to 50 y) 2,000 mg/day (51 to >70 y)
Copper	0.240	0.415	0.197	0.341	5 mg/day	10,000 µg/day
Iron	0.777	1.346	0.806	1.397	N/A	45 mg/day
Magnesium	59.024	102.238	55.138	95.506	250 mg/day	350 mg/day

[2] Note: The reference protein pattern of essential amino acids is based on the amino acid requirements of 2 to 5 year old preschool aged children as determined by the FAO/WHO/UNU (see Table 6.5.3-1).

Table 6.4.4-1 Estimated Exposure to Minerals from the Partially Defatted Almond Protein Flour in the Total Population (90th Percentile)

Mineral	Partially Defatted Almond Protein Flour (Blanched Almonds)		Partially Defatted Almond Protein Flour (Natural Almonds)		Tolerable Upper Limit (EFSA, 2018)	Tolerable Upper Limit (IOM, 2006)
	Children (mg/day)	Female Adults (mg/day)	Children (mg/day)	Female Adults (mg/day)		
Manganese	0.418	0.724	0.531	0.920	N/A	11 mg/day
Molybdenum	0.009	0.015	0.009	0.015	0.6 mg/day	2,000 µg/day
Nickel	0.021	0.036	0.022	0.038	N/A	1.0 mg/day
Phosphorus	110.118	190.741	102.312	177.219	N/A	4 g/day
Sodium	6.362	11.019	0.197	0.341	N/A	2.3 g/day
Zinc	0.623	1.079	0.613	1.061	N/A	40 mg/day

N/A = not available.

6.5 GRAS Panel Evaluation

Blue Diamond has concluded partially defatted almond protein flour, as described herein, is GRAS for use in conventional food and beverage products, as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of partially defatted almond protein flour and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. George C. Fahey, Jr. (University of Illinois), and Dr. Robert J. Nicolosi (University of Massachusetts Lowell). For the purposes of the GRAS Panel's evaluation, "safe" or "safety" means there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use, as defined under 21 CFR §170.3(i) (U.S. FDA, 2019b).

The GRAS Panel, convened by Blue Diamond, independently and critically evaluated all data and information presented herein, and also concluded that partially defatted almond protein flour is GRAS based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses is presented in Appendix A.

6.6 Conclusion

Based on the above data and information presented herein, Blue Diamond has concluded that the intended uses of partially defatted almond protein flour as a plant-based protein source in conventional food and beverage products, as described in Section 1.3, is GRAS based on scientific procedures. General recognition of Blue Diamond's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training who similarly concluded that the intended use of partially defatted almond protein flour as a plant-based source of protein in conventional food and beverage products as described herein is GRAS.

Partially defatted almond protein flour 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.

Part 7. §170.255 List of Supporting Data and Information

Ahrens S, Venkatachalam M, Mistry AM, Lapsley K, Sathe SK (2005). Almond (*Prunus dulcis* L.) protein quality. Plant Foods Hum Nutr 60(3):123-128. DOI:10.1007/s11130-005-6840-2.

Arjariya S, Nitin N, Swati T (2013). Investigate the toxicological effect on aqueous extract of *Terminalia catappa* Linn in rat. Int J Res Dev Pharm Life Sci 2(5):596-601.

Bolling BW, Dolnikowski G, Blumberg JB, Chen CO (2010). Polyphenol content and antioxidant activity of California almonds depend on cultivar and harvest year. Food Chem 122(3):819-825. DOI:10.1016/j.foodchem.2010.03.068.

Bornhorst GM, Drechsler KC, Montoya CA, Rutherfurd SM, Moughan PJ, Singh RP (2016). Gastric protein hydrolysis of raw and roasted almonds in the growing pig. Food Chem 211:502-508. DOI:10.1016/j.foodchem.2016.05.085.

Boye J, Wijesinha-Bettoni R, Burlingame B (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br J Nutr 108(Suppl. 2):S183-S211. DOI:10.1017/S0007114512002309.

CDC (2018a). *National Health and Nutrition Examination Survey (NHANES): 2015-2016*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015 [NHANES Home Page last reviewed: October 30, 2018].

CDC (2018b). *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: July 2018].

CFIA (2018). Calculating protein ratings. In: *Elements within the Nutrition Facts Table. Protein*. (Food Labelling for Industry). Ottawa (ON): Canadian Food Inspection Agency (CFIA). Available at: http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/labelling/for-industry/nutrition-labelling/elements-within-the-nutrition-facts-table/eng/1389206763218/1389206811747?chap=7#s9c7 [Date modified: 2018-05-11].

Chai W, Liebman M (2004). Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. J Urol 172(3):953-957. DOI:10.1097/01.ju.0000135918.00761.8a.

Chaouali N, Gana I, Dorra A, Khelifi F, Nouioui A, Masri W, et al. (2013). Potential toxic levels of cyanide in almonds (*Prunus amygdalus*), apricot kernels (*Prunus armeniaca*), and almond syrup. ISRN Toxicol 2013:Article ID 610648 [6pp]. DOI:10.1155/2013/610648.

Che H, Zhang Y, Lyu SC, Nadeau KC, McHugh T (2019). Identification of almond (*Prunus dulcis*) vicilin as a food allergen. J Agric Food Chem 67(1):425-432 [plus supplementary data]. DOI:10.1021/acs.jafc.8b05290.

