Substantive Information Requests

Cell Bank Establishment

Hazard Analysis and Process Controls

1. Information Requested

The March 16, 2022, supplementary confidential material describes the hazard analysis and process controls in place throughout the cell bank establishment phase. The disclosable safety narrative (the May 25, 2022, disclosable safety narrative and the March 6, 2023, amendment to the disclosable safety narrative) do not discuss the hazard analysis and process controls used during the preliminary cell bank establishment and proprietary media transition steps to mitigate possible contamination from non-animal sourced reagents, animal sourced reagents, or the production environment. For addition to the disclosable safety narrative, please provide a summary (omitting confidential commercial information or trade secrets) of the management strategies, other than testing at the manufacturing cell bank establishment step, that are used for preventing contamination of non-animal sourced reagents, animal sourced reagents, or from the production environment at all steps of the cell bank establishment phase.

Mission Barns' Response:

Mission Barns would like to clarify that all hazard analysis and process controls discussed in the disclosable safety narrative (the May 25, 2022 disclosable safety narrative and the March 6, 2023 amendment) with respect to cell isolation, cell line establishment and manufacturing cell banking steps are applicable during all steps of the cell bank establishment phase, including the preliminary cell bank establishment and proprietary media transition steps.

Mission Barns' management strategies for preventing contamination from non-animal sourced reagents and animal sourced reagents during all steps of the cell bank establishment phase are described in our responses to information requests #7, #11 and #27 of our March 6, 2023 amendment to the disclosable safety narrative. To summarize, all reagents used during the cell bank establishment phase, including animal-derived substances, are food grade, pharmaceutical grade, or high-quality chemical grade and are either sterile when purchased from a third party with accompanying certificates of analysis (COA) or sterilized using membrane filtration by Mission Barns. The company has implemented multiple levels of control as part of its food safety and quality management system that takes into account the potential risks associated with the use of these substances, including a materials risk assessment for each reagent, a supplier approval program and a material handling and positive release program.

Mission Barns' management strategies for preventing contamination from the production environment during all steps of the cell bank establishment phase are described in Sections 3.2.1 and 3.2.1.1 of the May 25, 2022 disclosable safety narrative and our responses to

information requests #1 and #30 of its March 6, 2023 amendment to the disclosable safety narrative. To summarize, all operations are conducted by personnel utilizing aseptic techniques equipped with appropriate personal protective equipment (e.g., gowns, hairnets, masks and gloves) under a Class II biosafety cabinet (BSC) or equivalent environment. All surfaces with primary or secondary contact to tissues/cells are sterile. All cell culture vessels are either sterilized on-site via pressurized steam or purchased pre-sterilized from a third party with accompanying COAs. As discussed further in our response to information request #5 below of the current response submission, Mission Barns also maintains a sanitation and environmental monitoring program that includes regular facility sanitation, testing and record-keeping.

Finally, to prevent microbial contamination introduced during operation, a combination of antimicrobial reagents, including antibiotics and antifungals, are used during the cell banking process. Mission Barns uses microscopy to regularly monitor the cell cultures throughout the cell bank establishment phase to identify instances of potential bacterial or fungal contamination.

Proprietary Media Transition

2. Information Requested

On page 13, section 2.1.4 of the March 16, 2022, supplementary confidential material, you describe strategies to monitor cell growth and proliferation rate during the proprietary media transition stage. For addition to the public safety narrative, please provide a summary of controls used during this stage to monitor for unintended effects of adaptation to culture and the relevance or utility of the monitored parameters.

Mission Barns' Response:

During the proprietary media transition, Mission Barns monitors a culture's proliferation rate via doubling time. Cultures experience a decrease in proliferation rate for a period of time during the transition, but later stabilize at a higher proliferation rate after a specified number of passages in Mission Barns' proprietary cell culture media. No drops in cell viability and no mass death events are observed during this transition. Stable cell proliferation rates are an indicator of phenotypic stability and a successful transition to new cell culture media is expected to converge on a steady cell proliferation rate. Inability to maintain a stable cell population proliferation rate, drops in cell viability, or mass death events would be indicators of unintended effects of adaptation to culture.

In addition, as discussed in Section 3.2.4 of the disclosable safety narrative, karyotyping is also used to ensure the cells in the manufacturing cell bank maintain a stable chromosomal structure.

Substances Used During Cell Culture

Additional Information about Processing Aids

3. Information Requested

On page 7 of the disclosable safety narrative, you describe your proprietary cell culture medium as containing "…common nutrients such as amino acids, vitamins and their derivatives, and minerals that are needed by the cells to proliferate." You also note on page 8 that "Mission Barns also uses other proprietary processing aids." Please provide, for the addition to the disclosable safety narrative, additional information on the classes (e.g., surfactants) and characteristics of substances used in the culture medium which are neither metabolized nor used for the fundamental nutritional requirements of the cells.

Mission Barns' Response:

The three classes of substances used in Mission Barns' cell culture medium that are neither metabolized nor used for cellular nutrition requirements are surfactants, buffers, and antimicrobials. Surfactants are used to reduce the surface tension of the media, which helps to minimize shear stress. Buffers are added to cell culture media to maintain a stable pH within the optimal range for cell growth and function. Antimicrobial reagents include antibiotics and antifungals, which are used exclusively in the cell banking process to prevent microbial contamination. As discussed previously, each of these components has undergone a material risk assessment to establish appropriate product specifications based on identified risks. Additionally, multiple washing steps upon harvest substantially dilute all residuals of these components to levels well below appropriate safety thresholds.

Cell Culture Process

Sanitation and Environmental Monitoring Programs

5. Information Requested

Attachment 1 of the March 16, 2022, supplementary confidential material lists “sanitation and environmental monitoring programs” as one of the management strategies used to prevent contamination from the facility or environment for several steps of the cell culture food production process. For addition to the disclosable safety narrative, please provide the following:

a. The sanitation and environmental monitoring programs in place, including the steps during the production process where each process would be applied;
b. An explanation of the controls used to prevent adventitious agent contamination from inadequate sterilization of vessels; and
c. A discussion of the controls used to prevent contamination from the facility environment during transfer of cells between vessels/bioreactors at the cell culture expansion and cell fatting steps.

Please clarify if any testing is conducted for the environmental monitoring program, and whether the presence of any indicator microorganism is analyzed for. If so, please add this information to your disclosable safety narrative.

Mission Barns' Response:

a. As part of its food safety and quality management system, Mission Barns has implemented a comprehensive Environmental Monitoring Program (EMP) and a Facility and Equipment Cleaning and Sanitation procedures during all steps of the cell culture food production process.

- The Sanitation Policy creates a master sanitation schedule based on a risk and requirements assessment of each area of Mission Barns' manufacturing facilities and all equipment used in Mission Barns' manufacturing process. Per the terms of the Sanitation Policy, facilities and equipment are regularly sanitized using validated techniques.
- As part of the EMP, a hygiene zone map of Mission Barns' production facilities is utilized where zones are sampled and tested at appropriate frequencies based on a facility risk assessment of the applicable zone. Any sample result above predetermined limits triggers further investigative sampling, a root cause analysis to determine the source of the issue, and corrective actions as appropriate.

b. All cell culture vessels used by Mission Barns are either single use vessels that are purchased sterile from a third party with an accompanying COA or are stainless steel and are cleaned and sterilized using high temperature steam (>121°C). Environmental monitoring is used to evaluate the overall hygienic status of the manufacturing environment.

c. As with all steps of the cell cultivation process, transfers of cells between vessels/bioreactors at the cell culture and cell fattening steps are conducted by technicians utilizing aseptic techniques, wearing appropriate personal protective equipment (PPE) (e.g. gowns, hairnets, masks and gloves) in a positive air pressure filtration environment and under a Class II BSC or equivalent environment or are conducted through sterile tubing. Transfers of cells are performed with single-use serological pipettes that are purchased sterile from a third party with an accompanying COA or via sterile tubing. Media that is added to the culture vessel after transfer is sterilized using 0.2 µm filtration.

To clarify, Mission Barns does conduct testing as part of its environmental monitoring program, including for the presence of indicator microorganisms. Environmental monitoring includes active viable air monitoring and/or viable surface monitoring in processing areas and equipment such as biosafety cabinets and incubators. Further, spent media are tested for APC, *Enterobacteriaceae*, coliforms and yeast/mold.

Product Characterization

Fatty Acid Profile

6. Information Requested

On page 17-18, of the March 6, 2023, amendment to the disclosable safety narrative, you present fatty acid profiles for palmitic acid [16:0], stearic acid [18:0], oleic acid [18:1 cis], and linoleic acid [18:2 cis] from three non-consecutive batches of the harvested cell material (Table: "Mass fractions of common fatty acids from three representative batches (AOCS CE 1F-96)").

a. For addition to the disclosable safety narrative, please discuss the full fatty acid profile of the harvested cell material and your basis for determining that the presence of fatty acids typically not found in pork fat (e.g., elaidic acid, methyl nervonate) but identified in your analytical data would be consistent with a conclusion that your product would be as safe as comparable foods.

