From: Kurt Waananen
To: Hall, Karen
Subject: RE: Questions for GRN 918
Date: Wednesday, October 7, 2020 6:42:02 PM
Attachments: image001.png
Response to FDA - GRN 918 - Oct 6 2020 Final.pdf

Thank you, Karen.

We were able to finish the response today and it is attached here for your review. Please let me know if you need anything further during the review.

Best regards,
Kurt

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Wednesday, October 7, 2020 7:30 AM
To: Kurt Waananen <kwaananen@bdgrowers.com>
Subject: [EXTERNAL] - RE: Questions for GRN 918

Good Morning Kurt,

You may respond by October 12, 2020.

Kind Regards,
Karen

From: Kurt Waananen <kwaananen@bdgrowers.com>
Sent: Tuesday, October 6, 2020 3:46 PM
To: Hall, Karen <Karen.Hall@fda.hhs.gov>
Subject: RE: Questions for GRN 918

Hi Karen,

We have made good progress on our response are very close to completion. Can we please have an extension to respond by next Monday, October 12?

Thank you for your consideration.

Best regards,
Kurt

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers

1802 C Street I Sacramento, CA 95811
O: 916-446-8309 I C: 763-218-0495 I kwaananen@bdgrowers.com

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Monday, September 21, 2020 2:13 PM
To: Kurt Waananen <kwaananen@bdgrowers.com>
Subject: [EXTERNAL] - Questions for GRN 918

Good Afternoon Mr. Waananen,

During our review of GRN 918, which you submitted for partially defatted almond protein flour, we noted concerns that need to be addressed and are attached to this email. Please provide a response to the attached questions within 10 business days. If you are unable to complete the response within that time frame, please contact me to discuss further options. If you have questions or need further clarification, please feel free to contact me. Thank you in advance to your attention to our comments.

Kind Regards,
Karen

Karen Hall
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Karen.Hall@fda.hhs.gov

*****Communications may be monitored for quality assurance and security purposes. This correspondence may contain confidential information. Unless you are the addressee (or you are authorized to receive for the addressee), you may not copy, use, or distribute this information. If you have received this in error, please advise the sender immediately at Blue Diamond Growers and permanently delete all copies, electronic or other, you may have.*****

6 October 2020

Karen Hall
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740-3835 USA

Dear Karen Hall,

Re: GRAS Notice No. GRN 918 for Partially Defatted Almond Protein Flour

Please see find below responses to the United States (U.S.) Food and Drug Administration (FDA)'s queries on GRAS Notice (GRN) No. 918 pertaining to partially defatted almond protein flour (PDAPF).

Question 1. We acknowledge that you provided estimated daily intake of almond protein tables (Tables 3.1-2 and 3.1-3) which include a population group of infants and young children aged 0 to 2 years. Infants are under 12 months of age. Please clarify whether PDAPF proposed food uses include infant formula.

Response 1. The PDAPF ingredient is not intended for use in infant formula. The population group of infants (age 0 to 2 years) was removed, and the total U.S. population was updated to include only individuals 2 years of age and above. The updated dietary intakes are provided in Tables 1 and 2 below on an absolute basis (g/person/day) and body weight basis (mg/kg body weight/day), respectively.

Table 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
Young Children	2 to <3	2.9	7.0	84.4	156	3.5	7.8
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	2 and up	11.8	16.7	77.0	5,001	15.4	22.4

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

Table 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
Young Children	2 to <3	220	467	84.2	152	261	546
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	2 and up	170	295	76.9	4,951	221	388

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

Question 2. You state on page 26 that a major safety concern from the use of almonds is the presence of the cyanogenic glycoside, amygdalin, due to the release of hydrogen cyanide upon metabolization. You provided results from the analyses of amygdalin levels in PDAPF from five non-consecutive lots and four non-consecutive lots derived from blanched almonds and natural almonds, respectively. Please provide a specification for maximum amygdalin in PDAPF, list the method of analysis and provide data to demonstrate that PDAPF levels consistently meet the specification. Alternatively, you could provide your acceptance criteria for amygdalin for the source material.

Response 2. The amygdalin content of the PDAPF from blanched and natural almonds was measured using ultrahigh-pressure liquid chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (QqQ MS/MS) with electrospray ionization (ESI) as described by Lee *et al.* (2013)[1]. The mean levels of amygdalin across 5 non-consecutive lots of PDAPF from blanched almonds was 70.1±19.5 mg/kg and 143.3±35.8 mg/kg across 4 non-consecutive lots of PDAPF from natural almonds. Lee *et al.* (2013) measured the amygdalin content from 10 different varieties of sweet almonds sourced from 4 different growing regions in California. The mean amygdalin levels reported by Lee *et al.* ranged from 2.16±1.25 up to 157.44±54.01 mg/kg with an average content of 63.13±57.54 mg/kg. The highest reported amygdalin concentration was 229.72 mg/kg from Fritz varietal from the Stanislaus region. Considering the variation in amygdalin content of almonds of different varietals from different growing regions, Blue Diamond is proposing an upper value of 400 mg/kg for amygdalin in the PDAPF ingredient obtained from blanched and natural almonds to account for these variations. The analytical data on PDAPF from blanched and natural almond indicate the production batches to be below this upper value for amygdalin.

Question 3. You describe the different types of toxicity associated with chronic versus acute exposure to hydrogen cyanide, based on the tolerable daily intakes and acute reference doses established by JECFA and EFSA, respectively. However, on page 27, you use a single set of exposure estimates (i.e. chronic exposure) to assess the potential for both chronic and acute toxicity. Since it's inappropriate to compare chronic exposure estimates to acute reference doses, please provide the estimated cyanide exposures associated with acute

[1] Lee J, Zhang G, Wood E, Rogel Castillo C, Mitchell AE (2013). Quantification of amygdalin in nonbitter, semibitter, and bitter almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ MS/MS. J Agric Food Chem 61(32):7754-7759. DOI:10.1021/jf402295u. Epub 2013 Jul 31. PMID: 23862656.

exposure scenarios (e.g. ingestion of a large amount on a single eating occasion) and discuss the risk of acute toxicity within the context of those exposure levels.

Response 3. The acute cyanide exposure from the intended uses of PDAPF was estimated in two dietary exposure scenarios:

1. Exposure to hydrogen cyanide from the highest theoretical intake of PDAPF on a single eating occasion; and
2. Exposure to hydrogen cyanide from the total daily intake of PDAPF from all proposed food uses using only Day 1 consumption data from the 2015-2016 NHANES.

According to the Office of Food Additive Safety (OFAS) in FDA's Center for Food Safety and Applied Nutrition (CFSAN), both of the above scenarios are considered adequate in evaluating acute intake to contaminants that may be present in foods[2].

Amygdalin is the major cyanogenic glycoside present in almonds (JECFA, 1993[3]; Chaouali *et al.,* 2013[4]; EFSA, 2016[5]). Approximately 59 mg hydrogen cyanide is released following the complete hydrolysis of 1 g amygdalin. In both acute exposure scenarios, exposure to hydrogen cyanide from the consumption PDAPF was calculated based on the mean level of amygdalin in PDAPF produced from blanched and natural almonds (70.1 ± 19.5 and 143.3 ± 35.8 mg amygdalin/kg PDAFP, respectively) and the upper value of 400 mg/kg (see above response). The mean level of amygdalin in PDAPF correspond to a potential release of 2.99 to 10.57 mg of hydrogen cyanide per kg of PDAPF, while the upper value corresponds to a maximum potential release of 23.6 mg of hydrogen cyanide per kg of PDAPF.

Acute Exposure Scenario 1

The proposed use level of PDAPF is highest in 'Protein powders' for beverages (use level of 80% on a powder basis) and in 'Energy bars or protein bars' for foods (use level of 25%). When expressed on a serving basis, the use level remains highest for these food uses (56 g/serving for protein powder; 17 g/serving for protein energy bars or protein bars), as shown in Table 3. As protein powders and energy bars or protein bars could be reasonably consumed on the same eating occasion, it was assumed that the consumption of a single portion of both these foods containing PDAPF at the proposed use level would be representative of the highest potential intake of PDAPF on a single eating occasion, and consequently hydrogen cyanide from PDAPF on a single eating occasion.

---

[2] U.S. FDA (2006). *Guidance for Industry: Estimating Dietary Intake of Substances in Food*. (August 2006). Silver College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food.

[3] 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.

[4] 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.

[5] 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.

According to the U.S. EPA Exposure Factors Handbook, the lowest recommended value for body weight for ages 16 years and above in the U.S. is 71.6 kg[6]. This body weight value was used to calculate exposure to hydrogen cyanide from the highest potential intake of PDAPF in a single eating occasion on a body weight basis (µg/kg body weight) as it represents age groups with the lowest body weight (*i.e.,* lower than the recommended value for body weight for adults of 80 kg) likely to consume protein powder and energy bars or protein bars.

[6] U.S. EPA (2011). Body-weight studies (Chapter 8). In: *Exposure Factors Handbook 2011 Edition (Final)*. (EPA/600/R-090/052F). Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA). Available at: https://www.epa.gov/expobox/exposure-factors-handbook-chapter-8.

Table 3 **Summary of the Individual Proposed Food-Uses and Use-Levels for PDAPF in the U.S.**

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Proposed Food-Uses[a]	PDAPF Use-Level (%)	RACC (g)[b]	PDAPF Use-Level (g/serving)
Baked Goods and Baking Mixes	Biscuits	5	55	2.8
	Cakes	10	55 to 125	5.5 to 12.5
	Cookies	5	30	1.5
	Cornbread, Corn Muffins, or Tortillas	5	55	2.8
	Crackers	5	15 to 30	0.8 to 1.5
	Doughnuts	5	55	2.8
	French toast, pancakes, waffles	10	85 to 100	8.5 to 10
	Muffins	5	110	5.5
Beverages and Beverage Bases	Non-Milk-Based nutritional powders (Plant Based; incl. meal replacements)[c]	35	57[d]	20
	Protein powders	**80**	**70[e]**	**56**
Coffee and Tea	Ready-to-Drink Coffee Drinks	5	360	18
Grain Products and Pastas	Cereal and Granola Bars	5	40	2
	Energy Bars or Protein Bars	**25**	**68[f]**	**17**
	Meal Replacement Bars	10	50[g]	5.0
Milk Products	Milk-based smoothies	5	240	12
	Milk-based nutritional powders (incl. meal replacements)[c]	35	57[d]	20
Processed Fruits and Fruit Juices	Fruit Smoothies (RTD)	5	240	12

CFR = Code of Federal Regulations; incl. = including; PDAPF = partially defatted almond protein flour; RACC = Reference Amounts Customarily Consumed per Eating Occasion; 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] RACC based on values established in "U.S. FDA (2019). Part 101—Food labeling. §101.12—Reference amounts customarily consumed per eating occasion. 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).

[c] Includes ready-to-drink and powder forms.

[d] Highest serving size identified for a 'Nutritional powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Ensure Nutrition Powder: https://ensure.com/nutrition-products/ensure-powder).

[e] Highest serving size identified for a 'Protein powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Muscle Milk: https://shop.musclemilk.com/Protein-Powders/c/MuscleMilk@Powder).

[f] Highest serving size identified for a 'Energy bars or protein bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Clif Bar: https://www.clifbar.ca/products/clif/clif-bar/chocolate-chip).

[g] Highest serving size identified for a 'Meal Replacement Bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (South Beach Entree Bar: https://www.walmart.com/ip/South-Beach-Diet-Peanut-Butter-Bar-Entree-Bars-1-8-Oz-15-Count/907996791).

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Levels in PDAPF

Based on the mean levels of amygdalin in PDAPF, the potential amount of hydrogen released in in the range of 2.99 to 10.57 mg hydrogen cyanide/kg PDAPF. The resulting exposure to hydrogen cyanide from PDAPF is 0.167 to 0.592 mg/serving of protein powder and 0.051 to 0.180 mg hydrogen cyanide/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion is 0.772 mg/serving or **10.78 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Assuming a maximum potential release of 23.6 mg hydrogen cyanide/kg PDAPF, the resulting exposure to hydrogen cyanide was calculated to be 1.322 mg/serving of protein powder and 0.401 mg/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion in this case is 1.723 mg/serving or **24.06 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Exposure Scenario 2

Estimates for the total daily intake of PDAPF were re-calculated based on the intended conditions of use of PDAPF in combination with food consumption data for each individual who completed Day 1 only of the 24-hour dietary recall in the 2015-2016 NHANES cycle. The distribution of one-day intakes of PDAPF was established from which the mean and 90th percentile intake estimates for the cohort of interest were determined. Survey weights were incorporated to provide representative intakes for the entire U.S. population. A summary of the estimated mean and 90th percentile one-day intakes of PDAPF from all proposed food-uses is provided in Table 4. Intake estimates are provided on a body weight basis only (mg/kg body weight/day).

Exposure to hydrogen cyanide (μg/kg body weight/day) from one-day intakes of PDAPF at the 90th percentile was calculated for children, female adults (older population group with the highest consumer-only intakes of PDAPF) and the total U.S. population.

Table 4 Summary of the Estimated Daily Per Kilogram Body Weight Intake of PDAPF from Proposed Food-Uses in the U.S. by Population Group (2015-2016 NHANES Day 1 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
Young Children	2 to <3	244	517	73.2	155	333	**700**
Children	3 to 11	246	566	68.6	1,002	359	**700**
Female Teenagers	12 to 19	94	263	58.3	321	161	338
Male Teenagers	12 to 19	168	231	55.8	328	301	357
Female Adults	20 and up	210	302	58.9	1,523	356	**510**
Male Adults	20 and up	179	210	55.9	1,272	321	475
Total Population	2 and up	195	317	58.9	4,601	331	**531**

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

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Level in PDAPF

Based on the amount of hydrogen cyanide released from the mean upper limit of amygdalin in PDAPF (10.57 mg hydrogen cyanide/kg PDAPF), acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **7.40, 5.39 and 5.61 μg hydrogen cyanide/kg body weight/day**, respectively.

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Based on the amount of hydrogen cyanide released from the maximum theoretical amygdalin level in PDAPF (23.6 mg hydrogen cyanide/kg PDAPF), the highest potential acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **16.52, 12.04, and 12.53 μg hydrogen cyanide/kg body weight/day**, respectively.

Summary of the Acute Exposures to Hydrogen Cyanide from PDAPF

A summary of the acute exposures to hydrogen cyanide from both scenarios is provided in Table 5. The acute exposures to hydrogen cyanide were consistently below the acute reference dose of 20 μg/kg body weight/day established by EFSA (2016) and well below the acute reference dose of 90 μg/kg body weight/day established by JECFA (2011)[7] when estimated using the mean levels of amygdalin reported across 5 non-consecutive lots of PDAPF from blanched almonds and 4 non-consecutive lots of PDAPF from natural almonds. When the acute exposure was calculated using the upper value of 400 mg amygdalin/kg PDAPF, the acute exposure under Scenario 1 was 24.06 μg/kg body weight/day and up to 16.52 μg/kg body weight/day under Scenario 2, which is above the acute reference dose established by EFSA in Scenario 1 only, and below JECFA's acute reference dose in both Scenarios 1 and 2.

Blue Diamond notes that the upper value for amygdalin was established based on the amygdalin content of sweet almonds sourced from 10 different varieties from 4 different regions in California as reported by Lee *et al.* (2013), and is considered conservative to account for any variation due to differences in almond varieties and growing regions. As discussed in Response #1, the mean amygdalin levels reported by Lee *et al.* ranged from 2.16±1.25 up to 157.44±54.01 mg/kg with an average content of 63.13±57.54 mg/kg; the highest reported amygdalin concentration was 229.72 mg/kg. Similarly, Luo *et al.* (2017)[8] measured the amygdalin concentration of different sweet almond varieties (Aldrich, Avalon, Butte, Carmel, Fritz, Independence, Mission, Monterey, Nonpareil, Padre, Price, Sonora, Winters, and Wood Colony) obtained from the Colusa, Fresno, Kern, and Stanislaus growing regions of California from the 2014/2015 harvest year using the same analytical method as Lee *et al.* and reported an amygdalin concentration range of 1.62 to 76.50 mg/kg. Yildirim *et al.* (2014)[9] determined the mean amygdalin content of 9 cultivars of sweet almonds to be 734 mg/kg. Cressey *et al.* (2013)[10] also reported a mean hydrocyanic acid content of almond products (6 samples; included

[7] 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.