Chen L, Zhang S, Illa E, Song L, Wu S, Howad W, Arús P, van de Weg E, Chen K, Gao Z (2008). Genomic characterization of putative allergen genes in peach/almond and their synteny with apple. BMC Genomics 9:543 [15pp]. DOI:10.1186/1471-2164-9-543.

Costa J, Mafra I, Carrapatoso I, Oliveira MB (2012). Almond allergens: molecular characterization, detection, and clinical relevance. J Agric Food Chem 60(6):1337-1349. DOI:10.1021/jf2044923.

Cowan JW, Sabry ZI, Rinnu FJ, Campbell JA (1963). Evaluation of protein in Middle Eastern diets. J Nutr 81:235-240. DOI:10.1093/jn/81.3.235.

Das B, Ahmed N, Singh P (2011). *Prunus* diversity-early and present development: a review. Int J Biodivers Conserv 3(14):721-734. DOI:10.5897/ijbcx11.003.

Dazult Ltd. (2018). *DaDiet - The Dietary Intake Evaluation Tool [Software]*. (Version 17.04). Straffan, County Kildare, Ireland: Dazult Ltd. Available at: http://dadiet.daanalysis.com.

De Angelis E, Bavaro SL, Forte G, Pilolli R, Monaci L (2018). Heat and pressure treatments on almond protein stability and change in immunoreactivity after simulated human digestion. Nutrients 10(11):1679 [20pp]. DOI:10.3390/nu10111679.

Delaney B, Astwood JD, Cunny H, Conn RE, Herouet-Guicheney C, Macintosh S, et al. (2008). Evaluation of protein safety in the context of agricultural biotechnology [ILSI International Food Biotechnology Committee Task Force on Protein Safety]. Food Chem Toxicol 46(Suppl. 2):S71-S97. DOI:10.1016/j.fct.2008.01.045.

Duong QH, Clark KD, Lapsley KG, Pegg RB (2017). Quantification of inositol phosphates in almond meal and almond brown skins by HPLC/ESI/MS. Food Chem 229:84-92. DOI:10.1016/j.foodchem.2017.02.031.

EFSA (2016). Acute health risks related to the presence of cyanogenic glycosides in raw apricot kernels and products derived from raw apricot kernels (EFSA Panel on Contaminants in the Food Chain/CONTAM) Question no: EFSA-Q-2015-00225, adopted 1 March 2016 by European Food Safety Authority). EFSA J. 14(4):4426 [47pp]. DOI:10.2903/j.efsa.2016.4424. Available at: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2016.4424.

EFSA (2018). O*verview on Tolerable Upper Intake Levels as derived by the Scientific Committee on Food (SCF) and the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)*. (Version 4; September 2018). Parma, Italy: European Food Safety Authority (EFSA). Available at: https://www.efsa.europa.eu/sites/default/files/assets/UL_Summary_tables.pdf.

FAO/WHO (1991). *Protein Quality Evaluation. Report of the Joint FAO/WHO Expert Consultation*, Dec. 4-8 1989, Bethesda, Maryland. (FAO Food and Nutrition Paper, no 51). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: http://apps.who.int/iris/bitstream/10665/38133/1/9251030979_eng.pdf?ua=1.

FAO/WHO/UNU (1985). *Energy and Requirements in Human Nutrition*. Report of a Joint WHO/FAO/UNU Expert Consultation, Oct. 5-17, 1981, Rome. (WHO Technical Report Series no 724, Reprinted 1987, 1991). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO) / Tokyo, Japan: United Nations University (UNU). Available at: http://www.fao.org/docrep/003/AA040E/AA040E00.HTM.

FARRP (2019). *AllergenOnline Version 19: Home of the FARRP Allergen Protein Database*. Lincoln (NE): University of Nebraska-Lincoln, Food Allergy Research and Resource Program (FARRP). Available at: http://www.allergenonline.org [Released: February 10, 2019].

Gradziel TM (2011). Origin and dissemination of almond. In: Janick J, editor. *Origin and dissemination of Prunus Crops: Peach, Cherry, Apricot, Plum, Almond*. (Reprint of Horticultural Reviews, volume 38). American Pomological Society by permission of Hoboken (NJ): Wiley-Blackwell, pp. 187-241. DOI:10.1002/9780470872376.ch2.

Groven SL, Corwin C, Marx A, Messervy L, Hooshmand S, Kern M, et al. (2017). The effects of mixed nuts on lipid profiles, glucose, oxidative stress, and antioxidant capacity in atherogenic-diet fed rats FASEB J 31(Suppl. 1):lb321. DOI:10.1096/fasebj.31.1_supplement.lb321.

House JD, Hill K, Neufeld J, Franczyk A, Nosworthy MG (2019). Determination of the protein quality of almonds (*Prunus dulcis* L.) as assessed by *in vitro* and *in vivo* methodologies. Food Sci Nutr 7(9):2932-2938. DOI:10.1002/fsn3.1146.

IOM (2006). Dietary Reference Intakes (DRIs): tolerable upper intake levels, vitamins. In: *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington (DC): National Academies / The National Academy Press (NAP), Institute of Medicine (IOM). Available at: http://www.nationalacademies.org/hmd/~/media/Files/Activity%20Files/Nutrition/DRI-Tables/4_%20UL%20Values_Vitamins%20and%20Elements.pdf?la=en.