[redacted]

Mission Barns' Response:

a. The full fatty acid profile of the harvested cell material is shown in the table below.

Fatty Acid	Batch 1	Batch 2	Batch 3
Myristic acid (14:0)	0.0%	1.0%	0.0%
Palmitic acid (16:0)	14.7%	13.8%	11.7%
Palmitoleic acid (16:1 CIS)	0.0%	1.1%	0.0%
Stearic acid (18:0)	11.2%	10.1%	10.5%
Oleic acid (18:1 CIS)	48.1%	46.8%	48.5%
Elaidic acid (18:1 TRANS)	3.5%	3.2%	0.0%
Linoleic acid (18:2 CIS)	3.5%	3.4%	4.3%
Linolenic acid (18:3 CIS)	1.6%	1.4%	1.8%
Behenic acid (22:0)	0.0%	0.0%	1.7%
Arachidonic acid (20:4)	2.4%	1.7%	3.9%

Eicosapentaenoic acid (20:5)	2.2%	1.5%	0.0%
Nervonic acid (24:1)	3.3%	3.0%	4.2%

Mission Barns has conducted dietary exposure assessment for the two fatty acids the agency has highlighted - elaidic acid and nervonic acid. For elaidic acid, using the average fat content reported in information request #7 below of 5.32%, and the average and 90th percentile EDI for cultivated pork fat cells (discussed on page 17 of the May 25, 2022 disclosable safety narrative) of 6.93 g/day and 16.7 g/day, respectively, the daily intake of elaidic acid from our product's intended use can be calculated as follows:

Average EDI:

$$\frac{5.32\ g\ fat}{100\ g\ cultivated\ fat\ cells} \times \frac{2.2\ g\ elaidic\ acid}{100\ g\ fat} \times \frac{6.93\ g\ cultivated\ fat\ cells}{day} = \frac{8.11\ mg\ elaidic\ acid}{day}$$

90th percentile EDI:

$$\frac{5.32\ g\ fat}{100\ g\ cultivated\ fat\ cells} \times \frac{2.2\ g\ elaidic\ acid}{100\ g\ fat} \times \frac{16.7\ g\ cultivated\ fat\ cells}{day} = \frac{19.54\ mg\ elaidic\ acid}{day}$$

These intake levels assume that Mission Barns cultivated pork fat cells will replace 100% of conventional pork fat in the current marketplace. Even with this conservative assumption, an average EDI of 8.11 mg of elaidic acid per day is much lower than the elaidic acid intake from the consumption of a single link of sausage (containing 107 mg elaidic acid)[1] or a single slice of bacon (29 mg)[2], as reported in the USDA database.

The case is similar for nervonic acid (24:1)[3]. The daily intake of nervonic acid from our product's intended use can be calculated as follows:

[1] USDA FoodData Central, NDB Number:7074 Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/174584/nutrients (accessed May 31, 2023).
[2] USDA FoodData Central, NDB Number:10123 Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/168277/nutrients (accessed May 31, 2023).
[3] Nervonic acid is measured by third party testing laboratories through gas chromatography methodology. In order to perform such methods, nervonic acid is methylated and is reported by laboratories as the methylated form methyl nervonate. Such results do not indicate the presence of methyl nervonate in Mission Barns cultivated pork fat cells.

Average EDI:

$$\frac{5.32\ g\ fat}{100\ g\ cultivated\ fat\ cells} \times \frac{3.5\ g\ nervonic\ acid}{100\ g\ fat} \times \frac{6.93\ g\ cultivated\ fat\ cells}{day} = \frac{12.90\ mg\ nervonic\ acid}{day}$$

90th percentile EDI:

$$\frac{5.32\ g\ fat}{100\ g\ cultivated\ fat\ cells} \times \frac{3.5\ g\ nervonic\ acid}{100\ g\ fat} \times \frac{16.7\ g\ cultivated\ fat\ cells}{day} = \frac{31.10\ mg\ nervonic\ acid}{day}$$

The average daily intake of 12.9 mg per day of nervonic acid from Mission Barns cultivated pork fat cells would be comparable to the exposure from other commonly consumed foods, including, but not limited to, a 3 oz. serving of king salmon (containing 93.5 mg nervonic acid)[4], a 3 oz. serving of swordfish (65 mg)[5], a tablespoon of safflower oil (23 mg)[6], a 3 oz. ground turkey patty (19 mg)[7], or a 3 oz. serving of rainbow trout (17 mg)[8], a serving of flax seeds (14 mg)[9], a quarter cup of pesto (13 mg)[10], 5 chicken nuggets (12.5 mg)[11], two servings of potato chips (12 mg)[12], a tablespoon of yellow mustard (11.2 mg)[13], or a tablespoon of margarine (9 mg)[14], as reported in the USDA database.

To further demonstrate the analytical data support our conclusion that our product would be as safe as comparable foods, as shown in the table below, Mission Barns conducted a literature review of fatty acids (as a percentage of total fatty acids) present in pork and other commonly consumed meats and seafood products. The presence of fatty acids in Mission Barns' cultivated cells is generally consistent with or below levels reported in scientific literature to be present in comparable foods.

[4] USDA FoodData Central, NDB Number:35168. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167647/nutrients (accessed May 31, 2023).
[5] USDA FoodData Central, NDB Number:15110. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/173703/nutrients (accessed May 31, 2023).
[6] USDA FoodData Central, NDB Number:4511. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/1750350/nutrients (accessed May 31, 2023).
[7] USDA FoodData Central, NDB Number:5670. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/174495/nutrients (accessed May 31, 2023).
[8] USDA FoodData Central, NDB Number:15241. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/173718/nutrients (accessed May 31, 2023).
[9] USDA FoodData Central, NDB Number:12220. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169414/nutrients (accessed May 31, 2023).
[10] USDA FoodData Central, NDB Number:6626. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/171579/nutrients (accessed May 31, 2023).
[11] USDA FoodData Central, NDB Number:21309. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/173297/nutrients (accessed May 31, 2023).
[12] USDA FoodData Central, NDB Number:19411. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169677/nutrients (accessed May 31, 2023).
[13] USDA FoodData Central, NDB Number:2046, available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/326698/nutrients (accessed May 31, 2023).
[14] USDA FoodData Central, NDB Number:4611. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/172347/nutrients (accessed May 31, 2023).

Fatty Acid	Average	Conventional food comparator
Myristic acid (14:0)	0.3%	1.32% (pork)[15]
Palmitic acid (16:0)	13.4%	22.45% (pork)[16]
Palmitoleic acid (16:1 CIS)	0.4%	2.83% (pork)[17]
Stearic acid (18:0)	10.6%	12.25% (pork)[18]
Oleic acid (18:1 CIS)	47.8%	43.02% (pork)[19]
Elaidic acid (18:1 TRANS)	2.2%	2.63% (pork)[20] 3.1% (beef)[21] 3.2% (ovine/sheep)[22]
Linoleic acid (18:2 CIS)	3.7%	9.49% (pork)[23]
Linolenic acid (18:3 CIS)	1.6%	0.91% (pork)[24] 2.43% (pork)[25]
Behenic acid (22:0)	0.6%	0.490% (pork)[26]
Arachidonic acid (20:4)	2.7%	4.7% (pork)[27]
Eicosapentaenoic acid (20:5)	1.2%	0.73% (pork)[28]

[15] Calculated as a percentage of total lipid (fat) from USDA FoodData Central, NDB Number:10005. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167812/nutrients (accessed May 31, 2023).
[16] Id.
[17] Id.
[18] Id.
[19] Id.
[20] Lisitsyn, A., Chernukha, I. and Ivankin, A. (2013) 'Comparative study of fatty acid composition of meat material from various animal species', Scientific Journal of Animal Science, 2(5), pp. 124-131.
[21] Id.
[22] Id.
[23] Calculated as a percentage of total lipid (fat) from USDA FoodData Central, NDB Number:10005. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167812/nutrients (accessed May 31, 2023)
[24] Id.
[25] M. Enser, et al. "Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages." Meat Science, vol. 55, no. 2, Jun. 1999, p. 201.
[26] Kušec, Goran, et al. "Carcass Composition and Physicochemical Characteristics of Meat from Pork Chains Based on Native and Hybrid Pigs." Processes, vol. 10, no. 2, Feb. 2022, p. 370.
[27] Matthews, K R et al. "Effect of whole linseed (Linum usitatissimum) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues." The British journal of nutrition vol. 83,6 (2000): 637-43..
[28] M. Enser, et al. (1999).