[8] Luo KK, Kim DA, Mitchell-Silbaugh KC, Huang G, Mitchell AE (2017). Comparison of amygdalin and benzaldehyde levels in California almond (*Prunus dulcis*) varietals. In: Wirthensohn MG, editor. *Proceedings of the VII International Symposium on Almonds and Pistachios,* Nov. 5-9, 2017, Adelaide, Australia. (ISHS Acta Horticulturae, 1219). Leuven, Belgium: International Society for Horticultural Science (ISHS), pp. 1-8. DOI:0.17660/ActaHortic.2018.1219.1.

[9] Yildirim A, Akinci-Yildirim F, Polat M, Şan B, Selsi Y (2014). Amygdalin content in kernels of several almond cultivars grown in Turkey. HortScience 49(10):1268-1270. DOI:10.21273/HORTSCI.49.10.1268.

[10] Cressey P, Saunders D, Goodman J (2013). Cyanogenic glycosides in plant-based foods available in New Zealand. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 30(11):1946-1953. DOI:10.1080/19440049.2013.825819.

whole, flaked, ground, or butter) of 8.4 mg/kg (4.8 to 12.4 mg/kg), which would correspond to an average amygdalin content of approximately 210 mg/kg assuming that 1 g of amygdalin yields 59 mg of hydrogen cyanide.

The available data on amygdalin in the scientific literature indicate varietal and regional differences in amygdalin content of almonds. Blue Diamond notes that the almond starting material used in the production of PDAPF is sourced from California, and the company currently sources almond varieties that are consistent with the publications by Lee *et al.* (2013) and Luo *et al.* (2017). Therefore, the upper value of 400 mg/kg for amygdalin is considered sufficiently conservative (~2 times the average levels from batch data) to account for agricultural variation, and the analytical data support that the levels of amygdalin are well below this limit; thus, the acute exposure calculations using the upper value are not considered to be a "true" representation of acute exposure to hydrogen cyanide as a result of the intended uses of PDAPF.

Table 5 Summary of Acute Exposures to Hydrogen Cyanide from PDAPF Under Different Exposure Scenarios

Exposure Scenario	Exposure Value (µg/kg body weight/day)	Acute Reference Dose	
		EFSA (2016)	JECFA (2011)
Scenario 1			
Mean Levels[a]	10.78	20	90
Upper Value[b]	24.06	20	90
Scenario 2			
Mean Levels[a]	5.39 to 7.40	20	90
Upper Value[b]	12.04 to 16.52	20	90

[a] Mean level of amygdalin was 70.1 ± 19.5 and 143.3 ± 35.8 mg/kg in PDAPF from blanched and natural almonds, respectively.
[b] Upper value for amygdalin = 400 mg/kg.

Question 4. On page 26, you mention that the antinutrients, phytic acid and oxalic acid are present naturally in almonds. Please provide a narrative to discuss the levels of phytate/phytic acid and oxalate/oxalic acid in the context of safety of the PDAPF obtained from blanched and natural almonds for its intended uses.

Response 4. The Almond Board of California determined the phytic acid content of various varietals of natural almonds from the 2007/2008 growing year (Table 6). Although there were differences in phytic acid content across the different varietals, the levels of phytic acid were generally consistent with other commonly consumed tree nuts (*e.g.,* walnuts, cashews, macadamias, Brazil nuts, pistachios, pecans, hazelnuts, and pine nuts) and grains, cereals, and legumes (Duong *et al.,* 2017[11]). A summary of the phytic acid content of these foods is provided in Table 7 below.

Table 6 Phytic Acid Levels in Natural Almonds from the 2007/2008 Growing Year [CONFIDENTIAL]

Varietal	Phytic Acid (g/100 g)
Monterey	1.16
Sonora	1.08
Price	1.12

[11] 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.

Table 6 **Phytic Acid Levels in Natural Almonds from the 2007/2008 Growing Year [CONFIDENTIAL]**

Varietal	Phytic Acid (g/100 g)
Butte	1.06
Avalon	1.11
Carmel	1.16
Mission	1.11
Fritz	0.936
Nonpareil	1.14

Duong *et al.* (2017) measured the levels of myo-inositol phosphate (mono-, bi-, tri-, tetrakis- penta-, and hexakis-phosphate) in almond meal and almond brown skins from 6 different varieties (Mission, Aldrich, Nonpareil, Price, Butte, and Monterey) using HPLC/ESI/MS. Similar to the amygdalin concentration, levels of phytic acid varied across different varieties. The mean concentration of total phytic acid across the 6 different varieties was reported to be 11.92 µmol/g for almond meal and 10.99 µmol/g for almond brown skins, equivalent to 0.79 and 0.73 g/100 g, respectively. Accordingly, the reported phytic acid content in almond meal and almond brown skins was within the range of 5.3 to 32 µmol/g (0.35 to 2.11 g/100 g), which is consistent with other commonly consumed cereals, legumes, and tree nuts (Table 6). The results of Duong *et al.* (2017) suggest that phytic acid is primarily present in the skins of almonds; it is noted that Blue Diamond produces PDAPF from both blanched and natural almonds. Therefore, it is expected that the blanching step would significantly reduce the phytic acid content of PDAPF from blanched almonds. Nevertheless, the levels of phytic acid in almonds as determined by the Almond Board of California and reported in the scientific literature are generally consistent with other commonly consumed cereals, legumes, and tree nuts, and would therefore not pose any adverse effects on nutrients greater than these other components of the human diet.

Table 7 **Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.*, 2017)**

Food Product	Phytic Acid Content (µmol/g)
Cereals	
Barley	5.7 to 18.9
Maize	3.3 to 19.5
Millet	3.6 to 16.5
Oats	6.3 to 21.5
Rice	4.7 to 16.4
Rye	6.6 to 14.7
Sorghum	5.5 to 19.8
Triticale	3.5 to 15.2
Wheat	4.9 to 20.5
Legumes	
Chickpea	4.2 to 19.1
Common beans[a]	6.7 to 25.8
Cowpeas	4.4 to 13.9
Lentils	3.7 to 15.9
Mung beans	3.6 to 5.9
Peas	6.5 to 20.2
Peanuts	2.6 to 10.3

Table 7 **Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.*, 2017)**

Food Product	Phytic Acid Content (µmol/g)
Soybeans	13.3 to 28.8
Tree nuts	
Almond	5.3 to 32.0
English walnut	2.7 to 21.0
Cashew	2.3 to 29.8
Brazil nut	2.9 to 27.3
Macadamia	2.3 to 14.3
Pistachio	3.0 to 43.0
Pecan	1.8 to 28.9
Hazelnut	2.2 to 35.5
Pine nut	3.0 to 11.9

[a] Includes black, kidney, pinto, great northern, navy, and white beans.

The oxalic acid content of almonds was reported to be in the range of 131 to 503 mg/100 g (Brinkley *et al.*, 1990[12]; Hönow *et al.*, 2002[13]; Chai and Liebman, 2004[14]; Popova and Mihaylova, 2019[15]). The variation in oxalic acid levels was attributed by Chai and Liebman (2004)[14] to the extraction method and analysis. The Almond Board of California analyzed the oxalic acid content of various varietals of natural almonds from the 2007/2008 growing year, which is consistent with the reported range from the scientific literature (Table 8). Although the oxalic acid content of almonds is generally higher than legumes (~8 mg/kg), grains (35 to 270 mg/100 g), and tuber vegetables (0.4 to 2.3 mg/100 g), it is significantly less than leafy vegetables, such as spinach (751 to 832 mg/100 g dry weight basis) (Noonan and Savage, 1999[16]; Mou, 2008[17]; Popova and Mihaylova, 2019). The levels of oxalic acid in natural almonds obtained from the 2007/2008 growing year obtained by the Almond Board of California is reported to be in the range of 246 to 462 mg/100 g (Table 8). Noonan and Savage (1999) reported that tea is a significant source of oxalate in the English diet; the mean daily intake of oxalate was reported to be in the range of 70 to 150 mg. Furthermore, the same authors reported the minimum dose capable of causing death in an adult is 4 to 5 g. Based on the levels of oxalic acid in almonds and the intended uses of PDAPF, the highest possible exposure to oxalic acid would be approximately 103 mg/day in the total population.

[12] Brinkley LJ, Gregory J, Pak CY (1990). A further study of oxalate bioavailability in foods. J Urol 144(1):94-96. DOI:10.1016/s0022-5347(17)39377-1. Cited In: Chai and Liebman, 2004 [Ref. #4].

[13] Hönow R, Simon A, Hesse A (2002). Interference-free sample preparation for the determination of plasma oxalate analyzed by HPLC-ER: preliminary results from calcium oxalate stone-formers and non-stone-formers. Clin Chim Acta 318(12):19-24. DOI:10.1016/s0009-8981(01)00729-x.

[14] 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.

[15] Popova A, Mihaylova D (2019). Antinutrients in plant-based foods: a review. 13:68-76. DOI:10.2174/1874070701913010068.

[16] Noonan SC, Savage GP (1999). Oxalate content of foods and its effect on humans. Asia Pac J Clin Nutr 8(1):64-74.

[17] Mou B (2008). Evaluation of oxalate concentration in the U.S. spinach germplasm collection. HortScience 43(6):1690-1693. DOI:10.21273/HORTSCI.43.6.1690.

Table 8 **Oxalic Acid Levels in Natural Almonds from the 2007/2008 Growing Year [CONFIDENTIAL]**

Varietal	Oxalic Acid (g/100 g)
Monterey	0.246
Sonora	0.325
Price	0.270
Butte	0.461
Avalon	0.438
Carmel	0.311
Mission	0.322
Fritz	0.462
Nonpareil	0.246

Therefore, based on the available data, the low levels of antinutrients (oxalic acid, phytic acid) present in almonds, and as a result of exposure to Blue Diamond's PDAPF, are not expected to negatively affect the availability of other nutrients in foods to which the ingredient is added and is of no safety concern.

Question 5. On page 33 you provide information about true fecal protein digestibility of PDAPF from both blanched and natural almonds in rats (i.e., 93.78% and 90.87%, respectively). Please provide a reference source of these data and clarify whether these are unpublished data obtained from analysis of your ingredient. If the data are not published, please, describe how you used the information to support your safety conclusion (e.g. how they are corroborative of generally available information from peer reviewed studies such as the cited publications by Ahrens et al. and House et al. based on a discussion of the similarity/consistency of the methods used and results.)

Response 5. The applicant notes the discrepancy on page 30 of the notice. The true fecal protein digestibility of raw almonds from four different varieties (Monterey, Butte, Independence, and Nonpareil) were reported to be 80.6%, 78.3%, 78.9%, and 78.6%, respectively. In fact, these values were the *in vitro* protein digestibility and not the true fecal protein digestibility for these varieties, which were reported by House *et al.* (2019)[18] to be 89.9%, 86.2%, 88.9%, and 85.7%, respectively.

The true fecal protein digestibility of PDAPF from both blanched and natural almonds were determined internally using the same methodology as House *et al.* The digestibility values of PDAPF are consistent with the published *in vivo* digestibility values reported by Ahrens *et al.* (2005)[19] for three varieties of raw almonds (~83 to 92%) and the *in vivo* digestibility values reported by House *et al.* (2019) for four varieties of raw almonds (~86 to 90%). The resulting protein digestibility corrected amino acid score (PDCAAS) for PDAPF were 46.9% and 54.5% from blanched and natural almonds, respectively. These values are consistent with the published PDCAAS values of 44.3 to 47.8 as reported by House *et al.* (2019) further providing evidence on the protein

[18] 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.

[19] 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.

quality of almonds. The analytical data on PDAPF are corroborative to the generally available information from peer reviewed studies by Ahrens *et al.* (2005) and House *et al.* (2019).

The applicant notes that the safety of PDAPF was not based on the PDCAAS values as these values are indicators of the nutritional value of the ingredient. The safety of PDAPF was assessed using an adaptation 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[20]). In particular, the safety of PDAPF was supported by the long history of safe consumption of the ingredient (*i.e.,* almond protein that is derived from minimal processing of almonds); full characterization of the ingredient with respect to potential dietary exposure to natural toxins and anti-nutritional factors; nutritional aspects of the ingredient (*i.e.,* protein quality); and the absence of biological adverse effects from clinical studies.

Question 6. Please provide more details of the strategy used for the literature search described on page 24 by providing more information about the search terms used and publication time period covered.

Response 6. The literature search was conducted using ProQuest Dialog™ and the following databases: 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 search terms used to increase the relevancy and specificity of the literature include "almond" and "protein", including search modifiers to include these two terms within 5 words of each other. These substance terms were then searched with additional search terms to identify publications with relevant safety-related endpoints, specifically acute toxicity, repeated-dose toxicity, carcinogenicity, developmental/reproductive toxicity, genotoxicity, metabolism and digestibility terms. There were no limitations on publication date (*i.e.,* the literature search covered all publications published up to February 2020).

Question 7. Since you mention (page 26) that a major safety concern from toxins and antinutrients in almonds is the presence of cyanogenic glycosides due to hydrogen cyanide released when they are metabolized, please provide a specification for cyanogenic compound content in your ingredient.

Response 7. See response #2.

Question 8. You describe the different types of toxicity associated with chronic vs acute exposure to hydrogen cyanide, based on which the tolerable daily intakes and acute reference doses were established, respectively (by JECFA and EFSA). However, on page 27, you use a single set of exposure estimates (i.e. chronic exposure) to assess the potential for both chronic and acute toxicity. Since it's inappropriate to compare chronic exposure estimates to acute reference doses, please provide the estimated cyanide exposures associated with acute exposure scenarios (e.g. ingestion of a large amount on a single eating occasion), and discuss the risk of acute toxicity within the context of those exposure levels.

Response 8. See response #3.

Question 9. On page 26, you mention toxins and antinutrients present in almonds other than amygdalin. Since you concluded that your intended use of partially defatted almond protein flour will result in a considerable increase in dietary exposure to almond proteins please provide information about levels of the

[20] 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.

antinutrients (phytate/phytic acid and oxalate/oxalic) in your partially defatted almond protein flour from both blanched and natural almonds and discuss how those levels are consistent with your safety conclusion.

Response 9. See response #4.

Question 10. On page 33 you provide information about true fecal protein digestibility of partially defatted almond protein flour from both blanched and natural almonds in rats (93.78% and 90.87, respectively). Please provide a reference to the source of these data and clarify whether these are unpublished data obtained from analysis of your ingredient. If the data are not published, please, describe how you used the information to support your safety conclusion (e.g. how they are corroborative of generally available information from peer reviewed studies such as the cited publications by Ahrens et al. and House et al. based on a discussion of the similarity/consistency of the methods used and results.)

Response 10. See response #5.

Question 11. Please provide more details of the strategy used for the literature search described on page 24 by providing more information about the search terms used and publication time period covered.

Response 11. See response #6.

Sincerely,

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers
1802 C Street
Sacramento, CA 95811
kwaananen@bdgrowers.com
916-446-8309

6 October 2020

Karen Hall
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740-3835 USA

Dear Karen Hall,

Re: GRAS Notice No. GRN 918 for Partially Defatted Almond Protein Flour

Please see find below responses to the United States (U.S.) Food and Drug Administration (FDA)'s queries on GRAS Notice (GRN) No. 918 pertaining to partially defatted almond protein flour (PDAPF).

Question 1. We acknowledge that you provided estimated daily intake of almond protein tables (Tables 3.1-2 and 3.1-3) which include a population group of infants and young children aged 0 to 2 years. Infants are under 12 months of age. Please clarify whether PDAPF proposed food uses include infant formula.

Response 1. The PDAPF ingredient is not intended for use in infant formula. The population group of infants (age 0 to 2 years) was removed, and the total U.S. population was updated to include only individuals 2 years of age and above. The updated dietary intakes are provided in Tables 1 and 2 below on an absolute basis (g/person/day) and body weight basis (mg/kg body weight/day), respectively.