JECFA (1993). Cyanogenic glycosides. In: *Toxicological Evaluation of Certain Food Additives and Naturally Occurring Toxicants*. 39th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Feb. 3-12, 1992, Rome, Italy. (WHO Food Additives Series, no 30). Geneva, Switz.: World Health Organization (WHO) / International Programme on Chemical Safety (IPCS). Available at: http://www.inchem.org/documents/jecfa/jecmono/v30je18.htm.

JECFA (2011). Chapter 4.1. Cyanogenic glycosides. In: *Evaluation of Certain Food Additives and Contaminants*. Seventy-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 14-23, 2011, Rome. (WHO Technical Report Series, no 966). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO), pp. 55-70, 127-130. Available at: http://apps.who.int/iris/bitstream/10665/44788/1/WHO_TRS_966_eng.pdf.

JECFA (2012). Cyanogenic glycosides (addendum). In: *Safety Evaluation of Certain Food Additives and Contaminants*. Seventy-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Meeting Date, Meeting Place. (WHO Technical Report Series, no 65). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO), pp. 171-323, 821-823. Available at: http://apps.who.int/iris/bitstream/10665/44813/1/9789241660655_eng.pdf.

Jia X-Y, Zhang Q-A, Zhang Z-Q, Wang Y, Yuan J-F, Wang H-Y, et al. (2011). Hepatoprotective effects of almond oil against carbon tetrachloride induced liver injury in rats. Food Chem 125(2):673-678. DOI:10.1016/j.foodchem.2010.09.062.

Jin T, Albillos SM, Guo F, Howard A, Fu TJ, Kothary MH, et al. (2009). Crystal structure of prunin-1, a major component of the almond (*Prunus dulcis*) allergen amandin. J Agric Food Chem 57(18):8643-8651. DOI:10.1021/jf9017355.

Kim Y, Mistry Anahita M, Sathe Shridhar K (2003). Dietary almonds elevate plasma HDL and reduce plasma LDL cholesterol in rats. FASEB J 17(4/5):abstract 204.11.

Mandalari G, Mackie AR (2018). Almond allergy: an overview on prevalence, thresholds, regulations and allergen detection. Nutrients 10(11):1706 [12pp]. DOI:10.3390/nu10111706.

Mandalari G, Faulks RM, Rich GT, Lo Turco V, Picout DR, Lo Curto RB, et al. (2008). Release of protein, lipid, and vitamin E from almond seeds during digestion. J Agric Food Chem 56(9):3409-3416. DOI:10.1021/jf073393v.

O'Neil CE, Nicklas TA, Fulgoni VL III (2016). Almond consumption is associated with better nutrient intake, nutrient adequacy, and diet quality in adults: National Health and Nutrition Examination Survey 2001-2010. Food Nutr Sci 7:504-515. DOI:10.4236/fns.2016.77052.

Pacheco MT, Caballero-Córdoba GM, Sgarbieri VC (1997). Composition and nutritive value of yeast biomass and yeast protein concentrates. J Nutr Sci Vitaminol (Tokyo) 43(6):601-612. DOI:10.3177/jnsv.43.601.

Palacín A, Tordesillas L, Gamboa P, Sanchez-Monge R, Cuesta-Herranz J, Sanz ML, et al. (2010). Characterization of peach thaumatin-like proteins and their identification as major peach allergens. Clin Exp Allergy 40(9):1422-1430. DOI:10.1111/j.1365-2222.2010.03578.x.

Rietjens IMCM, Martena MJ, Boersma MG, Spiegelenberg W, Alink GM (2005). Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol Nutr Food Res 49(2):131-158. DOI:10.1002/mnfr.200400078.

Song Y, Wang W, Cui W, Zhang X, Zhang W, Xiang Q, et al. (2010). A subchronic oral toxicity study of almond skins in rats. Food Chem Toxicol 48(1):373-376. DOI:10.1016/j.fct.2009.10.025.

Sze-Tao KWC, Sathe SK (2000). Functional properties and in vitro digestibility of almond (*Prunus dulcis* L.) protein isolate. Food Chem 69(2):153-160. DOI:10.1016/S0308-8146(99)00244-7.

Tinus T, Damour M, Van Riel V, Sopade PA (2012). Particle size-starch-protein digestibility relationships in cowpea (*Vigna unguiculata*). J Food Eng 113:254-264. DOI:10.1016/j.jfoodeng.2012.05.041. Cited In: House et al., 2019 [Ref. #15].

Toomer OT, Do A, Pereira M, Williams K (2013). Effect of simulated gastric and intestinal digestion on temporal stability and immunoreactivity of peanut, almond, and pine nut protein allergens. J Agric Food Chem 61(24):5903-5913. DOI:10.1021/jf400953q.

U.S. EPA (2019). *Food Commodity Intake Database: What We Eat in America [2005-2010]*. [FCID]. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Office of Pesticide Programs. Available at: http://fcid.foodrisk.org/ ; http://fcid.foodrisk.org/dbc/ [© University of Maryland 2012 - 2019].