		4.14% (salmon)[29] 7.85% (white tuna)[30]
Nervonic acid (24:1)	3.5%	0.17% (pork)[31] 0.7% (chicken thigh)[32] 1.67% (chicken breast)[33] 3.6% (jersey yearling beef)[34] 8.84% (indian mackerel)[35]

[redacted]

[29] Calculated as a percentage of total lipid (fat) from USDA FoodData Central, NDB Number:15083. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/175138/nutrients (accessed May 31, 2023).

[30] Calculated as a percentage of total lipid (fat) from USDA FoodData Central, NDB Number:15126. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/175158/nutrients (accessed May 31, 2023).

[31] Kloareg, Maela et al. "Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs." The British journal of nutrition vol. 97,1 (2007): 35-44.

[32] Ali, M., Lee, S.-Y., Park, J.-Y. and Nam, K.-C. (2021) 'Evaluation of Meat from Native Chickens: Analysis of Biochemical Components, Fatty Acids, Antioxidant Dipeptides, and Microstructure at Two Slaughter Ages', Food Science of Animal Resources, 41(5), pp. 788.

[33] Id.

[34] Malau-Aduli, A. E., Siebert, B. D., Bottema, C. D. and Pitchford, W. S. (1998) 'Breed comparison of the fatty acid composition of muscle phospholipids in Jersey and Limousin cattle', Journal of Animal Science, 76(3), pp. 766-773.

[35] Alkuraieef, A. N., Alsuhaibani, A. M., Alshawi, A. H., Aljahani, A. H., Aljobair, M. O. and Albaridi, N. A. (2021) 'Proximate chemical composition and lipid profile of Indian mackerel fish', Food Science and Technology, 42.

[redacted]

Compositional Analysis

7. Information Requested

For addition to the disclosable safety narrative, please provide analytical data on proximates (e.g., moisture, protein, fat, ash), amino acids, vitamins, and minerals, preferably from three independent batches of the harvested cell material.

Mission Barns' Response:

The moisture, total protein, crude fat, ash, and carbohydrate content of Mission Barns cultivated pork fat cells are presented in the following table:

Parameter[37]	Units	Batch 1	Batch 2	Batch 3	Average
Moisture	g/100g	86.82	89.81	89.33	88.65
Fat	g/100g	5.54	5.85	4.56	5.32
Protein	g/100g	5.59	5.38	4.27	5.08
Ash	g/100g	1.01	1	0.88	0.96
Carbohydrates	g/100g	1.03	0	0.91	0.65

[37] Mission Barns utilizes a third party laboratory to conduct proximates analysis. Moisture content is determined using AOAC 990.20 methodology, fat is determined using AOAC 933.05 methodology, protein is determined using AOAC 991.20 methodology and ash is determined by AOAC 920.153 methodology. Carbohydrate content is determined by calculating the remainder of 100% following the subtraction of moisture, fat, protein and ash. In cases where moisture, fat, protein and ash sum to 100% or more, carbohydrates are reported as "0%".

Analytical data on the amino acid profile of Mission Barns cultivated pork fat cells are presented in the following table:

Amino acid[38]	Mission Barns' Cultivated Pork Fat Cells (g/100g)			Conventional pork fat (g/100g)[39]
	Batch 1	Batch 2	Batch 3	
Alanine	0.275	0.2	0.25	0.104 - 0.544
Arginine	0.325	0.225	0.35	0.182 - 0.617
Aspartic Acid, Asparagine	0.575	0.425	0.55	0.163 - 0.906
Cystine	0.15	0.1	0.125	0.015 - 0.107
Glutamic Acid, Glutamine	0.875	0.625	0.8	0.273 - 1.48
Glycine	0.3	0.225	0.275	0.08 - 0.418
Histidine	0.15	0.1	0.125	0.02 - 0.4
Isoleucine	0.3	0.225	0.275	0.046 - 0.456
Leucine	0.525	0.4	0.5	0.123 - 0.789
Lysine	0.5	0.375	0.475	0.146 - 0.859
Methionine	0.175	0.15	0.175	0.026 - 0.255
Phenylalanine	0.2	0.15	0.2	0.066 - 0.389
Proline	0.35	0.275	0.325	0.067 - 0.375
Serine	0.3	0.225	0.275	0.072 - 0.401
Threonine	0.275	0.2	0.25	0.058 - 0.416
Tryptophan	0.075	0.05	0.075	0.006 - 0.11
Tyrosine	0.25	0.225	0.25	0.029 - 0.366
Valine	0.375	0.275	0.35	0.084 - 0.484
Hydroxyproline	<0.025	<0.025	<0.025	0.024 - 0.032

[38] Mission Barns utilized a third party testing laboratory to conduct an amino acid profile *via* the USDA MSS2 (1993) method.

[39] Reported ranges are the lowest and highest reported value from USDA FoodData Central for NDB Number 10004 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167811/nutrients, accessed 11/29/22), NDB Number 10006 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167813/nutrients, accessed 11/30/22), NDB Number 10942 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169179/nutrients, accessed 11/30/22), NDB Number 10109 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167861/nutrients, accessed 11/30/22), and NDB Number 10007 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/168221/nutrients, accessed 5/29/23).

Analytical data on vitamins for Mission Barns cultivated pork fat cells are presented in the following table:

Vitamin[40]	Unit	Mission Barns' Cultivated Pork Fat Cells			Conventional pork fat[41]
		Batch 1	Batch 2	Batch 3	
Vitamin A	mcg RAE/100 g	<10	<10	<10	0 - 26
Thiamin (B1)	mg/100g	0.45	0.275	0.325	0.084 - 0.21
Riboflavin (B2)	mg/100g	0.325	0.3	0.325	0.051 - 0.2
Niacin (B3)	mg/100g	3.625	3	3.625	0.985 - 3.23
Pantothenic Acid (B5)	mg/100g	0.6	0.55	0.575	0 - 0.611
Pyridoxine (B6)	mg/100g	0.5	0.475	0.475	0.03 - 0.275
Vitamin E	mg a-tocoph/100g	1.25	1.25	1.25	0 - 0.42

[40] The vitamins were analyzed by a third-party laboratory according to the following methods: Vitamin A was assayed according to Analyst(1984) 109:489 (an accredited ISO method), Vitamin B1 was assayed according to AOAC 942.23, Vitamin B2 was assayed according to AOAC 970.65, Vitamin B3 was assayed according to AOAC 985.34 with associated VitaFast kits, Vitamins B5 and B6 were assayed according to AOAC 960.46 with associated VitaFast kits, and Vitamin E was assayed according to AOAC 992.03.

[41] Reported ranges are the lowest and highest reported value from USDA FoodData Central for NDB Number 10004 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167811/nutrients, accessed 11/29/22), NDB Number 10006 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167813/nutrients, accessed 11/30/22), NDB Number 10942 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169179/nutrients, accessed 11/30/22), NDB Number 10109 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167861/nutrients, accessed 11/30/22), and NDB Number 10007 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/168221/nutrients, accessed 5/29/23).

Analytical data on minerals for Mission Barns cultivated pork fat cells are presented in the following table:

Mineral[42]	Unit	Mission Barns' Cultivated Pork Fat Cells			Conventional pork fat[43]
		Batch 1	Batch 2	Batch 3	
Calcium	mg per 100g	<0.25	<0.25	<0.25	1 - 22
Copper	mg per 100g	<0.0025	<0.0025	<0.0025	0.009 - 0.09
Iron	mg per 100g	<0.125	<0.125	0.15	0.09 - 0.47
Magnesium	mg per 100g	4.3	7.1	6.125	1 - 9
Manganese	mg per 100g	0.0175	0.02	0.0225	0 - 0.006
Phosphorus	mg per 100g	116	144	154	19 - 121
Potassium	mg per 100g	42	79	61	31 - 333
Selenium	mg per 100g	<0.025	<0.025	<0.025	0.008 - 0.0121
Sodium[44]	mg per 100g	507	530	522	5 - 81
Zinc	mg per 100g	0.49	0.78	0.63	0.18 - 0.9

[42] The minerals were analyzed by a third-party laboratory according to AOAC 2015.01 Mod<2232>.
[43] Reported ranges are the lowest and highest reported value from USDA FoodData Central for NDB Number 10004 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167811/nutrients, accessed 11/29/22), NDB Number 10006 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167813/nutrients, accessed 11/30/22), NDB Number 10942 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169179/nutrients, accessed 11/30/22), NDB Number 10109 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167861/nutrients, accessed 11/30/22), and NDB Number 10007 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/168221/nutrients, accessed 5/29/23).
[44] Please note the cells are aseptically washed with a saline solution containing sodium after harvest.