Table 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
Young Children	2 to <3	2.9	7.0	84.4	156	3.5	7.8
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	2 and up	11.8	16.7	77.0	5,001	15.4	22.4

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

Table 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
Young Children	2 to <3	220	467	84.2	152	261	546
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	2 and up	170	295	76.9	4,951	221	388

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

Question 2. You state on page 26 that a major safety concern from the use of almonds is the presence of the cyanogenic glycoside, amygdalin, due to the release of hydrogen cyanide upon metabolization. You provided results from the analyses of amygdalin levels in PDAPF from five non-consecutive lots and four non-consecutive lots derived from blanched almonds and natural almonds, respectively. Please provide a specification for maximum amygdalin in PDAPF, list the method of analysis and provide data to demonstrate that PDAPF levels consistently meet the specification. Alternatively, you could provide your acceptance criteria for amygdalin for the source material.

Response 2. The amygdalin content of the PDAPF from blanched and natural almonds was measured using ultrahigh-pressure liquid chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer (QqQ MS/MS) with electrospray ionization (ESI) as described by Lee *et al.* (2013)[1]. The mean levels of amygdalin across 5 non-consecutive lots of PDAPF from blanched almonds was 70.1±19.5 mg/kg and 143.3±35.8 mg/kg across 4 non-consecutive lots of PDAPF from natural almonds. Lee *et al.* (2013) measured the amygdalin content from 10 different varieties of sweet almonds sourced from 4 different growing regions in California. The mean amygdalin levels reported by Lee *et al.* ranged from 2.16±1.25 up to 157.44±54.01 mg/kg with an average content of 63.13±57.54 mg/kg. The highest reported amygdalin concentration was 229.72 mg/kg from Fritz varietal from the Stanislaus region. Considering the variation in amygdalin content of almonds of different varietals from different growing regions, Blue Diamond is proposing an upper value of 400 mg/kg for amygdalin in the PDAPF ingredient obtained from blanched and natural almonds to account for these variations. The analytical data on PDAPF from blanched and natural almond indicate the production batches to be below this upper value for amygdalin.

Question 3. You describe the different types of toxicity associated with chronic versus acute exposure to hydrogen cyanide, based on the tolerable daily intakes and acute reference doses established by JECFA and EFSA, respectively. However, on page 27, you use a single set of exposure estimates (i.e. chronic exposure) to assess the potential for both chronic and acute toxicity. Since it's inappropriate to compare chronic exposure estimates to acute reference doses, please provide the estimated cyanide exposures associated with acute

[1] Lee J, Zhang G, Wood E, Rogel Castillo C, Mitchell AE (2013). Quantification of amygdalin in nonbitter, semibitter, and bitter almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ MS/MS. J Agric Food Chem 61(32):7754-7759. DOI:10.1021/jf402295u. Epub 2013 Jul 31. PMID: 23862656.

exposure scenarios (e.g. ingestion of a large amount on a single eating occasion) and discuss the risk of acute toxicity within the context of those exposure levels.

Response 3. The acute cyanide exposure from the intended uses of PDAPF was estimated in two dietary exposure scenarios:

1. Exposure to hydrogen cyanide from the highest theoretical intake of PDAPF on a single eating occasion; and
2. Exposure to hydrogen cyanide from the total daily intake of PDAPF from all proposed food uses using only Day 1 consumption data from the 2015-2016 NHANES.

According to the Office of Food Additive Safety (OFAS) in FDA's Center for Food Safety and Applied Nutrition (CFSAN), both of the above scenarios are considered adequate in evaluating acute intake to contaminants that may be present in foods[2].

Amygdalin is the major cyanogenic glycoside present in almonds (JECFA, 1993[3]; Chaouali *et al.,* 2013[4]; EFSA, 2016[5]). Approximately 59 mg hydrogen cyanide is released following the complete hydrolysis of 1 g amygdalin. In both acute exposure scenarios, exposure to hydrogen cyanide from the consumption PDAPF was calculated based on the mean level of amygdalin in PDAPF produced from blanched and natural almonds (70.1 ± 19.5 and 143.3 ± 35.8 mg amygdalin/kg PDAFP, respectively) and the upper value of 400 mg/kg (see above response). The mean level of amygdalin in PDAPF correspond to a potential release of 2.99 to 10.57 mg of hydrogen cyanide per kg of PDAPF, while the upper value corresponds to a maximum potential release of 23.6 mg of hydrogen cyanide per kg of PDAPF.

Acute Exposure Scenario 1

The proposed use level of PDAPF is highest in 'Protein powders' for beverages (use level of 80% on a powder basis) and in 'Energy bars or protein bars' for foods (use level of 25%). When expressed on a serving basis, the use level remains highest for these food uses (56 g/serving for protein powder; 17 g/serving for protein energy bars or protein bars), as shown in Table 3. As protein powders and energy bars or protein bars could be reasonably consumed on the same eating occasion, it was assumed that the consumption of a single portion of both these foods containing PDAPF at the proposed use level would be representative of the highest potential intake of PDAPF on a single eating occasion, and consequently hydrogen cyanide from PDAPF on a single eating occasion.

---

[2] U.S. FDA (2006). *Guidance for Industry: Estimating Dietary Intake of Substances in Food*. (August 2006). Silver College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food.

[3] 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.

[4] 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.

[5] 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.

According to the U.S. EPA Exposure Factors Handbook, the lowest recommended value for body weight for ages 16 years and above in the U.S. is 71.6 kg[6]. This body weight value was used to calculate exposure to hydrogen cyanide from the highest potential intake of PDAPF in a single eating occasion on a body weight basis (μg/kg body weight) as it represents age groups with the lowest body weight (*i.e.,* lower than the recommended value for body weight for adults of 80 kg) likely to consume protein powder and energy bars or protein bars.

[6] U.S. EPA (2011). Body-weight studies (Chapter 8). In: *Exposure Factors Handbook 2011 Edition (Final)*. (EPA/600/R-090/052F). Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA). Available at: https://www.epa.gov/expobox/exposure-factors-handbook-chapter-8.

Table 3 Summary of the Individual Proposed Food-Uses and Use-Levels for PDAPF in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Proposed Food-Uses[a]	PDAPF Use-Level (%)	RACC (g)[b]	PDAPF Use-Level (g/serving)
Baked Goods and Baking Mixes	Biscuits	5	55	2.8
	Cakes	10	55 to 125	5.5 to 12.5
	Cookies	5	30	1.5
	Cornbread, Corn Muffins, or Tortillas	5	55	2.8
	Crackers	5	15 to 30	0.8 to 1.5
	Doughnuts	5	55	2.8
	French toast, pancakes, waffles	10	85 to 100	8.5 to 10
	Muffins	5	110	5.5
Beverages and Beverage Bases	Non-Milk-Based nutritional powders (Plant Based; incl. meal replacements)[c]	35	57[d]	20
	Protein powders	**80**	**70[e]**	**56**
Coffee and Tea	Ready-to-Drink Coffee Drinks	5	360	18
Grain Products and Pastas	Cereal and Granola Bars	5	40	2
	Energy Bars or Protein Bars	**25**	**68[f]**	**17**
	Meal Replacement Bars	10	50[g]	5.0
Milk Products	Milk-based smoothies	5	240	12
	Milk-based nutritional powders (incl. meal replacements)[c]	35	57[d]	20
Processed Fruits and Fruit Juices	Fruit Smoothies (RTD)	5	240	12

CFR = Code of Federal Regulations; incl. = including; PDAPF = partially defatted almond protein flour; RACC = Reference Amounts Customarily Consumed per Eating Occasion; 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] RACC based on values established in "U.S. FDA (2019). Part 101—Food labeling. §101.12—Reference amounts customarily consumed per eating occasion. 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).
[c] Includes ready-to-drink and powder forms.
[d] Highest serving size identified for a 'Nutritional powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Ensure Nutrition Powder: https://ensure.com/nutrition-products/ensure-powder).
[e] Highest serving size identified for a 'Protein powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Muscle Milk: https://shop.musclemilk.com/Protein-Powders/c/MuscleMilk@Powder).
[f] Highest serving size identified for a 'Energy bars or protein bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Clif Bar: https://www.clifbar.ca/products/clif/clif-bar/chocolate-chip).
[g] Highest serving size identified for a 'Meal Replacement Bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (South Beach Entree Bar: https://www.walmart.com/ip/South-Beach-Diet-Peanut-Butter-Bar-Entree-Bars-1-8-Oz-15-Count/907996791).

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Levels in PDAPF

Based on the mean levels of amygdalin in PDAPF, the potential amount of hydrogen released in in the range of 2.99 to 10.57 mg hydrogen cyanide/kg PDAPF. The resulting exposure to hydrogen cyanide from PDAPF is 0.167 to 0.592 mg/serving of protein powder and 0.051 to 0.180 mg hydrogen cyanide/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion is 0.772 mg/serving or **10.78 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Assuming a maximum potential release of 23.6 mg hydrogen cyanide/kg PDAPF, the resulting exposure to hydrogen cyanide was calculated to be 1.322 mg/serving of protein powder and 0.401 mg/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion in this case is 1.723 mg/serving or **24.06 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Exposure Scenario 2

Estimates for the total daily intake of PDAPF were re-calculated based on the intended conditions of use of PDAPF in combination with food consumption data for each individual who completed Day 1 only of the 24-hour dietary recall in the 2015-2016 NHANES cycle. The distribution of one-day intakes of PDAPF was established from which the mean and 90th percentile intake estimates for the cohort of interest were determined. Survey weights were incorporated to provide representative intakes for the entire U.S. population. A summary of the estimated mean and 90th percentile one-day intakes of PDAPF from all proposed food-uses is provided in Table 4. Intake estimates are provided on a body weight basis only (mg/kg body weight/day).

Exposure to hydrogen cyanide (μg/kg body weight/day) from one-day intakes of PDAPF at the 90th percentile was calculated for children, female adults (older population group with the highest consumer-only intakes of PDAPF) and the total U.S. population.

Table 4 Summary of the Estimated Daily Per Kilogram Body Weight Intake of PDAPF from Proposed Food-Uses in the U.S. by Population Group (2015-2016 NHANES Day 1 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
Young Children	2 to <3	244	517	73.2	155	333	**700**
Children	3 to 11	246	566	68.6	1,002	359	**700**
Female Teenagers	12 to 19	94	263	58.3	321	161	338
Male Teenagers	12 to 19	168	231	55.8	328	301	357
Female Adults	20 and up	210	302	58.9	1,523	356	**510**
Male Adults	20 and up	179	210	55.9	1,272	321	475
Total Population	2 and up	195	317	58.9	4,601	331	**531**

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

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Level in PDAPF

Based on the amount of hydrogen cyanide released from the mean upper limit of amygdalin in PDAPF (10.57 mg hydrogen cyanide/kg PDAPF), acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **7.40, 5.39 and 5.61 µg hydrogen cyanide/kg body weight/day**, respectively.

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Based on the amount of hydrogen cyanide released from the maximum theoretical amygdalin level in PDAPF (23.6 mg hydrogen cyanide/kg PDAPF), the highest potential acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **16.52, 12.04, and 12.53 µg hydrogen cyanide/kg body weight/day**, respectively.

<u>Summary of the Acute Exposures to Hydrogen Cyanide from PDAPF</u>

A summary of the acute exposures to hydrogen cyanide from both scenarios is provided in Table 5. The acute exposures to hydrogen cyanide were consistently below the acute reference dose of 20 µg/kg body weight/day established by EFSA (2016) and well below the acute reference dose of 90 µg/kg body weight/day established by JECFA (2011)[7] when estimated using the mean levels of amygdalin reported across 5 non-consecutive lots of PDAPF from blanched almonds and 4 non-consecutive lots of PDAPF from natural almonds. When the acute exposure was calculated using the upper value of 400 mg amygdalin/kg PDAPF, the acute exposure under Scenario 1 was 24.06 µg/kg body weight/day and up to 16.52 µg/kg body weight/day under Scenario 2, which is above the acute reference dose established by EFSA in Scenario 1 only, and below JECFA's acute reference dose in both Scenarios 1 and 2.

Blue Diamond notes that the upper value for amygdalin was established based on the amygdalin content of sweet almonds sourced from 10 different varieties from 4 different regions in California as reported by Lee *et al.* (2013), and is considered conservative to account for any variation due to differences in almond varieties and growing regions. As discussed in Response #1, the mean amygdalin levels reported by Lee *et al.* ranged from 2.16±1.25 up to 157.44±54.01 mg/kg with an average content of 63.13±57.54 mg/kg; the highest reported amygdalin concentration was 229.72 mg/kg. Similarly, Luo *et al.* (2017)[8] measured the amygdalin concentration of different sweet almond varieties (Aldrich, Avalon, Butte, Carmel, Fritz, Independence, Mission, Monterey, Nonpareil, Padre, Price, Sonora, Winters, and Wood Colony) obtained from the Colusa, Fresno, Kern, and Stanislaus growing regions of California from the 2014/2015 harvest year using the same analytical method as Lee *et al.* and reported an amygdalin concentration range of 1.62 to 76.50 mg/kg. Yildirim *et al.* (2014)[9] determined the mean amygdalin content of 9 cultivars of sweet almonds to be 734 mg/kg. Cressey *et al.* (2013)[10] also reported a mean hydrocyanic acid content of almond products (6 samples; included

[7] 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.

[8] Luo KK, Kim DA, Mitchell-Silbaugh KC, Huang G, Mitchell AE (2017). Comparison of amygdalin and benzaldehyde levels in California almond (*Prunus dulcis*) varietals. In: Wirthensohn MG, editor. *Proceedings of the VII International Symposium on Almonds and Pistachios,* Nov. 5-9, 2017, Adelaide, Australia. (ISHS Acta Horticulturae, 1219). Leuven, Belgium: International Society for Horticultural Science (ISHS), pp. 1-8. DOI:0.17660/ActaHortic.2018.1219.1.

[9] Yildirim A, Akinci-Yildirim F, Polat M, Şan B, Selsi Y (2014). Amygdalin content in kernels of several almond cultivars grown in Turkey. HortScience 49(10):1268-1270. DOI:10.21273/HORTSCI.49.10.1268.

[10] Cressey P, Saunders D, Goodman J (2013). Cyanogenic glycosides in plant-based foods available in New Zealand. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 30(11):1946-1953. DOI:10.1080/19440049.2013.825819.

whole, flaked, ground, or butter) of 8.4 mg/kg (4.8 to 12.4 mg/kg), which would correspond to an average amygdalin content of approximately 210 mg/kg assuming that 1 g of amygdalin yields 59 mg of hydrogen cyanide.

The available data on amygdalin in the scientific literature indicate varietal and regional differences in amygdalin content of almonds. Blue Diamond notes that the almond starting material used in the production of PDAPF is sourced from California, and the company currently sources almond varieties that are consistent with the publications by Lee *et al.* (2013) and Luo *et al.* (2017). Therefore, the upper value of 400 mg/kg for amygdalin is considered sufficiently conservative (~2 times the average levels from batch data) to account for agricultural variation, and the analytical data support that the levels of amygdalin are well below this limit; thus, the acute exposure calculations using the upper value are not considered to be a "true" representation of acute exposure to hydrogen cyanide as a result of the intended uses of PDAPF.

Table 5 Summary of Acute Exposures to Hydrogen Cyanide from PDAPF Under Different Exposure Scenarios

Exposure Scenario	Exposure Value (μg/kg body weight/day)	Acute Reference Dose	
		EFSA (2016)	JECFA (2011)
Scenario 1			
Mean Levels[a]	10.78	20	90
Upper Value[b]	24.06	20	90
Scenario 2			
Mean Levels[a]	5.39 to 7.40	20	90
Upper Value[b]	12.04 to 16.52	20	90

[a] Mean level of amygdalin was 70.1 ± 19.5 and 143.3 ± 35.8 mg/kg in PDAPF from blanched and natural almonds, respectively.
[b] Upper value for amygdalin = 400 mg/kg.

Question 4. On page 26, you mention that the antinutrients, phytic acid and oxalic acid are present naturally in almonds. Please provide a narrative to discuss the levels of phytate/phytic acid and oxalate/oxalic acid in the context of safety of the PDAPF obtained from blanched and natural almonds for its intended uses.

Response 4. The Almond Board of California determined the phytic acid content of various varietals of natural almonds from the 2007/2008 growing year (Table 6). Although there were differences in phytic acid content across the different varietals, the levels of phytic acid were generally consistent with other commonly consumed tree nuts (*e.g.*, walnuts, cashews, macadamias, Brazil nuts, pistachios, pecans, hazelnuts, and pine nuts) and grains, cereals, and legumes (Duong *et al.*, 2017[11]). A summary of the phytic acid content of these foods is provided in Table 7 below.