U.S. FDA (1993). Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: *Priority Based Assessment of Food Additives (PAFA) Database*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), p. 58. Available at: http://www.fda.gov/ohrms/DOCKETS/DOCKETS/95s0316/95s-0316-Rpt0272-36-Appendix-D-Reference-F-FDA-vol205.pdf.

U.S. FDA (2004). *Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II) [FALCPA]*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106187.htm [Page Last Updated: 05/02/2016].

U.S. FDA (2017). *Agency Response Letter GRAS Notice No. GRN 000684 [San Francisco (CA): Hampton Creek, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=684 [Aug. 4, 2017].

U.S. FDA (2019a). Part 164—Tree nut and peanut products. §164.110—Mixed nuts [almonds]. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

U.S. FDA (2019b). Part 170—Food additives. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

U.S. FDA (2019b). Part 170—Food additives. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

U.S. FDA (2019c). Part 164—Tree nut and peanut products. Section §164.110—Mixed nuts. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

USDA (2018a). Full report (all nutrients): 45285557, California almonds, UPC: 034952587395. In: *USDA Branded Food Products Database*. (Release July, 2018). Beltsville (MD): U.S. Department of Agriculture (USDA). Available at: https://ndb.nal.usda.gov/ndb/foods/show/45285557?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=natural+california+almonds&ds=&qt=&qp=&qa=&qn=&q=&ing=.

USDA (2018b). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2015-2016.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: July 31, 2018].

USDA (2018c). *Fruit and Tree Nut Yearbook Tables*. Washington (DC): U.S. Department of Agriculture (USDA), Economic Research Service (ERS). Available at: https://www.ers.usda.gov/data-products/fruit-and-tree-nut-data/fruit-and-tree-nut-yearbook-tables/#40907 [Last updated: Wednesday, October 31, 2018].

USDA-ARS (2019). *USDA Food and Nutrient Database for Dietary Studies 2015-2016 [FNDDS]*. Beltsville (MD): U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Food Surveys Research Group. Available at: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds/ [Last Modified: 05/21/2019].

WHO/IUIS (2019). Search results for '*Prunus dulcis* (Almond)'. In: *Allergen Nomenclature [Database]*. Geneva, Switz.: World Health Organization (WHO) / International Union of Immunological Societies (IUIS). Available at: http://www.allergen.org/search.php?allergenname=&allergensource=almond&TaxSource=&TaxOrder=&foodallerg=all&bioname= [Last accessed: February 28, 2018].

Appendix A
GRAS Panel Consensus Statement

GRAS Panel Evaluation of Partially Defatted Almond Protein Flour for Uses in Conventional Food and Beverage Products

25 April 2019

INTRODUCTION

Blue Diamond Growers ("Blue Diamond" hereinafter) convened a panel of independent scientists (GRAS 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 data and information pertinent to the safety of the company's partially defatted almond protein flour (PDAPF) and to determine whether the intended uses of PDAPF in various conventional food and beverage products as described in Table A-1 would be Generally Recognized as Safe (GRAS) based on scientific procedures. The GRAS Panel consisted of the below-signed qualified scientific experts: Professor Emeritus Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Professor Emeritus George C. Fahey, Jr. (University of Illinois), and Professor Robert J. Nicolosi (University of Massachusetts Lowell).

The GRAS Panel, independently and collectively, critically evaluated a comprehensive package of publicly available scientific data and information compiled from the literature and summarized in a dossier titled "*Evaluation of Partially Defatted Almond Protein Flour as Generally Recognized as Safe (GRAS) for Use in Conventional Food and Beverage Products*" (dated 1 April 2019), which included an evaluation of available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended food-uses of PDAPF. This information was prepared in part from a comprehensive search of the scientific literature performed by Blue Diamond and included information characterizing the identity and purity of the ingredient, the manufacture of the ingredient, product specifications, supporting analytical data, intended conditions of use, estimated exposure under the intended uses, and the safety of PDAPF.

Following its independent and collective critical evaluation, and on the basis of scientific procedures, the GRAS Panel unanimously concluded that PDAPF, meeting food-grade specifications and manufactured in accordance with current Good Manufacturing Practice (cGMP), is GRAS for use in conventional food and beverage products as described in Table A-1. A summary of the information reviewed by the GRAS Panel is presented below.

SUMMARY AND BASIS FOR GRAS

PDAPF manufactured by Blue Diamond is obtained from almonds through a series of mechanical processes without the use of any processing aids or solvents. The final ingredients (PDAPF from blanched almonds and PDAPF from natural almonds) is comprised of protein (41.95 to 45.86%), carbohydrates (35.30 to 38.98%), moisture (4.65 to 4.76%), fat (7.83 to 8.00%), and ash (6.25 to 6.43%). Blue Diamond intends to market PDAPF in the United States (U.S.) marketplace as a food ingredient in various conventional food and beverage products (Table A-1).

The GRAS Panel individually and collectively critically evaluated details of the manufacturing process for PDAPF, which utilizes a series of mechanical processing steps, including crushing of pasteurized almonds with a mechanical press to remove the oil, crumbling the crushed almond cake that is then pneumatically conveyed to a mill where it is powdered into a flour. After milling, the almond flour is pneumatically conveyed through a thermal treatment and packaged and sealed into 55-lb multi-wall bags prior to storage and distribution. The product is manufactured in accordance with the principles of cGMP and include critical control steps to limit the introduction of foreign materials and microbiological contaminant into the final product.