Characterization of Differentiated Cells

9. Information Requested

Please provide, for addition to the disclosable safety narrative, a general description of the analytical testing strategy and results described on pages 16-17, section 2.2.2 of the March 16, 2022, supplementary confidential material.

Mission Barns' Response:

Once enough proliferative cells are obtained from cell culture expansion, they are induced to form lipid droplets within the cells. Proprietary food-grade reagents are added to the cell culture media to conduct this process. Mission Barns has verified the accumulation of lipid droplets that are determined to be desirable for culinary applications in two manners. First, Mission Barns has applied a commonly used fluorescent stain to the cell population following induction and observed the stained cells using microscopy. Microscopic observation revealed an increase in cell size and an accumulation of intracellular lipid droplets. Second, Mission Barns has quantified the amount of lipids present in induced cells using an assay sensitive to the lipids expected to accumulate in the induced cells. Assay results verified a measurable increase in

lipid levels in the cells following induction for a specified time period in Mission Barns' proprietary cell culture media relative to a control.

Adventitious Agent Hazard Analysis and Testing

10. Information Requested

In the March 6, 2023, amendment to the disclosable safety narrative, you describe updated adventitious agent tests performed and batch release testing specifications (page 12-13; Table: "Mission Barns' Cultivated Pork Fat Cells Batch Release Criteria"). For addition to the disclosable safety narrative, please provide the results from the analysis of the new adventitious agent tests performed (i.e., aerobic plate count, *Enterobacteriaceae*, coliforms, and yeast and mold) from at least three independent batches of the harvested cell material.

Mission Barns' Response:

The results from Mission Barns' new cultivated pork fat cells batch release adventitious agent testing from the three independent batches using spent media are provided in the table below:

Adventitious Agent Batch Test Results				
Test	**Specification**	**Batch 1**	**Batch 2**	**Batch 3**
Aerobic Plate Count	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL
Enterobacteriaceae	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL
Coliforms	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL
Yeast and Mold	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL	<10 CFU/mL

11. Information Requested

On pages 15-16 of the March 6, 2023, amendment to the disclosable safety narrative, you identify foodborne pathogens associated with conventional pork by referencing "Fresh Pork from Farm to Table" from the United States Department of Agriculture, Food Safety Inspection Service. You list several microorganisms associated with conventional pork including the parasite *Trichinella spiralis* and additional species of bacteria (*Escherichia coli*, *Salmonella* serovars, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Listeria monocytogenes*) and state, "Mission Barns' process is different from a microbiological perspective than traditional meat processing because its products do not involve animal slaughtering, during which meat products may be exposed to common pathogens present in an animal's digestive tract and fecal matter or through the cross-contamination of food contact surfaces during meat processing." However, in Attachment 1 of the March 16, 2022, supplementary confidential material, you

identify two genera of microorganisms as potential biological hazards resulting from "contamination from environment or human sources."

Please clarify whether the two genera of microorganisms identified in Attachment 1 of the March 16, 2022, supplementary confidential material are relevant to your production process. If so, for addition to the disclosable safety narrative, please identify and list the relevant species of concern, their potential source, and clarify whether any further analyses are performed to assess for the presence of these microorganisms.

Mission Barns' Response:

Mission Barns would like to clarify the relevance of two genera of microorganisms (i.e., *Listeria monocytogenes* and *Staphylococcus*) identified in Attachment 1 of the March 16, 2022, supplementary confidential material. Mission Barns has updated its assessment to remove *Listeria monocytogenes* as a microorganism of concern for the following reasons:

- *Listeria monocytogenes* is commonly found in moist environments, soil, water, decaying vegetation and animals.[45] Mission Barns' manufacturing process does not occur in a wet or moist environment such as that commonly found in conventional meat processing facilities. Rather, Mission Barns produces its cultivated cells within a dry cleanroom environment supplied with pressurized, HEPA-filtered air, making *Listeria* contamination events highly unlikely.
- Regular EMP test data for Mission Barns' GMP manufacturing facility collected over more than a year-long period has resulted in zero occurrences of *Listeria* contamination.[46]

Mission Barns continues to identify *Staphylococcus* as a potential microorganism of concern given that people are a common source of *Staphylococcus* contamination. Mission Barns' manufacturing process utilizes contamination control measures such as hygiene training, the use of PPE and aseptic techniques to mitigate the potential of human-sourced *Staphylococcus* contamination. In addition to these control measures, Mission Barns' EMP regularly conducts Aerobic Plate Count (APC) testing to monitor the presence of indicator organisms in our manufacturing environment and APC testing of spent media as part of cultivated fat cells batch release criteria. While APC testing does not specifically target *Staphylococcus*, it covers a broad range of microorganisms including *Staphylococcus*. Following any presumptive positive test result for APC from EMP testing, Mission Barns sterilizes the affected area following standard operating procedures (SOPs) and resamples the area following sterilization. Following any presumptively positive test result from APC testing of spent media, Mission Barns performs further analysis to identify the species of the microbe(s), using methods such as gene sequencing (e.g., QA-0095-3000 GeneSeq) and/or mass spectroscopy (e.g., MALDI-TOF).

[45] Listeria (Listeriosis), U.S. Food & Drug Administration, available at https://www.fda.gov/food/foodborne-pathogens/listeria-listeriosis (accessed May 31, 2023).
[46] Please note Mission Barns no longer conducts regular testing for listeria as part of its EMP as it is currently not considered a microorganism of concern.

12. Information Requested

In the March 6, 2023, amendment to the disclosable safety narrative, you list specifications for microorganisms including *Enterobacteriaceae* and coliforms (pages 8, 12, 17), but do not provide specifications for other common, notable foodborne pathogen analyses, such as *Salmonella* serovars. For addition to the disclosable safety narrative, please clarify if further analysis is performed to identify the genera or species of any presumptive positive result from the analysis of *Enterobacteriaceae* or coliforms. If further analysis is not performed, please describe why analysis of *Enterobacteriaceae* and coliforms is sufficient.

Mission Barns' Response:

Mission Barns clarifies that common, notable foodborne pathogens, including *Salmonella*, would be detected by the *Enterobacteriaceae*, coliforms or APC testing that Mission Barns conducts as part of its batch release criteria. For any non-conforming batches that fail to pass the microbial testing plan acceptance criteria, Mission Barns performs further analysis to identify the species of the microbe(s), using methods such as gene sequencing (e.g. QA-0095-3000 GeneSeq) and/or mass spectroscopy (e.g. MALDI-TOF). Quality Assurance personnel will then conduct a detailed investigation and risk/impact assessments, which include a root cause analysis (RCA) to determine the source of the issue, and corrective and preventative actions (CAPA), as needed.

13. Information Requested

On page 16 of the March 6, 2023, amendment to the disclosable safety narrative, you state, "As a final check in our production process, we also test our product after-harvest for fungal and bacterial contamination via Aerobic Plate Counts, coliforms, enterobacteria, mycoplasma, and yeast/mold testing;" however, the table presented on page 17 of the same amendment identified the substance being analyzed as the spent media, rather than the harvested cell material.

a. For addition to the disclosable safety narrative, please clarify whether the spent media or the harvested cell material is analyzed at this stage in your production process.
b. If the spent media is the substance being analyzed, please describe how it is a sufficient proxy for analysis of the harvested cell material with respect to control of microbial food safety risks.
c. Finally, please also clarify whether the presence of *Enterobacteriaceae* or *Enterobacteria* spp. are analyzed at this stage in your production process (*Enterobacteriaceae* are included in the tables presented on pages 8, 12, and 17, while *Enterobacteria* spp. are mentioned on pages 2, 6, 7, and 16 of the same amendment).

Mission Barns' Response:

a. Mission Barns would like to clarify that the spent media in contact with cells immediately prior to harvest is analyzed for fungal and bacterial contamination.

b. Mission Barns maintains that spent media is a sufficient proxy for analysis of the harvested cell material with respect to control of microbial food safety risks. For easy reference, we have copied the updated testing plan on page 17 of the March 6, 2023, amendment to the disclosable safety narrative, below:

Bacterial and Fungal Batch Release Testing Plan			
Test	**Method**	**Sample**	**Specification**
Aerobic Plate Count	APHA CMMEF CHP 8 or equivalent	25 mL of spent media	Negative (<10 CFU/mL)
Enterobacteriaceae	APHA CMMEF CHP 9 or equivalent	25 mL of spent media	Negative (<10 CFU/mL)
Coliforms	FDA BAM ONLINE CHP 4 or equivalent	25 mL of spent media	Negative (<10 CFU/mL)
Mycoplasma	Mycoplasma Genus TaqMan® PCR, or equivalent	200 µL of spent media	Negative
Yeast and Mold	FDA-BAM, 7th ed., AOAC Official Method 2014.05, or equivalent	50 mL of spent media	Negative (<10 CFU/mL)

Nutrient-rich culture media is circulated throughout the cultivator throughout the cell culture process and, as such, is in direct contact with cell material. Any microbial contamination, if present, is expected to overtake animal cell cultures due to the absence of any antibiotics in the cell culture media during the cell proliferation and fattening stages. Microbial contamination of the cell material that may occur during the course of the cell culture process would likewise be expected to be present in the surrounding media. Spent media is sampled immediately prior to commencing the harvest and is aseptically placed into single-use sterile tubes to avoid cross contamination.