Table 6 Phytic Acid Levels in Natural Almonds from the 2007/2008 Growing Year [CONFIDENTIAL]

Varietal	Phytic Acid (g/100 g)
Monterey	1.16
Sonora	1.08
Price	1.12

[11] 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.

Table 6 Phytic Acid Levels in Natural Almonds from the 2007/2008 Growing Year [CONFIDENTIAL]

Varietal	Phytic Acid (g/100 g)
Butte	1.06
Avalon	1.11
Carmel	1.16
Mission	1.11
Fritz	0.936
Nonpareil	1.14

Duong *et al.* (2017) measured the levels of myo-inositol phosphate (mono-, bi-, tri-, tetrakis- penta-, and hexakis-phosphate) in almond meal and almond brown skins from 6 different varieties (Mission, Aldrich, Nonpareil, Price, Butte, and Monterey) using HPLC/ESI/MS. Similar to the amygdalin concentration, levels of phytic acid varied across different varieties. The mean concentration of total phytic acid across the 6 different varieties was reported to be 11.92 µmol/g for almond meal and 10.99 µmol/g for almond brown skins, equivalent to 0.79 and 0.73 g/100 g, respectively. Accordingly, the reported phytic acid content in almond meal and almond brown skins was within the range of 5.3 to 32 µmol/g (0.35 to 2.11 g/100 g), which is consistent with other commonly consumed cereals, legumes, and tree nuts (Table 6). The results of Duong *et al.* (2017) suggest that phytic acid is primarily present in the skins of almonds; it is noted that Blue Diamond produces PDAPF from both blanched and natural almonds. Therefore, it is expected that the blanching step would significantly reduce the phytic acid content of PDAPF from blanched almonds. Nevertheless, the levels of phytic acid in almonds as determined by the Almond Board of California and reported in the scientific literature are generally consistent with other commonly consumed cereals, legumes, and tree nuts, and would therefore not pose any adverse effects on nutrients greater than these other components of the human diet.

Table 7 Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.*, 2017)

Food Product	Phytic Acid Content (µmol/g)
Cereals	
Barley	5.7 to 18.9
Maize	3.3 to 19.5
Millet	3.6 to 16.5
Oats	6.3 to 21.5
Rice	4.7 to 16.4
Rye	6.6 to 14.7
Sorghum	5.5 to 19.8
Triticale	3.5 to 15.2
Wheat	4.9 to 20.5
Legumes	
Chickpea	4.2 to 19.1
Common beans[a]	6.7 to 25.8
Cowpeas	4.4 to 13.9
Lentils	3.7 to 15.9
Mung beans	3.6 to 5.9
Peas	6.5 to 20.2
Peanuts	2.6 to 10.3

Table 7 Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.*, 2017)

Food Product	Phytic Acid Content (µmol/g)
Soybeans	13.3 to 28.8
Tree nuts	
Almond	5.3 to 32.0
English walnut	2.7 to 21.0
Cashew	2.3 to 29.8
Brazil nut	2.9 to 27.3
Macadamia	2.3 to 14.3
Pistachio	3.0 to 43.0
Pecan	1.8 to 28.9
Hazelnut	2.2 to 35.5
Pine nut	3.0 to 11.9

[a] Includes black, kidney, pinto, great northern, navy, and white beans.

The oxalic acid content of almonds was reported to be in the range of 131 to 503 mg/100 g (Brinkley *et al.*, 1990[12]; Hönow *et al.*, 2002[13]; Chai and Liebman, 2004[14]; Popova and Mihaylova, 2019[15]). The variation in oxalic acid levels was attributed by Chai and Liebman (2004)[14] to the extraction method and analysis. The Almond Board of California analyzed the oxalic acid content of various varietals of natural almonds from the 2007/2008 growing year, which is consistent with the reported range from the scientific literature (Table 8). Although the oxalic acid content of almonds is generally higher than legumes (~8 mg/kg), grains (35 to 270 mg/100 g), and tuber vegetables (0.4 to 2.3 mg/100 g), it is significantly less than leafy vegetables, such as spinach (751 to 832 mg/100 g dry weight basis) (Noonan and Savage, 1999[16]; Mou, 2008[17]; Popova and Mihaylova, 2019). The levels of oxalic acid in natural almonds obtained from the 2007/2008 growing year obtained by the Almond Board of California is reported to be in the range of 246 to 462 mg/100 g (Table 8). Noonan and Savage (1999) reported that tea is a significant source of oxalate in the English diet; the mean daily intake of oxalate was reported to be in the range of 70 to 150 mg. Furthermore, the same authors reported the minimum dose capable of causing death in an adult is 4 to 5 g. Based on the levels of oxalic acid in almonds and the intended uses of PDAPF, the highest possible exposure to oxalic acid would be approximately 103 mg/day in the total population.

[12] Brinkley LJ, Gregory J, Pak CY (1990). A further study of oxalate bioavailability in foods. J Urol 144(1):94-96. DOI:10.1016/s0022-5347(17)39377-1. Cited In: Chai and Liebman, 2004 [Ref. #4].

[13] Hönow R, Simon A, Hesse A (2002). Interference-free sample preparation for the determination of plasma oxalate analyzed by HPLC-ER: preliminary results from calcium oxalate stone-formers and non-stone-formers. Clin Chim Acta 318(12):19-24. DOI:10.1016/s0009-8981(01)00729-x.

[14] 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.

[15] Popova A, Mihaylova D (2019). Antinutrients in plant-based foods: a review. 13:68-76. DOI:10.2174/1874070701913010068.

[16] Noonan SC, Savage GP (1999). Oxalate content of foods and its effect on humans. Asia Pac J Clin Nutr 8(1):64-74.

[17] Mou B (2008). Evaluation of oxalate concentration in the U.S. spinach germplasm collection. HortScience 43(6):1690-1693. DOI:10.21273/HORTSCI.43.6.1690.

Table 8 **Oxalic Acid Levels in Natural Almonds from the 2007/2008 Growing Year [CONFIDENTIAL]**

Varietal	Oxalic Acid (g/100 g)
Monterey	0.246
Sonora	0.325
Price	0.270
Butte	0.461
Avalon	0.438
Carmel	0.311
Mission	0.322
Fritz	0.462
Nonpareil	0.246

Therefore, based on the available data, the low levels of antinutrients (oxalic acid, phytic acid) present in almonds, and as a result of exposure to Blue Diamond's PDAPF, are not expected to negatively affect the availability of other nutrients in foods to which the ingredient is added and is of no safety concern.

Question 5. On page 33 you provide information about true fecal protein digestibility of PDAPF from both blanched and natural almonds in rats (i.e., 93.78% and 90.87%, respectively). Please provide a reference source of these data and clarify whether these are unpublished data obtained from analysis of your ingredient. If the data are not published, please, describe how you used the information to support your safety conclusion (e.g. how they are corroborative of generally available information from peer reviewed studies such as the cited publications by Ahrens et al. and House et al. based on a discussion of the similarity/consistency of the methods used and results.)

Response 5. The applicant notes the discrepancy on page 30 of the notice. The true fecal protein digestibility of raw almonds from four different varieties (Monterey, Butte, Independence, and Nonpareil) were reported to be 80.6%, 78.3%, 78.9%, and 78.6%, respectively. In fact, these values were the *in vitro* protein digestibility and not the true fecal protein digestibility for these varieties, which were reported by House *et al.* (2019)[18] to be 89.9%, 86.2%, 88.9%, and 85.7%, respectively.

The true fecal protein digestibility of PDAPF from both blanched and natural almonds were determined internally using the same methodology as House *et al.* The digestibility values of PDAPF are consistent with the published *in vivo* digestibility values reported by Ahrens *et al.* (2005)[19] for three varieties of raw almonds (~83 to 92%) and the *in vivo* digestibility values reported by House *et al.* (2019) for four varieties of raw almonds (~86 to 90%). The resulting protein digestibility corrected amino acid score (PDCAAS) for PDAPF were 46.9% and 54.5% from blanched and natural almonds, respectively. These values are consistent with the published PDCAAS values of 44.3 to 47.8 as reported by House *et al.* (2019) further providing evidence on the protein

[18] 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.

[19] 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.

quality of almonds. The analytical data on PDAPF are corroborative to the generally available information from peer reviewed studies by Ahrens *et al.* (2005) and House *et al.* (2019).

The applicant notes that the safety of PDAPF was not based on the PDCAAS values as these values are indicators of the nutritional value of the ingredient. The safety of PDAPF was assessed using an adaptation 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[20]). In particular, the safety of PDAPF was supported by the long history of safe consumption of the ingredient (*i.e.,* almond protein that is derived from minimal processing of almonds); full characterization of the ingredient with respect to potential dietary exposure to natural toxins and anti-nutritional factors; nutritional aspects of the ingredient (*i.e.,* protein quality); and the absence of biological adverse effects from clinical studies.

Question 6. Please provide more details of the strategy used for the literature search described on page 24 by providing more information about the search terms used and publication time period covered.

Response 6. The literature search was conducted using ProQuest Dialog™ and the following databases: 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 search terms used to increase the relevancy and specificity of the literature include "almond" and "protein", including search modifiers to include these two terms within 5 words of each other. These substance terms were then searched with additional search terms to identify publications with relevant safety-related endpoints, specifically acute toxicity, repeated-dose toxicity, carcinogenicity, developmental/reproductive toxicity, genotoxicity, metabolism and digestibility terms. There were no limitations on publication date (*i.e.,* the literature search covered all publications published up to February 2020).

Question 7. Since you mention (page 26) that a major safety concern from toxins and antinutrients in almonds is the presence of cyanogenic glycosides due to hydrogen cyanide released when they are metabolized, please provide a specification for cyanogenic compound content in your ingredient.

Response 7. See response #2.

Question 8. You describe the different types of toxicity associated with chronic vs acute exposure to hydrogen cyanide, based on which the tolerable daily intakes and acute reference doses were established, respectively (by JECFA and EFSA). However, on page 27, you use a single set of exposure estimates (i.e. chronic exposure) to assess the potential for both chronic and acute toxicity. Since it's inappropriate to compare chronic exposure estimates to acute reference doses, please provide the estimated cyanide exposures associated with acute exposure scenarios (e.g. ingestion of a large amount on a single eating occasion), and discuss the risk of acute toxicity within the context of those exposure levels.

Response 8. See response #3.

Question 9. On page 26, you mention toxins and antinutrients present in almonds other than amygdalin. Since you concluded that your intended use of partially defatted almond protein flour will result in a considerable increase in dietary exposure to almond proteins please provide information about levels of the

---

[20] 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.

antinutrients (phytate/phytic acid and oxalate/oxalic) in your partially defatted almond protein flour from both blanched and natural almonds and discuss how those levels are consistent with your safety conclusion.

Response 9. See response #4.

Question 10. On page 33 you provide information about true fecal protein digestibility of partially defatted almond protein flour from both blanched and natural almonds in rats (93.78% and 90.87, respectively). Please provide a reference to the source of these data and clarify whether these are unpublished data obtained from analysis of your ingredient. If the data are not published, please, describe how you used the information to support your safety conclusion (e.g. how they are corroborative of generally available information from peer reviewed studies such as the cited publications by Ahrens et al. and House et al. based on a discussion of the similarity/consistency of the methods used and results.)

Response 10. See response #5.

Question 11. Please provide more details of the strategy used for the literature search described on page 24 by providing more information about the search terms used and publication time period covered.

Response 11. See response #6.

Sincerely,

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers
1802 C Street
Sacramento, CA 95811
kwaananen@bdgrowers.com
916-446-8309

From:	Kurt Waananen
To:	Hall, Karen
Subject:	Follow-up to two email questions and the conference call concerns - GRN 000918
Date:	Thursday, December 17, 2020 4:11:42 PM
Attachments:	image003.png Response to FDA - GRN 918 - December 17 2020.pdf

Hi Karen,

Attached is our follow-up from the two email questions and the concerns raised on the conference call from December 3.

Thank you for your review and consideration, and please keep us posted on additional information needed or next steps.

Best regards,
Kurt

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers
1802 C Street I Sacramento, CA 95811
O: 916-446-8309 I C: 763-218-0495 I kwaananen@bdgrowers.com

From: Kurt Waananen
Sent: Friday, November 20, 2020 3:49 PM
To: Hall, Karen <Karen.Hall@fda.hhs.gov>
Subject: RE: Regarding GRN 000918

Thank you, Karen. I have held that time and set up a meeting notice with the Webex details for our other Blue Diamond and Intertek attendees.

Best regards,
Kurt

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Friday, November 20, 2020 9:21 AM
To: Kurt Waananen <kwaananen@bdgrowers.com>
Subject: [EXTERNAL] - RE: Regarding GRN 000918

Good Afternoon Kurt,

December 3, 2020 at 2:00 pm has been confirmed. Below is the WebEx information. Thank you.

Kind Regards,
Karen

-- Do not delete or change any of the following text. --

When it's time, join your Webex meeting here.

Meeting number (access code): 199 921 7050
Meeting password: jRMJFAJB323

Join meeting

Tap to join from a mobile device (attendees only)
+1-210-795-0506,,1999217050## US Toll
+1-877-465-7975,,1999217050## US Toll Free

Join by phone
+1-210-795-0506 US Toll
+1-877-465-7975 US Toll Free
Global call-in numbers | Toll-free calling restrictions

If you are a host, click here to view host information.

Need help? Go to http://help.webex.com

From: Kurt Waananen <kwaananen@bdgrowers.com>
Sent: Thursday, November 19, 2020 5:59 PM
To: Hall, Karen <Karen.Hall@fda.hhs.gov>
Subject: RE: Regarding GRN 000918

Hi Karen,

The Dec. 3rd time slot works for our team. Will you schedule it and then I can forward to our other team members, or would you prefer I send something through?

Thanks,
Kurt

From: Kurt Waananen
Sent: Thursday, November 19, 2020 12:26 PM
To: Hall, Karen <Karen.Hall@fda.hhs.gov>
Subject: RE: Regarding GRN 000918

Hi again, Karen.

Also confirming receipt of this request for a teleconference. I will follow-up with our team and get back on availability for one of these time slots.

Best,
Kurt

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers
1802 C Street I Sacramento, CA 95811
O: 916-446-8309 I C: 763-218-0495 I kwaananen@bdgrowers.com

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Thursday, November 19, 2020 12:03 PM
To: Kurt Waananen <kwaananen@bdgrowers.com>
Subject: [EXTERNAL] - Regarding GRN 000918

Dear Kurt,

We have reviewed your amendment for GRAS notice GRN 000918 for the intended use of PDAPF. We request a teleconference with you to discuss your response to Question 3. We wish to discuss the basis for a conclusion regarding the safety of PDAPF in light of your estimated acute cyanide exposure. Please let me know if you are available for a teleconference during any of the following time slots.

December 3, 2020 at 2:00 pm ET
December 4, 2020 at 1:00 pm ET
December 4, 2020 at 2:00 pm ET

Kind Regards,
Karen

Karen Hall
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Karen.Hall@fda.hhs.gov

*****Communications may be monitored for quality assurance and security purposes. This correspondence may contain confidential information. Unless you are the addressee (or you are authorized to receive for the addressee), you may not copy, use, or distribute this information. If you have received this in error, please advise the sender immediately at Blue Diamond Growers and permanently delete all copies, electronic or other, you may have.*****

December 17, 2020

Karen Hall
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740-3835 USA

Dear Karen,

Re: Additional Questions for GRAS Notice No. GRN 918 for Partially Defatted Almond Protein Flour

Please find responses below to the additional questions on GRAS Notice (GRN) No. 918 pertaining to partially defatted almond protein flour (PDAPF).

1) **On pages 8-10 of the amendment dated October 6, 2020 you discuss the phytic acid contents of almonds, almond meal and almond brown skins. Please explain how much phytic acid or phytate is in PDAPF and provide a rationale for your conclusion that dietary exposure to phytate from the intended uses of PDAPF is safe, for example by comparing it to dietary phytate exposure from other plant based foods.**

   The phytic acid content of PDAPF has not been analysed, but it is expected to be similar to the levels present in the whole almonds, which were used as the starting materials, given the fact that PDAPF is minimally processed using only mechanical processes. As presented in Table 1 below, the phytic acid content of almonds as reported in the scientific literature (Duong *et al.,* 2018[1]) ranges from 5.3 to 32.0 µmol/g. In comparison, other nuts, pistachios and hazelnuts, contain higher levels of phytic acid, ranging from 3.0 and 43.0 µmol/g and 2.2 and 35.5 µmol/g, respectively.