Blue Diamond has established food-grade specifications for PDAPF which include parameters related to chemical properties and heavy metals and microbiological contaminants. All analytical methods are internationally recognized. The GRAS Panel reviewed the results from 5 batches of PDAPF from blanched almonds and 4 batches of PDAPF from natural (unblanched) almonds and concluded that the manufacturing process produces a consistent product in conformance with the established specifications. PDAPF from blanched and unblanched almond sources were analyzed for their mineral and amino acid profile. The amino acid profiles were consistent and balanced across all lots and the levels of minerals did not raise safety concerns under the conditions of intended use. The levels of amygdalin in PDAPF from both blanched and unblanched almonds also did not raise safety concerns.

The GRAS Panel reviewed the accelerated 12-month shelf-life stability of PDAPF from blanched and unblanched almonds under frozen (30°F/-16.1°C), ambient (68 to 72°F/20 to 22°C) and accelerated (104°F/40°C) storage conditions. The samples were analyzed at 0, 2, 4, 6, 8, 10, and 12-week intervals for moisture, water activity, hexanal, flavor, aroma, and overall difference from control. The results indicate no variance from the product specifications for moisture and other attributes compared to the control, and the product is stable and in compliance with the labeled shelf-life of 12 months in unopened bags when stored between 65 to 75°F (18 to 24°C) in a dry, odor-free area away from direct sunlight.

There was limited safety data available on almond and/or almond protein conducted in animal models or humans. Several studies conducted in rats evaluated efficacy-related endpoints, such as hepatoprotective effects of almond oil (Jia *et al.,* 2011), lipid profile, glucose, or antioxidant capacity of almonds (Groven *et al.,* 2017), and modulation of high-density lipoprotein (HDL) or low-density lipoprotein (LDL) cholesterol (Kim *et al.,* 2003) but no safety endpoints. Song *et al.,* (2010) and Arjariya *et al.*, (2013) reported on safety-related endpoints in rats. These studies were conducted with partially defatted almond skins (Song *et al.,* 2010) or aqueous extracts of the Indian almond plant (Arjariya *et al.,* 2013); since test articles were compositionally distinct from PDAPF, they are not relevant to the safety assessment of PDAFP. Several clinical studies evaluated the effects of almond consumption on risk factors of cardiovascular disease (HDL-cholesterol or LDL-cholesterol, lipid profile, blood pressure), vascular function, biomarkers of inflammation and lipid peroxidation, antioxidant effects, insulin resistance, or post-prandial metabolic response in pregnant women. Findings from these studies did not identify any biological effects that suggest that use of PDAPF as a food ingredient would be unsafe.

Since PDAPF is derived from a whole food with a long-history of safe consumption, the safety assessment can be evaluated using general principles of the 2-tiered weight of evidence approach described by the International Life Sciences Institute (ILSI) for the safety assessment of proteins produced in genetically engineered agricultural products (Delaney *et al.,* 2008). In the first step (Tier I), hazard identification of the ingredient can be considered sufficient to characterize the safety of the ingredient for food-use without the need for animal toxicology data provided that the following criteria can be established: (1) there is a long-history of safe use of the ingredient in the food supply by a substantial population group that is representative of the expected population of consumers (*e.g.*, children, elderly, healthy and unhealthy subjects); (2) the ingredient is fully characterized with respect to exposure to natural toxins and anti-nutritional factors under the proposed conditions of use; (3) the nutritional implications of the ingredient are fully addressed, *e.g.*, protein quality, levels of nutrients and minerals; and (4) there are no biological effects associated with the ingredient from clinical studies to suggest that its use in food may be unsafe. In the event that data gaps exist at the Tier I stage, additional information may be necessary for evaluation under Tier II. This information includes toxicological studies or other hypothesis-based testing strategies to address specific safety-related questions (*e.g.*, animal toxicology studies), clinical studies, and nutritional studies (*e.g.*, protein quality if uses in children and infants are desired). The approach described by ILSI has been used to support the GRAS status of protein ingredients such as mung bean protein isolate [GRAS Notice (GRN) 684; U.S. FDA, 2017].

Almonds have a long history of safe use in the diet. Based on the history of safe use of almonds and the fact that PDAPF derived from almonds is minimally processed, e.g. does not involve the use of solvents, it can be concluded that PDAPF is unlikely to contain unknown constituents of toxicological concern. Safety concerns related to the use of PDAPF as an ingredient can be predicted by an understanding of the manufacturing process and its impact on the ingredient composition (*i.e*., the potential to concentrate natural toxins, minerals, or anti-nutrients factors), characterization of the allergenicity potential, and consideration of nutritional impacts for food applications where protein quality may impact the growth and development of the consumer (*e.g*., foods intended for use by infants or growing children). Each of these safety considerations are discussed in the following paragraphs.

Based upon the outcome of the Tier 1 evaluation, it was concluded that *in vivo* toxicology testing was not necessary to characterize the hazard of PDAPF for use as a food ingredient. Chemical analysis of PDAPF demonstrated that it does not present toxicological, nutritional, or microbiological hazards originating from current agricultural and cultivation practices, or that may arise through the production process. These analyses included data characterizing the content of natural toxic constituents [*e.g.*, anti-nutritional factors (amygdalin)], and external contaminants (*e.g.*, pesticides and heavy metals).