While in theory the cells can also be exposed to microbial contaminants once the nutrient-rich feed source of the cell culture media is removed, the risk of microbial contamination is very low during the harvesting step where multiple rounds of washing take place to remove the residual cell culture media. Mission Barns also continues to implement rigorous controls during harvest to prevent contamination. For example, all equipment and reagents used during this process are sterile, and the operations are conducted in an aseptic environment by operators wearing appropriate PPE and practicing aseptic techniques.

Therefore, bacterial and fungal testing of spent media provides a sufficient proxy for analysis of the harvested cell material with respect to control of microbial food safety risks.

To further control microbial food safety risks, harvested cells are cooked for at least 4 minutes at a minimum internal temperature of 165 °F once mixed with plant-based ingredients to formulate final food products such as sausage and bacon alternatives.

c. Mission Barns would like to clarify that the presence of *Enterobacteriaceae*, not *Enterobacter* spp, is tested for in spent media.

Points of Clarification

Adventitious Agent Hazard Analysis and Testing

14. Information Requested

Throughout the March 6, 2023, amendment to the disclosable safety narrative, you identify the method used for the analysis of yeast and mold as "FDA-BAM, 7th ed., AOAC Official Method 2014.05, or equivalent;" however, you do not identify the chapter of the FDA *Bacteriological Analytical Manual* (BAM) that is used for these analyses. For addition to the disclosable safety narrative, please provide the chapter of the BAM used for the analysis of yeast and mold.

Mission Barns' Response:

With respect to yeast and mold testing, Mission Barns' references to "FDA-BAM, 7th ed." refer to chapter 18 of the FDA *Bacteriological Analytical Manual* (BAM).

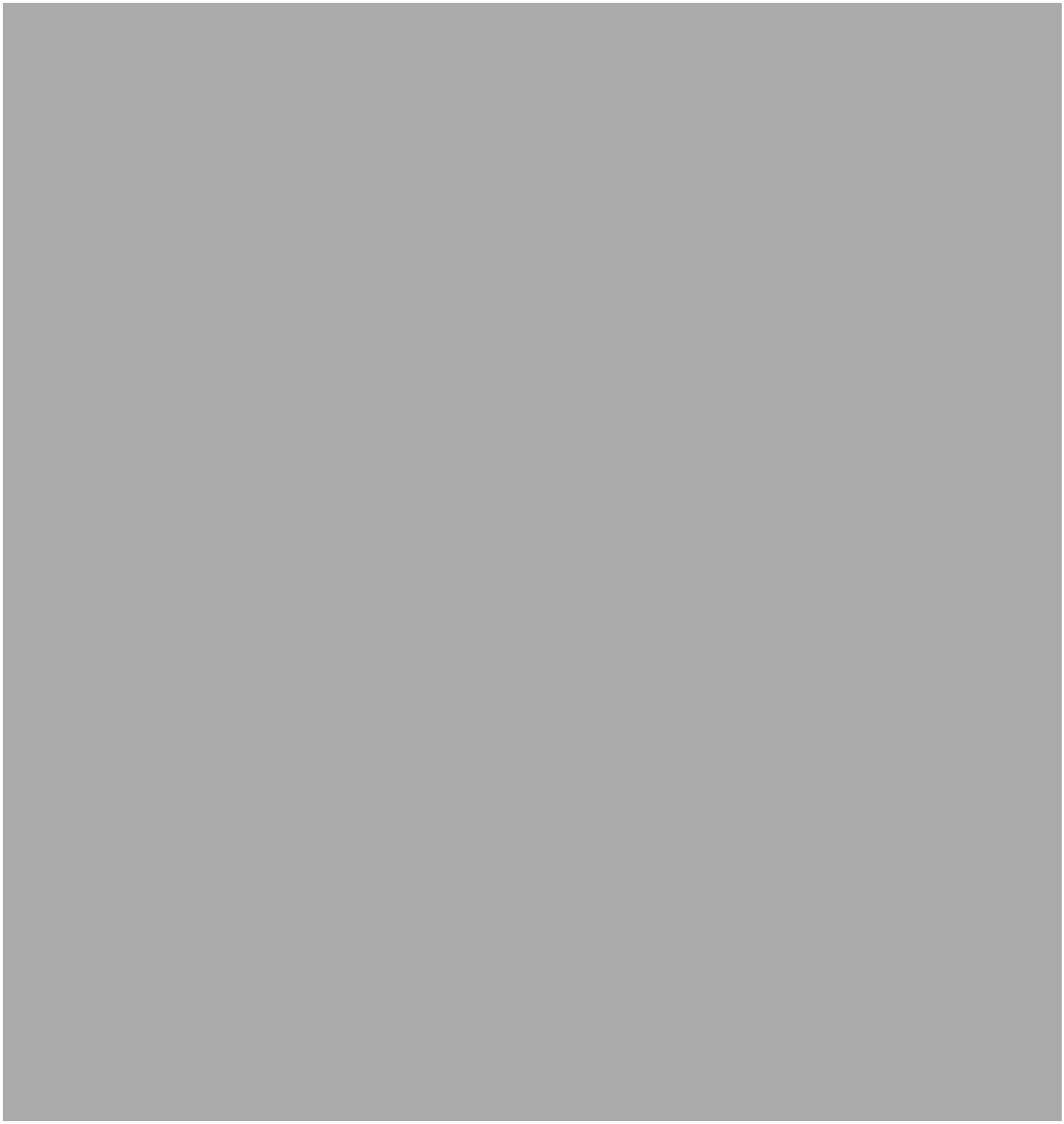
Allergens

15. Information Requested

On pages 8 (section 1.3.5) and 31 (section 4.2) of the disclosable safety narrative, you discuss the allergenicity potential of cell culture basal media ingredients, supplements, and other proprietary processing aids. You state, "None of these are or contain major food allergens as identified under the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA)." As of January 1, 2023, sesame has been identified as a major food allergen defined in the law as the result of the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act. For addition to the disclosable safety narrative, please provide an updated statement to clarify whether the substances used in the production process are, or contain, any of the major food allergens, including sesame.

Mission Barns' Response:

Mission Barns confirms that none of the substances used in the production process are or contain any of the major food allergens, including sesame.



[redacted]

TO Andrew Zajac
Division Director
Office of Food Additive Safety, CFSAN
U.S. Food and Drug Administration

Ashley E. Nazario-Toole, Ph.D.
Biologist
Office of Food Additive Safety, CFSAN
U.S. Food and Drug Administration

FROM [redacted] **TELEPHONE** [redacted]

DATE August 23, 2023

Privileged And Confidential
By Electronic Mail

SUBJECT **Mission Barns New COAs on Fatty Acid Profile re: CCC 000008**

Dear Andy and Ashley,

On behalf of our client Mission Barns, Inc. (Mission Barns, the Company), we would like to report that by replacing the chemically defined mixture of various lipids prepared by a third party supplier with an "in-house" version prepared by Mission Barns itself with the same ingredients, the Company is able to produce new batches of cultivated cells with the elaidic acid levels ranging from 0.6% to 0.7%.

Fatty acid profiles in conventional pork fat can be impacted by a variety of factors including pig breed, sex, and diets of the animals. While in our previous response to FDA's follow-up request for information, dated June 5, 2023, we identified elaidic acid level as high as 2.63% in pork as reported by scientific literature, we also note per the USDA database (links provided below), the elaidic acid levels in Mission Barns pork fat cells samples are below or comparable to the USDA reported range of 0.73% to 0.8%:

- USDA FoodData Central, NDB Number:10006 "Pork, fresh, separable fat, raw" (*available at* https://fdc.nal.usda.gov/fdc-app.html#/food-details/167813/nutrients, accessed August 21, 2023)

- USDA FoodData Central, NDB Number:10942 "Pork, fresh, composite of separable fat, with added solution, raw" (*available at* https://fdc.nal.usda.gov/fdc-app.html#/food-details/169179/nutrients, accessed August 21, 2023).