Table 1 Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.,* 2018)

Food Product	Phytic Acid Content (µmol/g)
Cereals	
Barley	5.7 to 18.9
Maize	3.3 to 19.5
Millet	3.6 to 16.5
Oats	6.3 to 21.5
Rice	4.7 to 16.4

[1] Duong QH, Lapsley KG, Pegg RB (2018). Inositol phosphates: health implications, methods of analysis, and occurrence in plant foods. J. Food Bioact;1:41–55.

Table 1 **Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.*, 2018)**

Food Product	Phytic Acid Content (µmol/g)
Rye	6.6 to 14.7
Sorghum	5.5 to 19.8
Triticale	3.5 to 15.2
Wheat	4.9 to 20.5
Legumes	
Chickpea	4.2 to 19.1
Common beans[a]	6.7 to 25.8
Cowpeas	4.4 to 13.9
Lentils	3.7 to 15.9
Mung beans	3.6 to 5.9
Peas	6.5 to 20.2
Peanuts	2.6 to 10.3
Soybeans	13.3 to 28.8
Tree nuts	
Almond	5.3 to 32.0
English walnut	2.7 to 21.0
Cashew	2.3 to 29.8
Brazil nut	2.9 to 27.3
Macadamia	2.3 to 14.3
Pistachio	3.0 to 43.0
Pecan	1.8 to 28.9
Hazelnut	2.2 to 35.5
Pine nut	3.0 to 11.9

[a] Includes black, kidney, pinto, great northern, navy, and white beans.

Based on the mean and 90th percentile intakes of these tree nuts obtained from the USDA FCID[2], pistachios, which contain higher levels of phytic acid, contribute the highest dietary exposures of phytic acid of 0.577 g/day (mean) or 1.362 g/day (90th percentile) (see Table 2). In comparison, phytic acid consumption from almonds is about 0.103 g/day (mean) or 0.281 g/day (90th percentile), which is much less than phytic acid exposures from pistachios. Assuming that all of the phytic acid present in the starting materials, whole almonds (*i.e.*, 32 µmol phytic acid/g almonds or 0.021 g phytic acid/g almonds taking into account a MW of 660.04 g/mol for phytic acid), is carried over into the final PDAPF product, the potential dietary exposure to phytic acid based on the intended uses of PDAPF, after correcting for the concentration effect of fat removal (approximately 40%) is approximately 0.783 g/day for the total population (90th percentile) (based on the 90th percentile intakes of 22.4 g PDAPF/day from Table 2 of the amendment). This is nearly 2-fold lower than the 90th percentile intakes of phytic acid from pistachios.

[2] https://fcid.foodrisk.org/percentiles

Table 2 Phytic Acid Exposure from Tree Nuts

Tree Nut	Tree Nut Consumption (g/day)[a]		Phytic Acid Consumption (g/day)[b]	
	Mean	90th Percentile	Mean	90th Percentile
Pistachio	20.33	48	0.577	1.362
Hazelnut	1.04	2.9	0.024	0.068
Almond	4.87	13.3	0.103	0.281
Walnut	2.43	5.6	0.034	0.078
Cashew	8.56	21.1	0.168	0.415
Brazil nut	1.84	4.2	0.033	0.076
Macadamia nut	15.57	22.4	0.147	0.211
Pecan	1.87	5.2	0.036	0.099
Pine nut	8.5	22.7	0.067	0.178

[a] Consumption rates were taken from USDA FCID: https://fcid.foodrisk.org/percentiles
[b] Highest phytic acid content taken from Duong *et al.* (2018) as presented in Table 1.
Duong QH, Lapsley KG, Pegg RB (2018). Inositol phosphates: health implications, methods of analysis, and occurrence in plant foods. J. Food Bioact;1:41–55.

2) On page 10 of the amendment dated October 6, 2020 you state that "Based on the levels of oxalic acid in almonds and the intended uses of PDAPF, the highest possible exposure to oxalic acid would be approximately 103 mg/ day in the total population." Table 8 and the preceding text discusses oxalate levels in almonds but not in PDAPF. Please explain how you arrived at the estimate of 103 mg/day.

The content of oxalic acid in PDAPF was not analysed. The exposure to oxalic acid from PDAPF is estimated based on the assumption that all oxalic acid present in whole almonds (used as the starting material) is carried over into the final PDAPF product, considering that the PDAPF product is minimally processed using mechanical processes. The intake estimate of oxalic acid of 172.5 mg/day was calculated based on the highest oxalic content reported in almonds after correcting for the concentration effect of removing lipids to produce PDAPF (0.462 g/100 g for Fritz variety, Table 8 of the amendment) and the 90th percentile intake of PDAPF of 22.4 g/day (from Table 2 of the amendment).

Estimation of Acute Exposure to Hydrogen Cyanide from Proposed Uses of PDAPF

During a follow-up call on December 3, 2020 with Blue Dimond Growers (BDG), FDA pointed out that the upper value of 400 mg/kg that was set by BDG for amygdalin was not representative of the mean level of amygdalin in PDAPF produced from blanched and natural almonds, which was reported as 70.1±19.5 and 143.3±35.8 mg amygdalin/kg PDAPF, respectively. As such, FDA requested that BDG considers revising the upper limit for amygdalin and re-calculate the acute cyanide exposure from the intended uses of PDAPF, taking into account the minimal risk level of 0.05 mg CN^-/kg/day for intermediate duration oral exposure that was set by the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2006[3]).

Accordingly, BDG has revised the upper value of amygdalin in the PDAPF to 330 mg/kg, based on the highest reported mean amygdalin value in almonds of up to 211.45 mg/kg (157.44±54.01 mg/kg) reported in Aldrich varieties of almonds (Lee *et al.,* 2013[4]). All amygdalin values reported by Lee *et al.* (2013) were obtained from almonds from the Fall 2010 harvest year. The almonds used as raw materials in the production of PDAPF are a mixture of various almond varieties from different growing regions in California. For example, the Fritz variety may be mixed with Butte or Mission varieties, amongst other combinations.

Considering different mixtures of almond varieties are used as source materials for PDAPF, in order to set up an upper limit for amygdalin levels, as a worst case scenario, it was assumed that the PDAPF ingredient was obtained from a 50/50 blend of Aldrich and Fritz, as these two varieties have the highest reported mean amygdalin values of 157.44±54.01 mg/kg and 144.87±36.44 mg/kg (see Table 3 below). Taking the mean amygdalin levels in Fritz and Aldrich varieties into account and a 50/50 mixture of the two varieties, the potential amygdalin content in PDAPF was calculated to be approximately 330 mg/kg after correcting for the lipid removal of almonds (approximately 40%), which could potentially concentrate the amygdalin content. The calculation is as follows: [(0.5 x 181.31) + (0.5 x 211.45)] / 0.6.

It should be noted that this estimate is considered conservative in that it assumed a 50/50 blend with Aldrich variety; in reality, Aldrich variety is an insignificant portion of the total almond production in California. Production data from the Almond Board of California[5] indicate that the Aldrich variety comprised approximately 4% or less of the total almond production in California from 2010 to 2020. In comparison, the Nonpareil varieties comprised approximately 40% of total almond production in the same time period, while other varieties, such as Monterey, Butte, Carmel, and Fritz, collectively comprise up to 40%. According to Lee *et al.* (2013), the mean amygdalin content of Nonpareil varieties was approximately 12.23±4.41 mg/kg, which is approximately 10-fold lower than Aldrich varieties.

The theoretical amygdalin levels in PDAPF were estimated assuming a 50/50 mixture of the Aldrich variety with other almond varieties; for example, blends of Aldrich/Butte, Aldrich/Mission, Aldrich/Price, *etc.* using the mean values of amygdalin reported by Lee *et al.* (2013). The results are summarized in Table 3 below. An example calculation for PDAPF derived from a 50/50 mixture of Fritz/Butte is as follows: [(0.5 x 181.31) + (0.5 x 3.41)] / 0.6 = 153.93 mg amygdalin/kg. As indicated above, the highest possible amygdalin content in PDAPF

---

[3] ATSDR (2006). *Toxicological Profile for Cyanide*. (PB/2007-100674). Atlanta (GA): Agency for Toxic Substances and Disease Registry (ATSDR). Available at: https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=72&tid=19.

[4] Lee J, Zhang G, Wood E, Rogel Castillo C, Mitchell AE (2013). Quantification of amygdalin in nonbitter, semibitter, and bitter almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ MS/MS. J Agric Food Chem 61(32):7754-7759. DOI:10.1021/jf402295u. Epub 2013 Jul 31. PMID: 23862656.

[5] https://www.almonds.com/tools-and-resources/crop-reports/almond-almanac

would arise from a mixture of Fritz and Aldrich varieties (*i.e.,* 327.30 mg/kg or approximately 330 mg/kg set as the upper limit for amygdaline). BDG also utilizes blends of Nonpareil, Carmel, Sonora, Monterey, Butte, Mission, and Wood Colony; however, the levels of amygdalin in PDAPF would be less than the upper limit when obtained from these mixtures (see Table 3 below).

Table 3 Theoretical Levels of Amygdalin in PDAPF Based on a Mixture of Aldrich Variety with Other Almond Varieties (50/50) as Reported in Lee *et al.* (2013)

Variety	Mean Amygdalin Content from Lee *et al.* (2013) (mg/kg)		Total Amygdalin in PDAPF from Mean Value (mg/kg)
Butte	2.16±1.25	3.41	153.93
Price	4.32±2.45	6.77	156.73
Sonora	7.76±6.04	13.8	162.59
Nonpareil	12.23±4.41	16.64	164.96
Monterey	62.47±27.19	89.66	225.81
Wood Colony	75.03±8.07	83.1	220.34
Carmel	76.97±15.22	92.19	227.92
Mission	89.6±32.34	121.94	252.71
Fritz	144.87±36.44	181.31	302.18
Aldrich	157.44±54.01	211.45	**327.30**

In a recent study, Luo *et al.* (2018[6]) reported the mean amygdalin levels in 14 different almond varieties from the 2014/2015 harvest year, but from the same growing regions in California as reported by Lee *et al.* (2013). Using the same approach described above, the total possible amygdalin content in the final PDAPF product were calculated using data reported by Luo *et al.* (2013). The results are presented in Table 4. The amygdalin content in the same almond varieties as reported by Luo *et al.* (2018) were considerably less than those reported by Lee *et al.* (2013). The difference was attributed to seasonal differences due to the growing year; the findings suggest that batches of almonds from recent growing years have much less amygdalin content than prior years. Using the values reported by Luo *et al.* (2018), in the same Fritz/Aldrich blend, PDAPF obtained from this blend, assuming a 50/50 mixture and correction factor of 0.6 (to account for the concentration effect of removing lipids), would potentially have a total amygdalin content of 143.81 mg/kg (Table 4).

Table 4 Theoretical Levels of Amygdalin in PDAPF Based on a Mixture of Aldrich Variety with Other Almond Varieties (50/50) as Reported in Luo *et al.* (2018)

Variety	Mean Amygdalin Content from Luo *et al.* (2018) (mg/kg)		Total Amygdalin in PDAPF (mg/kg)
Aldrich	76.50±23.99	100.49	**143.81**
Avalon	3.00±4.17	7.17	66.04
Butte	18.56±20.77	39.33	92.84
Carmel	26.43±14.30	40.73	94.01
Fritz	59.71±12.37	72.08	120.13
Independence	2.07±1.66	3.73	63.18

[6] Luo KK, Kim DA, Mitchell-Silbaugh KC, Huang G, Mitchell AE (2017). Comparison of amygdalin and benzaldehyde levels in California almond (*Prunus dulcis*) varietals. In: Wirthensohn MG, editor. *Proceedings of the VII International Symposium on Almonds and Pistachios,* Nov. 5-9, 2017, Adelaide, Australia. (ISHS Acta Horticulturae, 1219). Leuven, Belgium: International Society for Horticultural Science (ISHS), pp. 1-8. DOI:0.17660/ActaHortic.2018.1219.1.

Table 4 Theoretical Levels of Amygdalin in PDAPF Based on a Mixture of Aldrich Variety with Other Almond Varieties (50/50) as Reported in Luo *et al.* (2018)

Variety	Mean Amygdalin Content from Luo *et al.* (2018) (mg/kg)		Total Amygdalin in PDAPF (mg/kg)
Mission	40.24±18.40	58.64	108.93
Monterey	46.76±15.21	61.97	111.71
Nonpareil	9.11±4.42	13.53	71.34
Padre	53.24±16.74	69.98	118.38
Price	1.77±1.74	3.51	62.99
Sonora	5.56±2.20	7.76	66.53
Winters	1.62±2.10	3.72	63.17
Wood Colony	41.49±14.41	55.9	106.65

Therefore, the revised upper limit for amygdalin in PDAPF of 330 mg/kg is considered sufficiently conservative to account for any differences in amygdalin content due to almond varieties, growing regions, as well as harvest year. The analytical data on PDAPF from blanched (mean values of 70.1±19.5 mg/kg) and natural (mean value of 143±35.8 mg/kg) almonds indicate the production batches to be below this upper value for amygdalin.

The acute cyanide exposure from the intended uses of PDAPF was estimated under two exposure scenarios:

1. Exposure to hydrogen cyanide from the highest theoretical intake of PDAPF on a single eating occasion; and
2. Exposure to hydrogen cyanide from the total daily intake of PDAPF from all proposed food uses using only Day 1 consumption data from the 2015-2016 NHANES.

According to the Office of Food Additive Safety (OFAS) in FDA's Center for Food Safety and Applied Nutrition (CFSAN), both of the above scenarios are considered adequate in evaluating acute intake to contaminants that may be present in foods[7].

Amygdalin is the major cyanogenic glycoside present in almonds (JECFA, 1993[8]; Chaouali *et al.,* 2013[9]; EFSA, 2016[10]). Approximately 59 mg hydrogen cyanide is released following the complete hydrolysis of 1 g amygdalin. In both acute exposure scenarios, the estimated acute cyanide exposure from the intended uses of PDAPF using the revised upper limit of 330 mg amygdalin/kg of PDAPF is discussed below. The mean level of

---

[7] U.S. FDA (2006). *Guidance for Industry: Estimating Dietary Intake of Substances in Food.* (August 2006). Silver College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food.

[8] 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.

[9] 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.

[10] 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.

amygdalin in PDAPF correspond to a potential release of 2.99 to 10.57 mg of hydrogen cyanide per kg of PDAPF, while the upper value corresponds to a maximum potential release of 19.47 mg of hydrogen cyanide per kg of PDAPF.

Acute Exposure Scenario 1

The proposed use level of PDAPF is highest in 'Protein powders' for beverages (use level of 80% on a powder basis) and in 'Energy bars or protein bars' for foods (use level of 25%). When expressed on a serving basis, the use level remains highest for these food uses (56 g/serving for protein powder; 17 g/serving for protein energy bars or protein bars), as shown in Table 5 below. As protein powders and energy bars or protein bars could be reasonably consumed during the same eating occasion, it was assumed that the consumption of a single portion of both these foods containing PDAPF at the proposed use level would be representative of the highest potential intake of PDAPF on a single eating occasion, and consequently hydrogen cyanide from PDAPF on a single eating occasion.

According to the U.S. EPA Exposure Factors Handbook, the lowest recommended value for body weight for ages 16 years and above in the U.S. is 71.6 kg[11]. This body weight value was used to calculate exposure to hydrogen cyanide from the highest potential intake of PDAPF on a single eating occasion on a body weight basis (µg/kg body weight) as it represents age groups with the lowest body weight (*i.e.,* lower than the recommended value for body weight for adults of 80 kg) likely to consume protein powder and energy bars or protein bars.

[11] U.S. EPA (2011). Body-weight studies (Chapter 8). In: *Exposure Factors Handbook 2011 Edition (Final).* (EPA/600/R-090/052F). Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA). Available at: https://www.epa.gov/expobox/exposure-factors-handbook-chapter-8.