PDAPF is a comminuted product of a whole food (almond) that has a history of widespread consumption as a staple food commodity in the U.S. and elsewhere. PDAPF is intended to be added as a food ingredient in a variety of conventional food and beverage products as a source of plant protein to substitute for other protein sources in the diet (see Table A-1). The GRAS Panel reviewed data related to the estimated dietary exposure to PDAPF based on an assessment of the anticipated intake of PDAPF as an ingredient under the intended conditions of use as described in Table A-1, and the assessment of the estimated intake of almond protein from the background diet. The dietary intakes of the ingredient were estimated using the information from the 2013-2014 cycle of the National Health and Nutrition Examination Survey (NHANES) based on the proposed food-uses and use-levels of PDAPF as described in Table A-1.

On consumer-only basis, the resulting mean and 90^{th} percentile intakes of PDAPF by the total U.S. population from proposed food-uses in the U.S., were estimated to be 15.2 g/person/day (221 mg/kg body weight/day) and 22.0 g/person/day (391 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean intakes of PDAPF were determined to be 19.3 g/person/day (212 mg/kg body weight/day), among male adults, and the highest 90^{th} percentile intakes of PDAPF were determined to be 29.5 g/person/day (399 mg/kg body weight/day), among female adults. While infants and young children had the lowest mean and 90^{th} percentile consumer-only intakes on an absolute basis of 3.0 and 7.4 g/person/day, respectively, when expressed on a body weight basis, this age group had the highest daily intakes of 249 and 555 mg/kg body weight/day at the mean and 90^{th} percentile intake, respectively. The mean calculated consumer-only intakes of PDAPF by the total U.S. population from all proposed food-uses is approximately 6 times higher than the background intake of the almond protein (15.2 g/person/day *versus* 2.51 g/person/day, respectively).

The U.S. Food and Drug Administration (FDA) has established a Daily Reference Value (DRV) for protein of 50 g in adults and in children 4 years of age or older. The 90th percentile all-user intakes of PDAPF by the total U.S. population from all proposed food-uses (*i.e.*, 22.0 g/day), corresponding to protein intakes ranging between 8.8 and 10.2 g/person/day, based on protein specifications for this ingredient (40.0% and 46.5%), is approximately 5 to 6 times lower than the DRV for protein. Note that PDAPF is proposed as an alternative source of protein and is therefore unlikely to increase consumer exposure to protein which is currently derived mostly from unprocessed foods, including meat, poultry, fish, and legumes.

Almond is considered a major food allergen under "tree nuts" and is subject to labelling under the Food Allergen Labeling and Consumer Protection Act (FALCPA), as regulated by the U.S. FDA (U.S. FDA, 2004). In the U.S., almond allergy is the third most commonly reported tree nut allergy (behind cashew and walnut) impacting 15% of individuals with tree-nut allergies (Costa *et al.,* 2012). Eight groups of proteins have been identified and characterized as allergenic in almonds, including pathogenesis-related (PR)-10 (Pru du 1), thaumatin-like proteins (TLP) (Pru du 2), prolamins (Pru du 2S albumin, Pru du 3), profilins (Pru du 4), 60sRP (Pru du 5), and cupin (Pru du 6, Pru du γ-conglutin) (Costa *et al.,* 2012; Mandalari and Mackie, 2018; Che *et al.,* 2019). Pru du 3, Pru du 4, Pru du 5, and Pru du 6 are listed as putative allergens in the World Health Organization and International Union of Immunological Societies (WHO/IUIS) list (WHO/IUIS, 2019). Pru du 6 has shown immunoglobin E (IgE)-binding activity *via* Western blot or enzyme-linked immunosorbent assay (ELISA) analyses that is characteristic of allergenic proteins. Pru 4 Profilin and Pru p 2 have been demonstrated experimentally to contain IgE-binding activity *via* Western blot or ELISA analysis and skin prick test, in addition to biological activity as measured by basophil activation (Chen *et al.*, 2008; Palacín *et al.*, 2010; Das *et al.*, 2011; Costa *et al.*, 2012). Foods containing PDAPF will be clearly labeled as containing almonds.

In vitro (Sze-Tao and Sathe, 2000; Mandalari *et al.,* 2008; Toomer *et al.,* 2013; Bornhorst *et al.,* 2016; De Angelis *et al.,* 2018) and *in vivo* (Boye *et al.,* 2012) digestibility studies suggest that PDAPF would be readily digested in the gastrointestinal tract into amino acids and small peptide components. The true fecal protein digestibility, indicating the amount of nutrients absorbed from the small intestine, was determined by Blue Diamond for PDAPF from both blanched and natural almonds in a rat model to be 93.78% and 90.87%, respectively, indicating that the protein is readily digestible *in vivo*. These values are consistent with those reported by Ahrens *et al.* (2005) for 3 varieties of almonds (Carmel, Mission, and Nonpareil) ranging from ~83 to 92%.