Even if we very conservatively assume that Mission Barns' cultivated pork fat cells will replace 100% conventional pork fat in the current market, our new test data demonstrate that the cultivated cells contain elaidic acid levels lower or comparable to the naturally occurring levels found in conventional pork fat. As such, there will be no expected increase of dietary intake to elaidic acid from the intended use of Mission Barn's cultivated pork fat cells. In addition, levels of other fatty acids from these new batches are also generally consistent with or below levels reported in pork and other commonly consumed comparable products by scientific literature.

For your easy reference, the fatty acid profiles from the three new batches of cultivated pork fat cells are summarized in the following table.

Fatty Acid	New Batch #1	New Batch #2	New Batch #3
Myristic acid (14:0)	0.8%	0.8%	0.8%
Palmitic acid (16:0)	12.6%	13.4%	12.1%
Palmitoleic acid (16:1 CIS)	2.8%	3.3%	3.3%
Stearic acid (18:0)	9.8%	9.6%	9.0%
Oleic acid (18:1 CIS)	58.5%	58.7%	58.2%
Elaidic acid (18:1 TRANS)	0.7%	0.6%	0.7%
Linoleic acid (18:2 CIS)	0%	0%	0%
Linolenic acid (18:3 CIS)	1.5%	1.5%	1.8%
Behenic acid (22:0)	0%	0%	0%
Arachidonic acid (20:4)	1.7%	1.4%	1.4%
Eicosapentaenoic acid (20:5)	3.7%	3.1%	3.2%
Nervonic acid (24:1)	1.9%	1.7%	1.9%

While the third party supplier for the chemically defined mixture of various lipids has not included the fatty acid profile in the COAs, the “in-house” version is made by Mission Barns with the same ingredients with COAs that attest to high purity. Going forward, Mission Barns intends to only use the new lipid mixture it prepares for future manufacturing operations. Mission Barns represents that the fatty acid profile reported above (including the elaidic acid level) is representative of all the future batches of its cultivated cells.

If you have any questions, please do not hesitate to contact us.

Sincerely,

Substantive Information Requests

Substances Used During Cell Culture

Lipid Concentrate Identity

1. Information Requested

In the August 23, 2023, amendment, you report replacement of the "… chemically defined mixture of various lipids prepared by a third-party supplier with an "in-house" version prepared by Mission Barns itself with the same ingredients". Please provide, for addition to the disclosable safety narrative, additional information about the lipid mixtures used during cell culture as follows:

a. Confirmation that the "chemically defined mixture of various lipids prepared by a third-party supplier" referenced in your August 23, 2023, amendment is referring to the "chemically defined lipid concentrate" manufactured by Sigma-Aldrich that was identified in your March 16, 2022, submission.
b. Identity of the components, certificates of analysis for the components, and levels of the components in the "in-house" version of lipids prepared by Mission Barns used in the manufacture of the three batches of cultivated pork fat cells presented in your August 23, 2023, amendment.
c. Further detail as to when the "in-house" version of lipids prepared by Mission Barns is incorporated into the manufacturing process for the cultivated pork fat cells.

Mission Barns' Response:

a. Yes, Mission Barns hereby confirms that the "chemically defined mixture of various lipids prepared by a third-party supplier" referenced in our August 23, 2023, amendment is referring to the "chemically defined lipid concentrate" that was identified in our March 16, 2022, submission.[1]

[redacted]

[1] [redacted]

Product Safety Assessment

Fatty Acid Profile

2. Information Requested

The analytical method used to provide fatty acid profile data in the March 16, 2022, submission differs from the method used in the August 23, 2023, amendment. AOCS CE 1F-96 was used for the March 16, 2022, submission, whereas AOCS CE 1J-07 was used for the August 23, 2023, amendment. For addition to the disclosable safety narrative, please explain why different analytical methods were used for the two different analyses. Further, please confirm that the new batch analyses presented in the August 23, 2023, amendment were carried out on batches manufactured using the new "in-house" lipid mixture.

Mission Barns' Response:

AOCS CE 1F-96 has been declared obsolete by the AOCS Uniform Methods Committee.[2] As such, Mission Barns employed the current AOCS method (i.e., AOCS CE 1J-07) for the fatty acid profile data presented in the August 23, 2023, amendment and will use it in substitute of AOCS CE 1F-96 for future fatty acid profile testing. We also confirm that the new batch analyses presented in the August 23, 2023, amendment were carried out on batches manufactured using the new "in-house" lipid mixture.

[2] American Oil Chemists' Society (AOCS). AOCS SURPLUS Method Ce 1f-96. Available at https://www.aocs.org/attain-lab-services/methods/methods/search-results?method=111773&SSO=True (accessed on 9/26/2023).

3. Information Requested

On page 11 of the June 5, 2023, amendment, in response to FDA's Question 7, you indicate that your cell culture product contains only 5.32% fat, on average. This concentration of fat is also incorporated into your exposure estimates for elaidic acid and nervonic acid (found on pages 7 and 8 of the June 5, 2023, amendment). The exposure estimates presume a 90th percentile pork fat consumption of 16.7 g/person (p)/d based on data obtained from the 2005-2010 WWEIA-Food Commodity Intake Database (FCID). The use of 16.7 g/p/d seems appropriate; however, we do not agree with the inclusion of the 5.32% factor in the calculation. If your cell culture product will be used as a replacement for animal fat in food products, which implies a one-to-one ratio of fat replacement, it does not seem appropriate to include the 5.32% factor in your exposure calculations. If you disagree with our conclusion, please discuss why you believe it is appropriate to include the 5.32% factor in your exposure estimates for elaidic and nervonic acid. Alternatively, please provide updated exposure assessments which do not include the 5.32% factor and which take into account the updated elaidic acid and nervonic acid levels from the August 23, 2023, amendment.

Mission Barns' Response:

As discussed in the June 5, 2023, amendment, Mission Barns respectfully submits that the inclusion of the 5.32% factor is appropriate in the exposure estimates for elaidic and nervonic acid. However, we present updated exposure assessments below, which do not include the 5.32% factor, and which take into account the updated elaidic acid and nervonic acid levels from the August 23, 2023, amendment. Without the 5.32% factor, the calculation represents the conservative "worst-case" scenario, which assumes that 100% of the composition of Mission Barns cultivated pork fat cells is fat.

Elaidic acid

Average level of updated elaidic acid reported in the August 23, 2023, amendment =

$$\frac{(0.7\ g\ +\ 0.6\ g +\ 0.7\ g)/3}{100\ g\ fat} = \frac{0.67\ g\ elaidic\ acid}{100\ g\ fat}$$

90th percentile EDI, "worst-case" scenario assuming cultivated pork fat cells are 100% fat

$$\frac{0.67\ g\ elaidic\ acid}{100\ g\ fat} \times \frac{16.7\ g\ cultivated\ pork\ fat\ cells}{day} = \frac{112\ mg\ elaidic\ acid}{day}$$

Nervonic acid

Average level of updated nervonic acid reported in the August 23, 2023, amendment =

$$\frac{(1.9\ g\ +\ 1.7\ g +\ 1.9\ g)/3}{100\ g\ fat} = \frac{1.83\ g\ nervonic\ acid}{100\ g\ fat}$$

90th percentile EDI, "worst-case" scenario assuming cultivated pork fat cells are 100% fat

$$\frac{1.83\ g\ nervonic\ acid}{100\ g\ fat} \times \frac{16.7\ g\ cultivated\ pork\ fat\ cells}{day} = \frac{306\ mg\ nervonic\ acid}{day}$$

The safety of nervonic acid is discussed further in our response to information request #4 below.

With respect to the dietary exposure of elaidic acid, as discussed in our response to information request #7 below, we propose a specification of ≤ 0.7 g total trans fat / 100 g harvested cultivated pork fat cells. This ensures any dietary exposure to trans fats from our intended use would be comparable to or lower than the consumption of conventional pork fat.

4. Information Requested

Please discuss the safety of nervonic acid and provide a safe dietary intake level. In the absence of traditional animal safety data on nervonic acid, at a minimum, this discussion should include the following data and information (additional data may be included):

Mission Barns' response:

As discussed in our response to information request #6 of our June 5, 2023 amendment, nervonic acid is present in many commonly consumed foods. As further discussed below, even assuming a "worst-case" scenario where 100% of Mission Barns cultivated pork fat cells are composed of fat, the resulting daily intake of 5.1 mg/kg bw/day of Mission Barns cultivated pork fat cells allows for an appropriate margin of safety based on the available toxicological data, and, therefore, does not present any human safety risk.

Below, we further discuss the safety of nervonic acid by responding to each of the agency's information requests.

a. Any safety data from efficacy studies on nervonic acid. Please do not discuss the efficacy results.