Table 5 Summary of the Individual Proposed Food-Uses and Use-Levels for PDAPF in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Proposed Food-Uses[a]	PDAPF Use-Level (%)	RACC (g)[b]	PDAPF Use-Level (g/serving)
Baked Goods and Baking Mixes	Biscuits	5	55	2.8
	Cakes	10	55 to 125	5.5 to 12.5
	Cookies	5	30	1.5
	Cornbread, Corn Muffins, or Tortillas	5	55	2.8
	Crackers	5	15 to 30	0.8 to 1.5
	Doughnuts	5	55	2.8
	French toast, pancakes, waffles	10	85 to 100	8.5 to 10
	Muffins	5	110	5.5
Beverages and Beverage Bases	Non-Milk-Based nutritional powders (Plant Based; incl. meal replacements)[c]	35	57[d]	20
	Protein powders	**80**	**70[e]**	**56**
Coffee and Tea	Ready-to-Drink Coffee Drinks	5	360	18
Grain Products and Pastas	Cereal and Granola Bars	5	40	2
	Energy Bars or Protein Bars	**25**	**68[f]**	**17**
	Meal Replacement Bars	10	50[g]	5.0
Milk Products	Milk-based smoothies	5	240	12
	Milk-based nutritional powders (incl. meal replacements)[c]	35	57[d]	20
Processed Fruits and Fruit Juices	Fruit Smoothies (RTD)	5	240	12

CFR = Code of Federal Regulations; incl. = including; PDAPF = partially defatted almond protein flour; RACC = Reference Amounts Customarily Consumed per Eating Occasion; 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] RACC based on values established in "U.S. FDA (2019). Part 101—Food labeling. §101.12—Reference amounts customarily consumed per eating occasion. 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).
[c] Includes ready-to-drink and powder forms.
[d] Highest serving size identified for a 'Nutritional powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Ensure Nutrition Powder: https://ensure.com/nutrition-products/ensure-powder).
[e] Highest serving size identified for a 'Protein powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Muscle Milk: https://shop.musclemilk.com/Protein-Powders/c/MuscleMilk@Powder).
[f] Highest serving size identified for a 'Energy bars or protein bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Clif Bar: https://www.clifbar.ca/products/clif/clif-bar/chocolate-chip).
[g] Highest serving size identified for a 'Meal Replacement Bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (South Beach Entree Bar: https://www.walmart.com/ip/South-Beach-Diet-Peanut-Butter-Bar-Entree-Bars-1-8-Oz-15-Count/907996791).

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Levels in PDAPF

Based on the mean levels of amygdalin in PDAPF, the potential amount of hydrogen released is in the range of 2.99 to 10.57 mg hydrogen cyanide/kg PDAPF. The resulting exposure to hydrogen cyanide from PDAPF is 0.167 to 0.592 mg/serving of protein powder and 0.051 to 0.180 mg hydrogen cyanide/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion is 0.772 mg/serving or **10.78 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Assuming a maximum potential release of 19.47 mg hydrogen cyanide/kg PDAPF, the resulting exposure to hydrogen cyanide was calculated to be 1.090 mg/serving of protein powder and 0.331 mg/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion in this case is 1.421 mg/serving or **19.85 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Exposure Scenario 2

Estimates for the total daily intake of PDAPF were re-calculated based on the intended conditions of use of PDAPF in combination with food consumption data for each individual who completed Day 1 only of the 24-hour dietary recall in the 2015-2016 NHANES cycle. The distribution of one-day intakes of PDAPF was established from which the mean and 90th percentile intake estimates for the cohort of interest were determined. Survey weights were incorporated to provide representative intakes for the entire U.S. population. A summary of the estimated mean and 90th percentile one-day intakes of PDAPF from all proposed food-uses is provided in Table 6. Intake estimates are provided on a body weight basis only (mg/kg body weight in a day).

Exposure to hydrogen cyanide (μg/kg body weight in a day) from one-day intakes of PDAPF at the 90th percentile was calculated for children, female adults (older population group with the highest consumer-only intakes of PDAPF) and the total U.S. population.

Table 6 Summary of the Estimated Daily Per Kilogram Body Weight Intake of PDAPF from Proposed Food-Uses in the U.S. by Population Group (2015-2016 NHANES Day 1 Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw in a day)		Consumer-Only Intake (mg/kg bw in a day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Young Children	2 to <3	244	517	73.2	155	333	**700**
Children	3 to 11	246	566	68.6	1,002	359	**700**
Female Teenagers	12 to 19	94	263	58.3	321	161	338
Male Teenagers	12 to 19	168	231	55.8	328	301	357
Female Adults	20 and up	210	302	58.9	1,523	356	**510**
Male Adults	20 and up	179	210	55.9	1,272	321	475
Total Population	2 and up	195	317	58.9	4,601	331	**531**

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

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Level in PDAPF

Based on the amount of hydrogen cyanide released from the mean upper limit of amygdalin in PDAPF (10.57 mg hydrogen cyanide/kg PDAPF), acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **7.40, 5.39 and 5.61 µg hydrogen cyanide/kg body weight in a day**, respectively.

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Based on the amount of hydrogen cyanide released from the maximum theoretical amygdalin level in PDAPF (19.47 mg hydrogen cyanide/kg PDAPF), the highest potential acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **13. 63, 9.93, and 10.34 µg hydrogen cyanide/kg body weight in a day**, respectively.

The ATSDR reported that humans ingesting 4.6 to 15 mg CN^-/kg as potassium cyanide had serious adverse effects in the nervous, respiratory, cardiovascular, gastrointestinal, renal, and musculoskeletal systems (ATSDR, 2006). Based on case reports, an oral LD_{50} of 1.52 mg/kg was estimated for humans. The ATSDR derived a minimal risk level (MRL) of 0.05 mg CN^-/kg/day for intermediate duration oral exposure (*i.e.,* 15 to 364 days) based on a NOAEL of 4.5 mg CN^-/kg/day and a LOAEL of 12.5 mg CN^-/kg/day from an NTP study in rats (NTP, 1993[12]; ATSDR, 2006[13]). It was noted that an MRL for acute duration oral exposures could not be calculated based on the serious effects observed at the lowest doses (ATSDR, 2006).

Summary of the Acute Exposures to Hydrogen Cyanide from PDAPF

A summary of the acute exposures to hydrogen cyanide from both scenarios is provided in Table 7 below.

Based on the estimated acute exposures to hydrogen cyanide from the proposed uses of PDAPF, the acute exposures were consistently below the acute reference dose of 20, 50, and 90 µg/kg body weight established by EFSA (2016), ATSDR (2006), and JECFA (2011)[14], respectively, when estimated using the mean levels of amygdalin reported across 5 non-consecutive lots of PDAPF from blanched almonds and 4 non-consecutive lots of PDAPF from natural almonds, as well as the proposed upper limit of 330 mg/kg for amygdalin, suggesting that the presence of amygdalin in PDAPF would not pose any safety concerns. It should be noted that this revised limit of 330 mg/kg is considered sufficiently conservative to account for differences in amygdalin content due to almond varieties and growing regions, as well as harvest year. The analytical data generated by BDG on PDAPF obtained from blanched and natural almonds indicate the production batches to be well below this proposed upper value.

---

[12] NTP (1993). *NTP Technical Report on Toxicity Studies of Sodium Cyanide (CAS No. 143-33-9) Administered in Drinking Water to F344/N Rats and B6C3F$_1$ Mice*. (Toxicity Report Series, No. 37, NIH Publication 94-3386). Research Triangle Park (NC): National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS). Available at: https://ntp.niehs.nih.gov/publications/reports/tox/000s/tox037/index.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tox037abs.

[13] ATSDR (2006). *Toxicological Profile for Cyanide*. (PB/2007-100674). Atlanta (GA): Agency for Toxic Substances and Disease Registry (ATSDR). Available at: https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=72&tid=19.

[14] 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.

Table 7 **Summary of Acute Exposures to Hydrogen Cyanide from PDAPF Under Different Exposure Scenarios**

Exposure Scenario	Exposure Value (μg/kg body weight)	Acute Reference Dose (μg/kg body weight)		Minimal Risk Level (μg/kg body weight)
		EFSA (2016)	JECFA (2011)	ATSDR (2006)
Scenario 1				
Mean Levels[a]	10.78	20	90	50
Upper Value[b]	19.85	20	90	
Scenario 2				
Mean Levels[a]	5.39 to 7.40	20	90	
Upper Value[b]	9.93 to 13.63	20	90	

[a] Mean level of amygdalin was 70.1 ± 19.5 and 143.3 ± 35.8 mg/kg in PDAPF from blanched and natural almonds, respectively.
[b] Upper value for amygdalin = 330 mg/kg.

Sincerely,

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers
1802 C Street
Sacramento, CA 95811
kwaananen@bdgrowers.com
916-446-8309

December 17, 2020

Karen Hall
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740-3835 USA

Dear Karen,

Re: Additional Questions for GRAS Notice No. GRN 918 for Partially Defatted Almond Protein Flour

Please find responses below to the additional questions on GRAS Notice (GRN) No. 918 pertaining to partially defatted almond protein flour (PDAPF).

1) **On pages 8-10 of the amendment dated October 6, 2020 you discuss the phytic acid contents of almonds, almond meal and almond brown skins. Please explain how much phytic acid or phytate is in PDAPF and provide a rationale for your conclusion that dietary exposure to phytate from the intended uses of PDAPF is safe, for example by comparing it to dietary phytate exposure from other plant based foods.**

   The phytic acid content of PDAPF has not been analysed, but it is expected to be similar to the levels present in the whole almonds, which were used as the starting materials, given the fact that PDAPF is minimally processed using only mechanical processes. As presented in Table 1 below, the phytic acid content of almonds as reported in the scientific literature (Duong *et al.,* 2018[1]) ranges from 5.3 to 32.0 µmol/g. In comparison, other nuts, pistachios and hazelnuts, contain higher levels of phytic acid, ranging from 3.0 and 43.0 µmol/g and 2.2 and 35.5 µmol/g, respectively.

Table 1 Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.,* 2018)

Food Product	Phytic Acid Content (µmol/g)
Cereals	
Barley	5.7 to 18.9
Maize	3.3 to 19.5
Millet	3.6 to 16.5
Oats	6.3 to 21.5
Rice	4.7 to 16.4

[1] Duong QH, Lapsley KG, Pegg RB (2018). Inositol phosphates: health implications, methods of analysis, and occurrence in plant foods. J. Food Bioact;1:41–55.

Table 1 Phytic Acid Levels in Various Plant-Based Foods (Taken from Duong *et al.*, 2018)

Food Product	Phytic Acid Content (μmol/g)
Rye	6.6 to 14.7
Sorghum	5.5 to 19.8
Triticale	3.5 to 15.2
Wheat	4.9 to 20.5
Legumes	
Chickpea	4.2 to 19.1
Common beans[a]	6.7 to 25.8
Cowpeas	4.4 to 13.9
Lentils	3.7 to 15.9
Mung beans	3.6 to 5.9
Peas	6.5 to 20.2
Peanuts	2.6 to 10.3
Soybeans	13.3 to 28.8
Tree nuts	
Almond	5.3 to 32.0
English walnut	2.7 to 21.0
Cashew	2.3 to 29.8
Brazil nut	2.9 to 27.3
Macadamia	2.3 to 14.3
Pistachio	3.0 to 43.0
Pecan	1.8 to 28.9
Hazelnut	2.2 to 35.5
Pine nut	3.0 to 11.9

[a] Includes black, kidney, pinto, great northern, navy, and white beans.

Based on the mean and 90th percentile intakes of these tree nuts obtained from the USDA FCID[2], pistachios, which contain higher levels of phytic acid, contribute the highest dietary exposures of phytic acid of 0.577 g/day (mean) or 1.362 g/day (90th percentile) (see Table 2). In comparison, phytic acid consumption from almonds is about 0.103 g/day (mean) or 0.281 g/day (90th percentile), which is much less than phytic acid exposures from pistachios. Assuming that all of the phytic acid present in the starting materials, whole almonds (*i.e.,* 32 μmol phytic acid/g almonds or 0.021 g phytic acid/g almonds taking into account a MW of 660.04 g/mol for phytic acid), is carried over into the final PDAPF product, the potential dietary exposure to phytic acid based on the intended uses of PDAPF, after correcting for the concentration effect of fat removal (approximately 40%) is approximately 0.783 g/day for the total population (90th percentile) (based on the 90th percentile intakes of 22.4 g PDAPF/day from Table 2 of the amendment). This is nearly 2-fold lower than the 90th percentile intakes of phytic acid from pistachios.

[2] https://fcid.foodrisk.org/percentiles

Table 2 **Phytic Acid Exposure from Tree Nuts**

Tree Nut	Tree Nut Consumption (g/day)[a]		Phytic Acid Consumption (g/day)[b]	
	Mean	90[th] Percentile	Mean	90[th] Percentile
Pistachio	20.33	48	0.577	1.362
Hazelnut	1.04	2.9	0.024	0.068
Almond	4.87	13.3	0.103	0.281
Walnut	2.43	5.6	0.034	0.078
Cashew	8.56	21.1	0.168	0.415
Brazil nut	1.84	4.2	0.033	0.076
Macadamia nut	15.57	22.4	0.147	0.211
Pecan	1.87	5.2	0.036	0.099
Pine nut	8.5	22.7	0.067	0.178

[a] Consumption rates were taken from USDA FCID: https://fcid.foodrisk.org/percentiles

[b] Highest phytic acid content taken from Duong *et al.* (2018) as presented in Table 1.

Duong QH, Lapsley KG, Pegg RB (2018). Inositol phosphates: health implications, methods of analysis, and occurrence in plant foods. J. Food Bioact;1:41–55.

2) On page 10 of the amendment dated October 6, 2020 you state that "Based on the levels of oxalic acid in almonds and the intended uses of PDAPF, the highest possible exposure to oxalic acid would be approximately 103 mg/ day in the total population." Table 8 and the preceding text discusses oxalate levels in almonds but not in PDAPF. Please explain how you arrived at the estimate of 103 mg/day.

The content of oxalic acid in PDAPF was not analysed. The exposure to oxalic acid from PDAPF is estimated based on the assumption that all oxalic acid present in whole almonds (used as the starting material) is carried over into the final PDAPF product, considering that the PDAPF product is minimally processed using mechanical processes. The intake estimate of oxalic acid of 172.5 mg/day was calculated based on the highest oxalic content reported in almonds after correcting for the concentration effect of removing lipids to produce PDAPF (0.462 g/100 g for Fritz variety, Table 8 of the amendment) and the 90th percentile intake of PDAPF of 22.4 g/day (from Table 2 of the amendment).

Estimation of Acute Exposure to Hydrogen Cyanide from Proposed Uses of PDAPF

During a follow-up call on December 3, 2020 with Blue Dimond Growers (BDG), FDA pointed out that the upper value of 400 mg/kg that was set by BDG for amygdalin was not representative of the mean level of amygdalin in PDAPF produced from blanched and natural almonds, which was reported as 70.1±19.5 and 143.3±35.8 mg amygdalin/kg PDAPF, respectively. As such, FDA requested that BDG considers revising the upper limit for amygdalin and re-calculate the acute cyanide exposure from the intended uses of PDAPF, taking into account the minimal risk level of 0.05 mg CN^-/kg/day for intermediate duration oral exposure that was set by the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2006[3]).

Accordingly, BDG has revised the upper value of amygdalin in the PDAPF to 330 mg/kg, based on the highest reported mean amygdalin value in almonds of up to 211.45 mg/kg (157.44±54.01 mg/kg) reported in Aldrich varieties of almonds (Lee *et al.,* 2013[4]). All amygdalin values reported by Lee *et al.* (2013) were obtained from almonds from the Fall 2010 harvest year. The almonds used as raw materials in the production of PDAPF are a mixture of various almond varieties from different growing regions in California. For example, the Fritz variety may be mixed with Butte or Mission varieties, amongst other combinations.

Considering different mixtures of almond varieties are used as source materials for PDAPF, in order to set up an upper limit for amygdalin levels, as a worst case scenario, it was assumed that the PDAPF ingredient was obtained from a 50/50 blend of Aldrich and Fritz, as these two varieties have the highest reported mean amygdalin values of 157.44±54.01 mg/kg and 144.87±36.44 mg/kg (see Table 3 below). Taking the mean amygdalin levels in Fritz and Aldrich varieties into account and a 50/50 mixture of the two varieties, the potential amygdalin content in PDAPF was calculated to be approximately 330 mg/kg after correcting for the lipid removal of almonds (approximately 40%), which could potentially concentrate the amygdalin content. The calculation is as follows: [(0.5 x 181.31) + (0.5 x 211.45)] / 0.6.