A comparison of the amino acid profile of PDAPF with the recommended scoring patterns for various population groups as proposed by the World Health Organization and Food and Agriculture Organization (WHO/FAO) (FAO, 2013) indicates that with the exception of lysine, PDAPF meets or exceeds the amino acid requirements of older children/adolescents and adults (4 to 18 years and >18 years, respectively). When compared with the recommended amino acid scoring for the population group of children (6 months to 3 years of age), PDAPF is deficient in lysine and the sulfur-containing amino acids, methionine and cysteine. With respect to the population groups of infants (birth to 6 months), PDAPF is deficient in all essential amino acids, excluding histidine. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) rating proposed by the FAO in 1989 and adopted by the U.S. FDA in 1993 as "the preferred best" method to evaluate protein quality (FAO/WHO, 1991; U.S. FDA, 1993), is a measure of the bioavailability of nutritionally essential amino acids from a given protein source in comparison to a reference protein. The PDCAAS rating for PDAPF from blanched and natural almonds was calculated to be 46.9% and 54.5%, respectively. These PDCAAS values are higher than the reported PDCAAS values for 3 varieties of almonds

(Carmel, Mission, and Nonpareil), which ranged from 0.22 to 0.24 for children (2 to 5 years) and 0.32 to 0.36 for adults ≥18 years (Ahrens *et al.,* 2005), suggesting that the protein extraction process likely resulted in a better digestibility and increased protein quality when compared to whole almonds. In comparison, the PDCAAS for the gold standard protein, casein, is 1.0 (Pacheco *et al.,* 1997). Based on the foregoing, PDAPF is not suitable for use as the sole source or principal source of protein in food products targeted to infants or young children.

CONCLUSION

We, the GRAS Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that partially defatted almond protein flour (PDAPF), meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is Generally Recognized as Safe (GRAS) based on scientific procedures for use in a variety of conventional food and beverage products as described in Table A-1.

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

[redacted]

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

29 April 2019
Date

[redacted]

Professor Emeritus George C. Fahey, Jr. Ph.D.,
University of Illinois

May 6, 2019
Date

[redacted]

Professor Emeritus Robert J. Nicolosi, Ph.D.
University of Massachusetts Lowell

05/03/2019
Date

REFERENCES

Ahrens S, Venkatachalam M, Mistry AM, Lapsley K, Sathe SK (2005). Almond (*Prunus dulcis* L.) protein quality. Plant Foods Hum Nutr 60(3):123-128. DOI:10.1007/s11130-005-6840-2.

Arjariya S, Nitin N, Swati T (2013). Investigate the toxicological effect on aqueous extract of *Terminalia catappa* Linn in rat. Int J Res Dev Pharm Life Sci 2(5):596-601.

Bornhorst GM, Drechsler KC, Montoya CA, Rutherfurd SM, Moughan PJ, Singh RP (2016). Gastric protein hydrolysis of raw and roasted almonds in the growing pig. Food Chem 211:502-508. DOI:10.1016/j.foodchem.2016.05.085.

Boye J, Wijesinha-Bettoni R, Burlingame B (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br J Nutr 108(Suppl. 2):S183-S211. DOI:10.1017/S0007114512002309.

Che H, Zhang Y, Lyu SC, Nadeau KC, McHugh T (2019). Identification of almond (*Prunus dulcis*) vicilin as a food allergen. J Agric Food Chem 67(1):425-432 [plus supplementary data]. DOI:10.1021/acs.jafc.8b05290.

Chen L, Zhang S, Illa E, Song L, Wu S, Howad W, Arús P, van de Weg E, Chen K, Gao Z (2008). Genomic characterization of putative allergen genes in peach/almond and their synteny with apple. BMC Genomics 9:543 [15pp]. DOI:10.1186/1471-2164-9-543.

Costa J, Mafra I, Carrapatoso I, Oliveira MB (2012). Almond allergens: molecular characterization, detection, and clinical relevance. J Agric Food Chem 60(6):1337-1349. DOI:10.1021/jf2044923.

Das B, Ahmed N, Singh P (2011). *Prunus* diversity-early and present development: a review. Int J Biodivers Conserv 3(14):721-734. DOI:10.5897/ijbcx11.003.

De Angelis E, Bavaro SL, Forte G, Pilolli R, Monaci L (2018). Heat and pressure treatments on almond protein stability and change in immunoreactivity after simulated human digestion. Nutrients 10(11):1679 [20pp]. DOI:10.3390/nu10111679.

Delaney B, Astwood JD, Cunny H, Conn RE, Herouet-Guicheney C, Macintosh S, et al. (2008). Evaluation of protein safety in the context of agricultural biotechnology [ILSI International Food Biotechnology Committee Task Force on Protein Safety]. Food Chem Toxicol 46(Suppl. 2):S71-S97. DOI:10.1016/j.fct.2008.01.045.

FAO (2013). *Dietary Protein Quality Evaluation in Human Nutrition. Report of an FAO Expert Consultation*, Mar. 31-Apr. 2, 2011, Auckland, New Zealand. (FAO Food and Nutrition Paper no 92). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). Available at: http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf.

FAO/WHO (1991). *Protein Quality Evaluation. Report of the Joint FAO/WHO Expert Consultation*, Dec. 4-8 1989, Bethesda, Maryland. (FAO Food and Nutrition Paper, no 51). Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) / Geneva, Switz.: World Health Organization (WHO). Available at: https://apps.who.int/iris/bitstream/handle/10665/38133/9251030979_eng.pdf;jsessionid=AEA9752E488D3C5D00340993B1CA4A34?sequence=1.