Mission Barns' response:

In the following table, we have summarized the safety discussion from several published efficacy studies on the application of nervonic acid to animals. No special toxicity effects were identified in these studies.

Subjects	Test Material and Study Duration	Summary of Safety Data/Discussion	Reference
C57Bl/6J mice	6 g/kg nervonic acid (0.6%) in an isocaloric normal or high fat diet for 12 weeks	Average food consumption between isocaloric normal and high fat diet supplemented with nervonic acid were similar to control over 12 weeks. Dietary nervonic acid supplementation "did not have an apparent effect on diet absorption."	Keppley et al. (2020)[3]

[3] Keppley, L. J. W., Walker, S. J., Gademsey, A. N., Smith, J. P., Keller, S. R., Kester, M., & Fox, T. E. (2020). Nervonic acid limits weight gain in a mouse model of diet-induced obesity. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 34(11), 15314–15326.

		The authors "did not observe any overt toxicities of animals fed a [nervonic acid]-enriched diet (eg [sic], ruffled fur, anorexia, cachexia, skin tenting, skin ulcerations, diarrhea, or death)."	
C57Bl/6 male mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced parkinsonism motor disorder	0, 20, 40, or 60 mg/kg bw nervonic acid via gavage for 10 days	Administration of nervonic acid at different doses did not impact levels of serum AST or ALT (markers to evaluate liver function) in male mice compared with controls. Liver and kidney histopathology analysis found no statistical difference among the treatment and control groups. The authors stated that nervonic acid "has no toxic effects on the mouse liver and kidney, even at the highest dose" of nervonic acid (60 mg/kg).	Hu et al. (2021)[4]
C57Bl/6 mice with experimental autoimmune encephalomyelitis	0, 197, 394, or 788 mg/kg bw nervonic acid via intragastric administration for 17 days	The authors noted that there were no unplanned animal deaths associated with nervonic acid treatment.	Liu et al. (2021)[5]
C57Bl/6 male Alzheimer's model mice	0, 10.95, or 43.93 mg/kg bw nervonic acid via gavage for 3 weeks.	No adverse events or unplanned animal deaths were noted.	Aihaiti et al. (2023)[6]
C57Bl/6J mice with dextran sodium sulfate-induced inflammation	5, 50, or 100 mg/kg bw nervonic acid via gavage for 7 days	Results demonstrated that nervonic acid concentrations lower than 50 µM had no statistically significant toxicity to RAW264.7 cells *in vitro*, in terms of cell viability. No adverse events or unplanned animal deaths were noted. The authors concluded that nervonic acid is a "natural and safe food resource."	Yuan et al. (2023)[7]

Based upon the studies summarized in the tables above, nervonic acid appears to be well-tolerated by mice at oral doses up to 0.6% of diet in studies of 12 weeks (Keppley et al., 2020) and up to 788 mg/kg bw/day for 14 days (Liu et al., 2021).

b. Read-across for other monounsaturated (cis) omega-9 fatty acids such as erucic acid, the safety of which was evaluated by EFSA and for which an ADI was established. If using an ADI and safety data from a read-across substance to support the safety discussion of nervonic acid, please briefly justify your choice of read-across substance.

[4] Hu, D., Cui, Y. & Zhang, J. (2021). Nervonic Acid Ameliorates Motor Disorder in Mice with Parkinson's Disease. *Neurochem. J.* 15, 317–324.

[5] Liu, S., Sun, H., Zhou, Q., Yu, G., Qin, D., Ma, Q. Nervonic acid regulates the oxidative imbalance in experimental allergic encephalomyelitis. *Food Science and Technology Research*, 27 (2), 269–280 (2021). https://www.jstage.jst.go.jp/article/fstr/27/2/27_269/_article

[6] Aihaiti, M., Shi, H., Liu, Y., Hou, C., Song, X., Li, M., Li, J. (2023). Nervonic acid reduces the cognitive and neurological disturbances induced by combined doses of D-galactose/AlCl3 in mice. *Food Science & Nutrition*.

[7] Yuan, S. N., Wang, M. X., Han, J. L., Feng, C. Y., Wang, M., Wang, M., Sun, J. Y., Li, N. Y., Simal-Gandara, J., & Liu, C. (2023). Improved colonic inflammation by nervonic acid via inhibition of NF-κB signaling pathway of DSS-induced colitis mice. *Phytomedicine: international journal of phytotherapy and phytopharmacology*, 112, 154702.

Mission Barns' response:

Nervonic acid is a monounsaturated very long-chain fatty acid (VLCFA). VLCFAs are constituents of many cellular lipids, including sphingolipids and glycerophospholipids, and also serve as precursors to lipid mediators. They account for ~1-5% of the fatty acids in most tissues.[8]

Monounsaturated fatty acids (MUFAs), including nervonic acid, have a single carbon-carbon double bond. Longer chain fatty acids can be formed endogenously through an elongation process where two carbons are added to the carboxyl end of a fatty acid. Elongation of monounsaturated fatty acids begins with oleic acid (C18:1), which can be converted to gondoic acid (C20:1), which can be in turn converted to erucic acid (C22:1), which can then be elongated to nervonic acid.[9] A schematic of the chain shortening and elongation process is shown below (**Figure 1**).

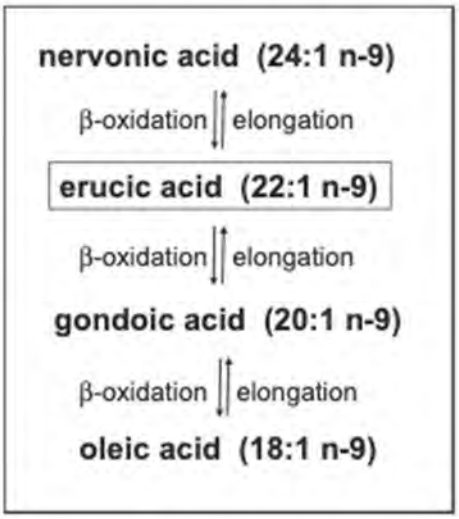


Figure 1.

Given nervonic acid's structural similarity to other MUFA's, and especially erucic acid[10], which can be elongated to nervonic acid[11], a read-across approach can be used to assess the safety of nervonic acid based on the available safety data of erucic acid (as depicted in **Figure 2** below).

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[8] Boles, D. J. and Rizzo, W. B. (1992) Dietary fatty acids temporarily alter liver very long-chain fatty acid composition in mice. *The Journal of nutrition*, 122(8), pp. 1662-1671.
[9] Sassa, T. and Kihara, A. (2014) Metabolism of very long-chain fatty acids: genes and pathophysiology. *Biomolecules & therapeutics*, 22(2), pp. 83.
[10] National Center for Biotechnology Information. PubChem Compound Summary for CID 5281116, Erucic Acid. Available at https://pubchem.ncbi.nlm.nih.gov/compound/5281116 (accessed 9/26/2023).
[11] National Center for Biotechnology Information. PubChem Compound Summary for CID 5281120, Nervonic Acid. Available at https://pubchem.ncbi.nlm.nih.gov/compound/5281120 (accessed 9/26/2023).

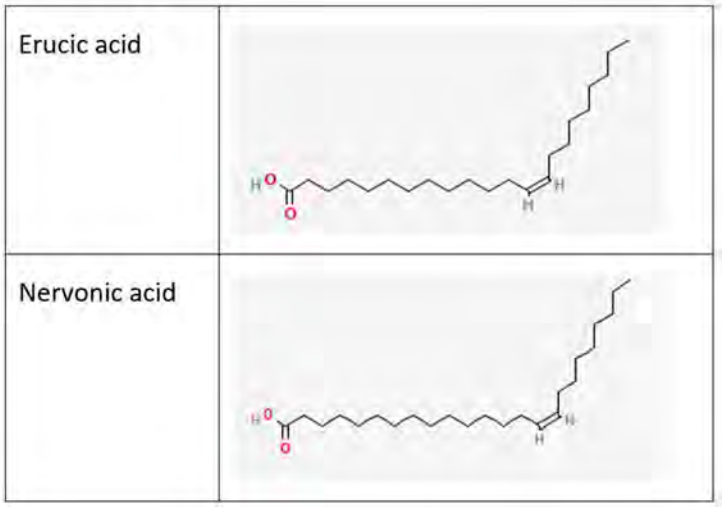

Figure 2.

c. Metabolism data on nervonic acid. (No discussion on the metabolism of nervonic acid in rare disease states is needed; this discussion should focus on healthy individuals). If using safety data for a read-across substance to support the safety of nervonic acid, please make sure to discuss the metabolism of the read-across substance and whether it is similar to the metabolism of nervonic acid.