It should be noted that this estimate is considered conservative in that it assumed a 50/50 blend with Aldrich variety; in reality, Aldrich variety is an insignificant portion of the total almond production in California. Production data from the Almond Board of California[5] indicate that the Aldrich variety comprised approximately 4% or less of the total almond production in California from 2010 to 2020. In comparison, the Nonpareil varieties comprised approximately 40% of total almond production in the same time period, while other varieties, such as Monterey, Butte, Carmel, and Fritz, collectively comprise up to 40%. According to Lee *et al.* (2013), the mean amygdalin content of Nonpareil varieties was approximately 12.23±4.41 mg/kg, which is approximately 10-fold lower than Aldrich varieties.

The theoretical amygdalin levels in PDAPF were estimated assuming a 50/50 mixture of the Aldrich variety with other almond varieties; for example, blends of Aldrich/Butte, Aldrich/Mission, Aldrich/Price, *etc*. using the mean values of amygdalin reported by Lee *et al.* (2013). The results are summarized in Table 3 below. An example calculation for PDAPF derived from a 50/50 mixture of Fritz/Butte is as follows: [(0.5 x 181.31) + (0.5 x 3.41)] / 0.6 = 153.93 mg amygdalin/kg. As indicated above, the highest possible amygdalin content in PDAPF

---

[3] ATSDR (2006). *Toxicological Profile for Cyanide*. (PB/2007-100674). Atlanta (GA): Agency for Toxic Substances and Disease Registry (ATSDR). Available at: https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=72&tid=19.

[4] Lee J, Zhang G, Wood E, Rogel Castillo C, Mitchell AE (2013). Quantification of amygdalin in nonbitter, semibitter, and bitter almonds (*Prunus dulcis*) by UHPLC-(ESI)QqQ MS/MS. J Agric Food Chem 61(32):7754-7759. DOI:10.1021/jf402295u. Epub 2013 Jul 31. PMID: 23862656.

[5] https://www.almonds.com/tools-and-resources/crop-reports/almond-almanac

would arise from a mixture of Fritz and Aldrich varieties (*i.e.,* 327.30 mg/kg or approximately 330 mg/kg set as the upper limit for amygdaline). BDG also utilizes blends of Nonpareil, Carmel, Sonora, Monterey, Butte, Mission, and Wood Colony; however, the levels of amygdalin in PDAPF would be less than the upper limit when obtained from these mixtures (see Table 3 below).

Table 3 Theoretical Levels of Amygdalin in PDAPF Based on a Mixture of Aldrich Variety with Other Almond Varieties (50/50) as Reported in Lee *et al.* (2013)

Variety	Mean Amygdalin Content from Lee *et al.* (2013) (mg/kg)		Total Amygdalin in PDAPF from Mean Value (mg/kg)
Butte	2.16±1.25	3.41	153.93
Price	4.32±2.45	6.77	156.73
Sonora	7.76±6.04	13.8	162.59
Nonpareil	12.23±4.41	16.64	164.96
Monterey	62.47±27.19	89.66	225.81
Wood Colony	75.03±8.07	83.1	220.34
Carmel	76.97±15.22	92.19	227.92
Mission	89.6±32.34	121.94	252.71
Fritz	144.87±36.44	181.31	302.18
Aldrich	157.44±54.01	211.45	**327.30**

In a recent study, Luo *et al.* (2018[6]) reported the mean amygdalin levels in 14 different almond varieties from the 2014/2015 harvest year, but from the same growing regions in California as reported by Lee *et al.* (2013). Using the same approach described above, the total possible amygdalin content in the final PDAPF product were calculated using data reported by Luo *et al.* (2013). The results are presented in Table 4. The amygdalin content in the same almond varieties as reported by Luo *et al.* (2018) were considerably less than those reported by Lee *et al.* (2013). The difference was attributed to seasonal differences due to the growing year; the findings suggest that batches of almonds from recent growing years have much less amygdalin content than prior years. Using the values reported by Luo *et al.* (2018), in the same Fritz/Aldrich blend, PDAPF obtained from this blend, assuming a 50/50 mixture and correction factor of 0.6 (to account for the concentration effect of removing lipids), would potentially have a total amygdalin content of 143.81 mg/kg (Table 4).

Table 4 Theoretical Levels of Amygdalin in PDAPF Based on a Mixture of Aldrich Variety with Other Almond Varieties (50/50) as Reported in Luo *et al.* (2018)

Variety	Mean Amygdalin Content from Luo *et al.* (2018) (mg/kg)		Total Amygdalin in PDAPF (mg/kg)
Aldrich	76.50±23.99	100.49	**143.81**
Avalon	3.00±4.17	7.17	66.04
Butte	18.56±20.77	39.33	92.84
Carmel	26.43±14.30	40.73	94.01
Fritz	59.71±12.37	72.08	120.13
Independence	2.07±1.66	3.73	63.18

[6] Luo KK, Kim DA, Mitchell-Silbaugh KC, Huang G, Mitchell AE (2017). Comparison of amygdalin and benzaldehyde levels in California almond (*Prunus dulcis*) varietals. In: Wirthensohn MG, editor. *Proceedings of the VII International Symposium on Almonds and Pistachios,* Nov. 5-9, 2017, Adelaide, Australia. (ISHS Acta Horticulturae, 1219). Leuven, Belgium: International Society for Horticultural Science (ISHS), pp. 1-8. DOI:0.17660/ActaHortic.2018.1219.1.

Table 4 Theoretical Levels of Amygdalin in PDAPF Based on a Mixture of Aldrich Variety with Other Almond Varieties (50/50) as Reported in Luo *et al.* (2018)

Variety	Mean Amygdalin Content from Luo *et al.* (2018) (mg/kg)		Total Amygdalin in PDAPF (mg/kg)
Mission	40.24±18.40	58.64	108.93
Monterey	46.76±15.21	61.97	111.71
Nonpareil	9.11±4.42	13.53	71.34
Padre	53.24±16.74	69.98	118.38
Price	1.77±1.74	3.51	62.99
Sonora	5.56±2.20	7.76	66.53
Winters	1.62±2.10	3.72	63.17
Wood Colony	41.49±14.41	55.9	106.65

Therefore, the revised upper limit for amygdalin in PDAPF of 330 mg/kg is considered sufficiently conservative to account for any differences in amygdalin content due to almond varieties, growing regions, as well as harvest year. The analytical data on PDAPF from blanched (mean values of 70.1±19.5 mg/kg) and natural (mean value of 143±35.8 mg/kg) almonds indicate the production batches to be below this upper value for amygdalin.

The acute cyanide exposure from the intended uses of PDAPF was estimated under two exposure scenarios:

1. Exposure to hydrogen cyanide from the highest theoretical intake of PDAPF on a single eating occasion; and
2. Exposure to hydrogen cyanide from the total daily intake of PDAPF from all proposed food uses using only Day 1 consumption data from the 2015-2016 NHANES.

According to the Office of Food Additive Safety (OFAS) in FDA's Center for Food Safety and Applied Nutrition (CFSAN), both of the above scenarios are considered adequate in evaluating acute intake to contaminants that may be present in foods[7].

Amygdalin is the major cyanogenic glycoside present in almonds (JECFA, 1993[8]; Chaouali *et al.,* 2013[9]; EFSA, 2016[10]). Approximately 59 mg hydrogen cyanide is released following the complete hydrolysis of 1 g amygdalin. In both acute exposure scenarios, the estimated acute cyanide exposure from the intended uses of PDAPF using the revised upper limit of 330 mg amygdalin/kg of PDAPF is discussed below. The mean level of

[7] U.S. FDA (2006). *Guidance for Industry: Estimating Dietary Intake of Substances in Food.* (August 2006). Silver College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food.

[8] 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.

[9] 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.

[10] 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.

amygdalin in PDAPF correspond to a potential release of 2.99 to 10.57 mg of hydrogen cyanide per kg of PDAPF, while the upper value corresponds to a maximum potential release of 19.47 mg of hydrogen cyanide per kg of PDAPF.

Acute Exposure Scenario 1

The proposed use level of PDAPF is highest in 'Protein powders' for beverages (use level of 80% on a powder basis) and in 'Energy bars or protein bars' for foods (use level of 25%). When expressed on a serving basis, the use level remains highest for these food uses (56 g/serving for protein powder; 17 g/serving for protein energy bars or protein bars), as shown in Table 5 below. As protein powders and energy bars or protein bars could be reasonably consumed during the same eating occasion, it was assumed that the consumption of a single portion of both these foods containing PDAPF at the proposed use level would be representative of the highest potential intake of PDAPF on a single eating occasion, and consequently hydrogen cyanide from PDAPF on a single eating occasion.

According to the U.S. EPA Exposure Factors Handbook, the lowest recommended value for body weight for ages 16 years and above in the U.S. is 71.6 kg[11]. This body weight value was used to calculate exposure to hydrogen cyanide from the highest potential intake of PDAPF on a single eating occasion on a body weight basis (µg/kg body weight) as it represents age groups with the lowest body weight (*i.e.,* lower than the recommended value for body weight for adults of 80 kg) likely to consume protein powder and energy bars or protein bars.

[11] U.S. EPA (2011). Body-weight studies (Chapter 8). In: *Exposure Factors Handbook 2011 Edition (Final).* (EPA/600/R-090/052F). Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA). Available at: https://www.epa.gov/expobox/exposure-factors-handbook-chapter-8.

Table 5 Summary of the Individual Proposed Food-Uses and Use-Levels for PDAPF in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2019)	Proposed Food-Uses[a]	PDAPF Use-Level (%)	RACC (g)[b]	PDAPF Use-Level (g/serving)
Baked Goods and Baking Mixes	Biscuits	5	55	2.8
	Cakes	10	55 to 125	5.5 to 12.5
	Cookies	5	30	1.5
	Cornbread, Corn Muffins, or Tortillas	5	55	2.8
	Crackers	5	15 to 30	0.8 to 1.5
	Doughnuts	5	55	2.8
	French toast, pancakes, waffles	10	85 to 100	8.5 to 10
	Muffins	5	110	5.5
Beverages and Beverage Bases	Non-Milk-Based nutritional powders (Plant Based; incl. meal replacements)[c]	35	57[d]	20
	Protein powders	**80**	**70[e]**	**56**
Coffee and Tea	Ready-to-Drink Coffee Drinks	5	360	18
Grain Products and Pastas	Cereal and Granola Bars	5	40	2
	Energy Bars or Protein Bars	**25**	**68[f]**	**17**
	Meal Replacement Bars	10	50[g]	5.0
Milk Products	Milk-based smoothies	5	240	12
	Milk-based nutritional powders (incl. meal replacements)[c]	35	57[d]	20
Processed Fruits and Fruit Juices	Fruit Smoothies (RTD)	5	240	12

CFR = Code of Federal Regulations; incl. = including; PDAPF = partially defatted almond protein flour; RACC = Reference Amounts Customarily Consumed per Eating Occasion; 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] RACC based on values established in "U.S. FDA (2019). Part 101—Food labeling. §101.12—Reference amounts customarily consumed per eating occasion. 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).
[c] Includes ready-to-drink and powder forms.
[d] Highest serving size identified for a 'Nutritional powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Ensure Nutrition Powder: https://ensure.com/nutrition-products/ensure-powder).
[e] Highest serving size identified for a 'Protein powder' product on the U.S market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Muscle Milk: https://shop.musclemilk.com/Protein-Powders/c/MuscleMilk@Powder).
[f] Highest serving size identified for a 'Energy bars or protein bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (Clif Bar: https://www.clifbar.ca/products/clif/clif-bar/chocolate-chip).
[g] Highest serving size identified for a 'Meal Replacement Bars' product on the U.S. market fitting the description of brands for representative food codes in the 2015-2016 NHANES (South Beach Entree Bar: https://www.walmart.com/ip/South-Beach-Diet-Peanut-Butter-Bar-Entree-Bars-1-8-Oz-15-Count/907996791).

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Levels in PDAPF

Based on the mean levels of amygdalin in PDAPF, the potential amount of hydrogen released is in the range of 2.99 to 10.57 mg hydrogen cyanide/kg PDAPF. The resulting exposure to hydrogen cyanide from PDAPF is 0.167 to 0.592 mg/serving of protein powder and 0.051 to 0.180 mg hydrogen cyanide/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion is 0.772 mg/serving or **10.78 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Assuming a maximum potential release of 19.47 mg hydrogen cyanide/kg PDAPF, the resulting exposure to hydrogen cyanide was calculated to be 1.090 mg/serving of protein powder and 0.331 mg/serving of energy bars or protein bars.

The total worst-case exposure to hydrogen cyanide from the consumption of a single portion of both foods on a single eating occasion in this case is 1.421 mg/serving or **19.85 μg/kg body weight** (assuming a standard body weight of 71.6 kg).

Acute Exposure Scenario 2

Estimates for the total daily intake of PDAPF were re-calculated based on the intended conditions of use of PDAPF in combination with food consumption data for each individual who completed Day 1 only of the 24-hour dietary recall in the 2015-2016 NHANES cycle. The distribution of one-day intakes of PDAPF was established from which the mean and 90th percentile intake estimates for the cohort of interest were determined. Survey weights were incorporated to provide representative intakes for the entire U.S. population. A summary of the estimated mean and 90th percentile one-day intakes of PDAPF from all proposed food-uses is provided in Table 6. Intake estimates are provided on a body weight basis only (mg/kg body weight in a day).

Exposure to hydrogen cyanide (μg/kg body weight in a day) from one-day intakes of PDAPF at the 90th percentile was calculated for children, female adults (older population group with the highest consumer-only intakes of PDAPF) and the total U.S. population.

Table 6 Summary of the Estimated Daily Per Kilogram Body Weight Intake of PDAPF from Proposed Food-Uses in the U.S. by Population Group (2015-2016 NHANES Day 1 Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw in a day)		Consumer-Only Intake (mg/kg bw in a day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Young Children	2 to <3	244	517	73.2	155	333	**700**
Children	3 to 11	246	566	68.6	1,002	359	**700**
Female Teenagers	12 to 19	94	263	58.3	321	161	338
Male Teenagers	12 to 19	168	231	55.8	328	301	357
Female Adults	20 and up	210	302	58.9	1,523	356	**510**
Male Adults	20 and up	179	210	55.9	1,272	321	475
Total Population	2 and up	195	317	58.9	4,601	331	**531**

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

Acute Hydrogen Cyanide Exposure from Mean Amygdalin Level in PDAPF

Based on the amount of hydrogen cyanide released from the mean upper limit of amygdalin in PDAPF (10.57 mg hydrogen cyanide/kg PDAPF), acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **7.40, 5.39 and 5.61 µg hydrogen cyanide/kg body weight in a day**, respectively.

Acute Hydrogen Cyanide Exposure from Upper Value for Amygdalin in PDAPF

Based on the amount of hydrogen cyanide released from the maximum theoretical amygdalin level in PDAPF (19.47 mg hydrogen cyanide/kg PDAPF), the highest potential acute exposure of hydrogen cyanide from 1-day intakes of PDAPF from all proposed food uses at the 90th percentile in children, female adults, and the total U.S. population were determined to be **13. 63, 9.93, and 10.34 µg hydrogen cyanide/kg body weight in a day**, respectively.

The ATSDR reported that humans ingesting 4.6 to 15 mg CN^-/kg as potassium cyanide had serious adverse effects in the nervous, respiratory, cardiovascular, gastrointestinal, renal, and musculoskeletal systems (ATSDR, 2006). Based on case reports, an oral LD_{50} of 1.52 mg/kg was estimated for humans. The ATSDR derived a minimal risk level (MRL) of 0.05 mg CN^-/kg/day for intermediate duration oral exposure (*i.e.,* 15 to 364 days) based on a NOAEL of 4.5 mg CN^-/kg/day and a LOAEL of 12.5 mg CN^-/kg/day from an NTP study in rats (NTP, 1993[12]; ATSDR, 2006[13]). It was noted that an MRL for acute duration oral exposures could not be calculated based on the serious effects observed at the lowest doses (ATSDR, 2006).

Summary of the Acute Exposures to Hydrogen Cyanide from PDAPF

A summary of the acute exposures to hydrogen cyanide from both scenarios is provided in Table 7 below.