Groven SL, Corwin C, Marx A, Messervy L, Hooshmand S, Kern M, et al. (2017). The effects of mixed nuts on lipid profiles, glucose, oxidative stress, and antioxidant capacity in atherogenic-diet fed rats FASEB J 31(Suppl. 1):lb321. DOI:10.1096/fasebj.31.1_supplement.lb321.

Jia X-Y, Zhang Q-A, Zhang Z-Q, Wang Y, Yuan J-F, Wang H-Y, et al. (2011). Hepatoprotective effects of almond oil against carbon tetrachloride induced liver injury in rats. Food Chem 125(2):673-678. DOI:10.1016/j.foodchem.2010.09.062.

Kim Y, Mistry Anahita M, Sathe Shridhar K (2003). Dietary almonds elevate plasma HDL and reduce plasma LDL cholesterol in rats. FASEB J 17(4/5):abstract 204.11.

Mandalari G, Faulks RM, Rich GT, Lo Turco V, Picout DR, Lo Curto RB, et al. (2008). Release of protein, lipid, and vitamin E from almond seeds during digestion. J Agric Food Chem 56(9):3409-3416. DOI:10.1021/jf073393v.

Mandalari G, Mackie AR (2018). Almond allergy: an overview on prevalence, thresholds, regulations and allergen detection. Nutrients 10(11):1706 [12pp]. DOI:10.3390/nu10111706.

Pacheco MT, Caballero-Córdoba GM, Sgarbieri VC (1997). Composition and nutritive value of yeast biomass and yeast protein concentrates. J Nutr Sci Vitaminol (Tokyo) 43(6):601-612. DOI:10.3177/jnsv.43.601.

Palacín A, Tordesillas L, Gamboa P, Sanchez-Monge R, Cuesta-Herranz J, Sanz ML, et al. (2010). Characterization of peach thaumatin-like proteins and their identification as major peach allergens. Clin Exp Allergy 40(9):1422-1430. DOI:10.1111/j.1365-2222.2010.03578.x.

Song Y, Wang W, Cui W, Zhang X, Zhang W, Xiang Q, et al. (2010). A subchronic oral toxicity study of almond skins in rats. Food Chem Toxicol 48(1):373-376. DOI:10.1016/j.fct.2009.10.025.

Sze-Tao KWC, Sathe SK (2000). Functional properties and in vitro digestibility of almond (*Prunus dulcis* L.) protein isolate. Food Chem 69(2):153-160. DOI:10.1016/S0308-8146(99)00244-7.

Toomer OT, Do A, Pereira M, Williams K (2013). Effect of simulated gastric and intestinal digestion on temporal stability and immunoreactivity of peanut, almond, and pine nut protein allergens. J Agric Food Chem 61(24):5903-5913. DOI:10.1021/jf400953q.

U.S. FDA (1993). Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: *Priority Based Assessment of Food Additives (PAFA) Database*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), p. 58.

U.S. FDA (2004). *Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II) [FALCPA]*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/ucm106187.htm [Page Last Updated: 05/02/2016].

U.S. FDA (2017). *Agency Response Letter GRAS Notice No. GRN 000684 [San Francisco (CA): Hampton Creek, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=684 [Aug. 4, 2017].

U.S. FDA (2018). Part 170—Food additives. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

WHO/IUIS (2019). Search results for '*Prunus dulcis* (Almond)'. In: *Allergen Nomenclature [Database]*. Geneva, Switz.: World Health Organization (WHO) / International Union of Immunological Societies (IUIS). Available at: http://www.allergen.org/search.php?allergenname=&allergensource=almond&TaxSource=&TaxOrder=&foodallerg=all&bioname= [Last accessed: February 28, 2018].

ATTACHMENT A1: INTENDED FOOD-USES AND USE-LEVELS FOR PARTIALLY DEFATTED ALMOND PROTEIN FLOUR IN THE UNITED STATES

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Partially Defatted Almond Protein Flour in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2018)	Proposed Food-Uses [a]	Partially Defatted Almond Protein Flour Use-Level (%)
Baked Goods and Baking Mixes	Biscuits	5
	Cakes	10
	Cookies	5
	Cornbread, Corn Muffins, or Tortillas	5
	Crackers	5
	Doughnuts	5
	French toast, pancakes, waffles	10
	Muffins	5
Beverages and Beverage Bases	Non-Milk-Based nutritional powders (Plant Based; incl. meal replacements)[b]	35
	Protein powders	80
Coffee and Tea	Ready-to-Drink Coffee Drinks	5
Grain Products and Pastas	Cereal and Granola Bars	5
	Energy Bars or Protein Bars	25
	Meal Replacement Bars	10
Milk Products	Milk-based smoothies	5
	Milk-based nutritional powders (incl. meal replacements)[b]	35
Processed Fruits and Fruit Juices	Fruit Smoothies (RTD)	5

CFR = Code of Federal Regulations; incl. = including; RTD = ready-to-drink; U.S. = United States.
[a] Partially Defatted almond protein flour is intended for use in unstandardized products where standards of identity, as established under 21 CFR §130 to 169, do not permit its addition in standardized products.
[b] Includes ready-to-drink and powder forms