Based on the estimated acute exposures to hydrogen cyanide from the proposed uses of PDAPF, the acute exposures were consistently below the acute reference dose of 20, 50, and 90 µg/kg body weight established by EFSA (2016), ATSDR (2006), and JECFA (2011)[14], respectively, when estimated using the mean levels of amygdalin reported across 5 non-consecutive lots of PDAPF from blanched almonds and 4 non-consecutive lots of PDAPF from natural almonds, as well as the proposed upper limit of 330 mg/kg for amygdalin, suggesting that the presence of amygdalin in PDAPF would not pose any safety concerns. It should be noted that this revised limit of 330 mg/kg is considered sufficiently conservative to account for differences in amygdalin content due to almond varieties and growing regions, as well as harvest year. The analytical data generated by BDG on PDAPF obtained from blanched and natural almonds indicate the production batches to be well below this proposed upper value.

---

[12] NTP (1993). *NTP Technical Report on Toxicity Studies of Sodium Cyanide (CAS No. 143-33-9) Administered in Drinking Water to F344/N Rats and B6C3F₁ Mice*. (Toxicity Report Series, No. 37, NIH Publication 94-3386). Research Triangle Park (NC): National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS). Available at: https://ntp.niehs.nih.gov/publications/reports/tox/000s/tox037/index.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tox037abs.

[13] ATSDR (2006). *Toxicological Profile for Cyanide*. (PB/2007-100674). Atlanta (GA): Agency for Toxic Substances and Disease Registry (ATSDR). Available at: https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=72&tid=19.

[14] 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.

Table 7 Summary of Acute Exposures to Hydrogen Cyanide from PDAPF Under Different Exposure Scenarios

Exposure Scenario	Exposure Value (µg/kg body weight)	Acute Reference Dose (µg/kg body weight)		Minimal Risk Level (µg/kg body weight)
		EFSA (2016)	JECFA (2011)	ATSDR (2006)
Scenario 1				
Mean Levels[a]	10.78	20	90	50
Upper Value[b]	19.85	20	90	
Scenario 2				
Mean Levels[a]	5.39 to 7.40	20	90	
Upper Value[b]	9.93 to 13.63	20	90	

[a] Mean level of amygdalin was 70.1 ± 19.5 and 143.3 ± 35.8 mg/kg in PDAPF from blanched and natural almonds, respectively.
[b] Upper value for amygdalin = 330 mg/kg.

Sincerely,

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers
1802 C Street
Sacramento, CA 95811
kwaananen@bdgrowers.com
916-446-8309

From:	Kurt Waananen
To:	Hall, Karen
Subject:	[EXTERNAL] RE: Regarding GRN 000918
Date:	Tuesday, April 20, 2021 6:14:33 PM
Attachments:	image001.png Accreditation Certificate 3885-01 rev 08-08-19.pdf BDG_GoodAgPractices_2021.pdf

Hi Karen,

Thank you for the option of responding with an email. Answers are covered below in red and the cited attachments.

1. Specifications:
   a. The *Salmonella* specification is given as negative in 2 x 375 g. We generally look for 25 g maximum samples for *Salmonella* serovar testing. Please clarify if the specification you have indicated refers to two sets of pooled-batches of tested 25 g samples (2x(15 x 25 g)) or to 2 x 375 g samples. If the latter is applicable, we request that you provide a specification with a smaller sample size (not more than 25 g) and provide results of batch analyses (minimum 3) to show your ingredient complies with the revised specification.

      We confirm that the specification indicated refers to two sets of pooled batches. The Blue Diamond QA Lab tests 25g samples (2 x (15 x 25g)).

   b. The heavy metal specifications for lead, arsenic, cadmium, mercury are all set to <0.5 mg/kg. These levels appear higher than the results provided for the batch analyses. We request that you lower the specifications and provide a revised specifications table.

      We agree that the specifications can be lowered based on analytical testing results.

      A summary of screening results for whole almonds (highest ppm value) is as follows:

Season	Arsenic	Cadmium	Lead	Mercury
20-21	0.02	0.03	<0.01	<0.01
19-20	0.06	0.03	0.11	<0.01
18-19	0.05	0.03	<0.01	<0.01

The analytical data on partially defatted almond flour from the original submission is below.

Production Lot Number	Type	Heavy Metal Arsenic ppm	Heavy Metal Cadmium ppm	Heavy Metal Lead ppm	Heavy Metal Mercury ppm
18179NABDB	Blanched	0.02	0.017	<0.01	<0.005
18198NABDB	Blanched	0.02	0.016	<0.01	<0.005
18241NABDB	Blanched	0.02	0.017	<0.01	<0.005
18288NABDB	Blanched	0.03	0.017	<0.01	<0.005

18302NABDB	Blanched	0.02	0.016	<0.01	<0.005
18179NAWDB	Natural	0.03	0.023	<0.01	<0.005
18200NAWDB	Natural	0.03	0.021	<0.01	<0.005
18248NAWDB	Natural	0.03	0.022	<0.01	<0.005
18274NAWDB	Natural	0.02	0.021	<0.01	<0.005

Based on the analytical results, below is the revised specifications table with limits reduced by 50% or more (lead was < 1 ppm) for the heavy metals.

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	<0.25 ppm	<0.25 ppm	EPA 3050/6020, USP 730 – ICP-MS
Arsenic	<0.25 ppm	<0.25 ppm	EPA 3050/6020, USP 730 – ICP-MS
Cadmium	<0.25 ppm	<0.25 ppm	EPA 3050/6020, USP 730 – ICP-MS
Mercury	<0.25 ppm	<0.25 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.

c. Please provide a statement that methods used to support specifications, including (but not limited to) heavy metals and *Salmonella*, are appropriate and fit for purpose.

Attached is a copy of the Blue Diamond QA Lab's accreditation that lists the approved methodologies.

2. For the record, please provide a statement that the almonds (starting material) are grown in accordance

with good agricultural practices.

Please see the second attachment confirming that almonds are grown in accordance with good agricultural practices.

3. You state that the methods used to detect yeast and to detect mold are "FDA BAM" (page 8 of the notice). For the administrative record, please provide the chapter number from the FDA Bacteriological Analytical Manual used for the referenced methods.

Additional details on the yeast and mold tests used by the Blue Diamond QA Lab are included in the first accreditation attachment. Petrifilm is used and AOAC method numbers are noted.

The 3M Standard and Rapid Yeast and Mold Petrifilm were validated against FDA-BAM Chapter 18 and/or ISO 21527 methods using 0.1% peptone as the sample diluent.

Table 2.3.2-1 could be updated to reflect this additional detail as shown below.

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, Chapter 18/AOAC 2014.05/AOAC 997.02
Mold	<500 CFU/g	<500 CFU/g	FDA BAM, Chapter 18/AOAC 2014.05/AOAC 997.02
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.

Please let us know if further clarification is needed, or if there are additional questions.

Sincerely,
Kurt Waananen

Kurt Waananen, Ph.D.
R&D Director
Blue Diamond Growers
1802 C Street I Sacramento, CA 95811
O: 916-446-8309 I C: 763-218-0495 I kwaananen@bdgrowers.com

From: Hall, Karen <Karen.Hall@fda.hhs.gov>
Sent: Monday, April 19, 2021 8:02 AM
To: Kurt Waananen <kwaananen@bdgrowers.com>
Subject: [EXTERNAL] - Regarding GRN 000918

Good Morning Kurt,

After reviewing Blue Diamond's GRAS Notice 000918 for the intended use of PDAPF, we have three additional concerns that need to be addressed. Responses may be sent in an email or in a separate document. Please do not send a revised copy of the notice. We respectively request a response within 5 business days. If you are unable to complete the response within that time frame or have questions, please contact me to discuss further options at 240-402-9195 or via email.

1. Specifications:
    a. The *Salmonella* specification is given as negative in 2 x 375 g. We generally look for 25 g maximum samples for *Salmonella* serovar testing. Please clarify if the specification you have indicated refers to two sets of pooled-batches of tested 25 g samples (2x(15 x 25 g)) or to 2 x 375 g samples. If the latter is applicable, we request that you provide a specification with a smaller sample size (not more than 25 g) and provide results of batch analyses (minimum 3) to show your ingredient complies with the revised specification.
    b. The heavy metal specifications for lead, arsenic, cadmium, mercury are all set to <0.5 mg/kg. These levels appear higher than the results provided for the batch analyses. We request that you lower the specifications and provide a revised specifications table.
    c. Please provide a statement that methods used to support specifications, including (but not limited to) heavy metals and *Salmonella*, are appropriate and fit for purpose.
2. For the record, please provide a statement that the almonds (starting material) are grown in accordance with good agricultural practices.
3. You state that the methods used to detect yeast and to detect mold are "FDA BAM" (page 8 of the notice). For the administrative record, please provide the chapter number from the FDA Bacteriological Analytical Manual used for the referenced methods.

Kind Regards,
Karen
Karen Hall
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Karen.Hall@fda.hhs.gov

permanently delete all copies, electronic or other, you may have.*****

BLUE DIAMOND GROWERS
QUALITY ASSURANCE LABORATORY
1802 C Street
Sacramento, CA 95811
Jeremy Scheeler Phone: 916-329-3311

BIOLOGICAL

Valid To: October 31, 2021 Certificate Number: 3885.01

In recognition of the successful completion of the A2LA evaluation process, accreditation is granted to this laboratory to perform the following tests on almonds, sponges and swabs:

Test Method Name and Technology	In-House Method	Reference Method
Quantitative Microbiology		
Escherichia coli – MPN	*E. coli* Testing	BAM Ch. 4
E. coli – Petrifilm	*E. coli* Testing	AOAC 991.14
Enterobacteriaceae – Petrifilm	Enterobacteriaceae Testing	AOAC 2003.01
Standard Plate Count – Petrifilm	Standard Plate Count Testing	AOAC 990.12
Staphylococcus aureus – Petrifilm	*S. aureus* Testing	AOAC 2003.07
Total Coliform – MPN	Coliform Testing	BAM Ch. 4
Total Coliform – Petrifilm	Coliform Testing	AOAC 991.14
Yeast & Mold – Petrifilm	Yeast and Mold Count Testing	AOAC 997.02
Yeast & Mold – Rapid Petrifilm	Yeast and Mold Count Testing	AOAC 2014.05
Qualitative Microbiology		
Listeria spp. – 3M MDS	*Listeria* Testing	AOAC 2016.07
Salmonella spp. – 3M MDS	*Salmonella* Testing	AOAC 2016.01

CHEMICAL

Test Method Name and Technology	In-House Method	Reference Method
Quantitative Chemistry		
Aflatoxin by HPLC	Determination of Aflatoxin by Gilson ASPEC System	AOAC 991.31, 999.07
Fat by CEM	Fat and Moisture Analysis by Oracle and Smart 6	AOAC 2008.06
Protein by CEM	Protein Analysis by Sprint	AOAC 2011.04

Accredited Laboratory

A2LA has accredited

BLUE DIAMOND GROWERS QUALITY ASSURANCE LABORATORY

Sacramento, CA

for technical competence in the field of

Biological Testing

This laboratory is accredited in accordance with the recognized International Standard ISO/IEC 17025:2017 *General requirements for the competence of testing and calibration laboratories*. This accreditation demonstrates technical competence for a defined scope and the operation of a laboratory quality management system (*refer to joint ISO-ILAC-IAF Communiqué dated April 2017*).

Presented this 8th day of August 2019.

Vice President, Accreditation Services
For the Accreditation Council
Certificate Number 3885.01
Valid to October 31, 2021

For the tests to which this accreditation applies, please refer to the laboratory's Biological Scope of Accreditation.

April 19, 2021

Re: Good Agricultural Practices

Blue Diamond recognizes that Good Agricultural Practices are an important component of a comprehensive food safety system. To achieve this goal, Blue Diamond supports the 8 basic principles established in the Almond Board Good Agricultural Practices document as follows:

1. Documentation and Traceability
2. Employee Training
3. Fertilizer and Soil Amendment Practices
4. Water Quality and Source
5. Field Sanitation and Worker Hygiene
6. Orchard Floor Management
7. Pest Control
8. Harvest and Delivery Sanitation

For additional detail, please refer to the entire document available on web at the following address:

https://www.almonds.com/sites/default/files/2020-04/gap-manual.pdf

If you have any questions concerning this information, please contact the Blue Diamond Quality Assurance staff.

Steven Phillips
Blue Diamond Growers
Sr. Manager – Corporate Food Safety and Quality

P.O. Box 1768, Sacramento, California 95812 (916) 442-0771
www.bluediamondgrowers.com
The Almond People®

SCOPE OF ACCREDITATION TO ISO/IEC 17025:2017

BLUE DIAMOND GROWERS
QUALITY ASSURANCE LABORATORY
1802 C Street
Sacramento, CA 95811
Jeremy Scheeler Phone: 916-329-3311

BIOLOGICAL

Valid To: October 31, 2021 Certificate Number: 3885.01

In recognition of the successful completion of the A2LA evaluation process, accreditation is granted to this laboratory to perform the following tests on almonds, sponges and swabs:

Test Method Name and Technology	**In-House Method**	**Reference Method**
Quantitative Microbiology		
Escherichia coli – MPN	*E. coli* Testing	BAM Ch. 4
E. coli – Petrifilm	*E. coli* Testing	AOAC 991.14
Enterobacteriaceae – Petrifilm	Enterobacteriaceae Testing	AOAC 2003.01
Standard Plate Count – Petrifilm	Standard Plate Count Testing	AOAC 990.12
Staphylococcus aureus – Petrifilm	*S. aureus* Testing	AOAC 2003.07
Total Coliform – MPN	Coliform Testing	BAM Ch. 4
Total Coliform – Petrifilm	Coliform Testing	AOAC 991.14
Yeast & Mold – Petrifilm	Yeast and Mold Count Testing	AOAC 997.02
Yeast & Mold – Rapid Petrifilm	Yeast and Mold Count Testing	AOAC 2014.05
Qualitative Microbiology		
Listeria spp. – 3M MDS	*Listeria* Testing	AOAC 2016.07
Salmonella spp. – 3M MDS	*Salmonella* Testing	AOAC 2016.01

CHEMICAL

Test Method Name and Technology	**In-House Method**	**Reference Method**
Quantitative Chemistry		
Aflatoxin by HPLC	Determination of Aflatoxin by Gilson ASPEC System	AOAC 991.31, 999.07
Fat by CEM	Fat and Moisture Analysis by Oracle and Smart 6	AOAC 2008.06
Protein by CEM	Protein Analysis by Sprint	AOAC 2011.04

5202 Presidents Court, Suite 220 | Frederick, MD 21703-8398 | Phone: 301 644 3248 | Fax: 240 454 9449 | www.A2LA.org

Accredited Laboratory

A2LA has accredited

BLUE DIAMOND GROWERS
QUALITY ASSURANCE LABORATORY

Sacramento, CA

for technical competence in the field of

Biological Testing

This laboratory is accredited in accordance with the recognized International Standard ISO/IEC 17025:2017 *General requirements for the competence of testing and calibration laboratories*. This accreditation demonstrates technical competence for a defined scope and the operation of a laboratory quality management system (*refer to joint ISO-ILAC-IAF Communiqué dated April 2017*).

Presented this 8th day of August 2019.

Vice President, Accreditation Services
For the Accreditation Council
Certificate Number 3885.01
Valid to October 31, 2021

For the tests to which this accreditation applies, please refer to the laboratory's Biological Scope of Accreditation.

April 19, 2021

Re: Good Agricultural Practices

Blue Diamond recognizes that Good Agricultural Practices are an important component of a comprehensive food safety system. To achieve this goal, Blue Diamond supports the 8 basic principles established in the Almond Board Good Agricultural Practices document as follows:

1. Documentation and Traceability
2. Employee Training
3. Fertilizer and Soil Amendment Practices
4. Water Quality and Source
5. Field Sanitation and Worker Hygiene
6. Orchard Floor Management
7. Pest Control
8. Harvest and Delivery Sanitation

For additional detail, please refer to the entire document available on web at the following address:

https://www.almonds.com/sites/default/files/2020-04/gap-manual.pdf

If you have any questions concerning this information, please contact the Blue Diamond Quality Assurance staff.

Steven Phillips
Blue Diamond Growers
Sr. Manager – Corporate Food Safety and Quality

P.O. Box 1768, Sacramento, California 95812 (916) 442-0771
www.bluediamondgrowers.com
The Almond People®