Mission Barns' response:

In their Scientific Opinion on dietary reference values for fats, EFSA noted that like other fatty acids, MUFAs including nervonic acid and erucic acids are almost completely absorbed in the intestine.[12] From there, these fatty acids are predominantly transported to the liver where they are then oxidized for energy production, converted to other fatty acids, or incorporated into tissue lipids.

A study by Carroll (1962) investigated the metabolism of ^{14}C-labeled nervonic and erucic acids in rats.[13] Radiolabeled fatty acids were administered by mouth and injection of the tail-vein and distribution in radioactivity in various body tissues were evaluated at intervals 6.5 and 24 hours post-administration. Nervonic and erucic acids were observed to be predominantly present in the gastrointestinal tract and in the liver at both measured time points and at comparable levels. Similarly, activity of respiratory CO_2 was measured in 15 minute intervals for a period of 6 hours following administration, and showed similar rates of oxidation for both erucic and nervonic acid.

In all, the studies we have identified indicate that nervonic acid will follow a similar absorption, metabolism, and distribution pathway as erucic acid.

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[12] EFSA (2010) 'Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol', Efsa Journal, 8(3), pp. 1461.

[13] Carroll, K. K. (1962). Levels of radioactivity in tissues and in expired carbon dioxide after administration of 1-C14-labelled palmitic acid, 2-C14-labelled erucic acid, or 2-C14-labelled nervonic acid to rats. *Canadian Journal of Biochemistry and Physiology* 40.9: 1229-1238.

d. Compare what is known about the metabolic fate and site of metabolism of nervonic acid in humans and animals describing the similarities and differences. Explain the significance of findings.

Mission Barns' response:

It has been demonstrated that both saturated and unsaturated (e.g., nervonic acid) VLCFAs undergo β-oxidization in rodent and human peroxisomes.[14] Nervonic acid was observed to be at levels 5 times higher in peroxisomes than in mitochondria in rat liver homogenate, which indicates preferential oxidation in the peroxisome. In normal human cultured skin fibroblasts, the mitochondrial β-oxidization-inhibitor, etomoxir, had no effect on oxidation of VLCFAs but did inhibit oxidation of shorter chain fatty acids which occur in mitochondria, further supporting the conclusion that peroxisomes are the site of VLCFA oxidation. Given that defects in peroxisomal β-oxidization in humans results in deficient nervonic acid oxidation, it can be concluded that nervonic acid oxidation also occurs in the peroxisome in humans. The observed similarity between β-oxidization in rodent and human peroxisomes indicates that nervonic acid metabolism is similar in humans and animals.

e. When calculating a margin of safety (MOS), it is customary to use a safety factor of 100 (10 for intraindividual variation and 10 for intraspecies variation). Please state your opinion on whether you think that during the calculation of a MOS between the intake of nervonic acid from your product and a NOAEL for a read-across substance, the use of 3 instead of the customary 10 for interspecies variation would be adequate/justifiable considering that nervonic acid is metabolized similarly in humans and animals.

References:
EFSA Panel on Contaminants in the Food Chain (CONTAM), Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., ... & Vleminckx, C. (2016). Erucic acid in feed and food. *EFSA Journal*, *14*(11), e04593.
Sandhir, R., Khan, M., Chahal, A., & Singh, I. (1998). Localization of nervonic acid β-oxidation in human and rodent peroxisomes: impaired oxidation in Zellweger syndrome and X-linked adrenoleukodystrophy. *Journal of Lipid Research*, *39*(11), 2161-2171.

Mission Barns' response:

Use of safety factors is based on the observation that toxic substances usually have thresholds below which toxic effects cannot be detected. The safety factor attempts to account for differences between animals and humans and differences in sensitivity among humans. While it is customary to use a safety factor of 100, exceptions are permitted in special circumstances where the available data may support a smaller safety factor. As discussed above, nervonic acid is a common fatty acid in foods, and data reviewed indicate nervonic acid metabolism is similar in humans and animals. As such, the use of 3 instead of the customary 10 for interspecies variation would be adequate/justifiable, and a safety factor of 30 can be developed.

[14] Sandhir, R., Khan, M., Chahal, A. and Singh, I. (1998). Localization of nervonic acid β-oxidation in human and rodent peroxisomes: impaired oxidation in Zellweger syndrome and X-linked adrenoleukodystrophy. *Journal of Lipid Research*, 39(11), pp. 2161-2171.

Following a comprehensive assessment of the available data in 2016, EFSA established a tolerable daily intake (TDI) of 7 mg/kg bw/day for erucic acid, which was based upon a NOAEL for lipidosis of 0.7 g/kg bw per day, observed in a 7-day feeding study in young rats and in a 2-week feeding study in newborn piglets.[15]

Assuming the "worst-case" scenario where cultivated pork fat cells are composed of 100% fat, the 90th percentile daily intake for nervonic acid from Mission Barns cultivated pork fat cells (calculated as 306 mg per day of nervonic acid in the response to information request #3 above) for a typical US adult body weight of 60 kg can be calculated as:

306 mg per day of nervonic acid ÷ 60 kg = 5.1 mg/kg bw/day

In light of the various conservative assumptions we have made, we expect the actual dietary exposure level to be much lower than 5.1 mg/kg bw/day. However, even using the 5.1 mg/kg bw/day as the dietary exposure for nervonic acid, and using the NOAEL for erucic as a read-across for nervonic acid, the MOS for 90th percentile intake can be calculated as:

0.7 g/kg bw/day ÷ 5.1 mg/kg bw/day = 137 > MOS of either 100 or 30

As such, the dietary intake of nervonic acid from the intended use of cultivated pork fat cells does not pose any human safety concern.

Product Characterization

Compositional Analysis

5. Information Requested

For addition to the disclosable safety narrative, please provide references for comparator data on proximates (e.g., moisture, protein, fat, ash). Please discuss differences between the levels of proximates, amino acids, minerals and vitamins reported in reference data for conventional comparators in relation to the levels found in independent batches of the harvested cell material. Please discuss whether such differences present food safety concerns.

Mission Barns' Response:

References for comparator data on proximates for conventional pork fat are presented in the following table:

[15] EFSA Panel on Contaminants in the Food Chain (CONTAM), Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., ... & Vleminckx, C. (2016). Erucic acid in feed and food. EFSA Journal, 14(11), e04593.

Parameter	Units	Mission Barns' cultivated pork fat cells (Average of 3 batches)	Conventional pork fat[16]
Moisture	g/100g	88.65	4.09 - 38.3
Fat	g/100g	5.32	52.3 - 94.2
Protein	g/100g	5.08	1.76 - 9.34
Ash	g/100g	0.96	0.1 - 0.72
Carbohydrates	g/100g	0.65	0[17]

As the above table indicates, moisture content is higher in cultivated pork fat cells compared to conventional pork fat, resulting in relatively lower levels of fat and protein in cultivated pork fat cells. A higher proportion of water content and the lower proportion of fat and protein in the cultivated pork fat cells does not present any food safety concerns. Levels of ash and carbohydrates are comparable to conventional pork fat and do not present any food safety concern.

Analytical data on amino acids, vitamins and minerals in cultivated pork fat cells and a comparison to the levels present in conventional pork fat were presented in our response to information request #7 of the June 5, 2023, amendment. Such data show that nutrient concentrations in cultivated pork fat cells are comparable to conventional pork fat. Vitamins and minerals that were present at concentrations slightly higher than the range typically reported for conventional pork fat (namely, thiamin (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), vitamin E, manganese, phosphorus and sodium) are all well within the daily values recommended by the FDA and do not present any food safety concern.[18]

6. Information Requested

In Table 3 of the May 25, 2023, submission, you indicate that your cultivated pork cells will be used in ground meat (up to 20%), formed products such as burgers and meatballs (up to 30%), and encased products such as hot dogs and sausages (up to 40%). Please provide, for addition

[16] Reported ranges are the lowest and highest reported value from USDA FoodData Central for NDB Number 10004 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167811/nutrients, accessed 9/22/2023), NDB Number 10006 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167813/nutrients, accessed 9/22/2023), NDB Number 10942 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169179/nutrients, accessed 9/22/2023), NDB Number 10109 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167861/nutrients, accessed 9/22/2023), and NDB Number 10007 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/168221/nutrients, accessed 9/22/2023), NDB Number 10005 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167812/nutrients, accessed 9/22/2023).

[17] Carbohydrate content is determined by calculating the remainder of 100% following the subtraction of moisture, fat, protein and ash. In cases where moisture, fat, protein and ash sum to 100% or more, carbohydrates are reported as "0%".

[18] U.S. Food and Drug Administration (FDA). Daily Value on the New Nutrition and Supplement Facts Labels. Available at https://www.fda.gov/food/new-nutrition-facts-label/daily-value-new-nutrition-and-supplement-facts-labels (accessed 9/22/2023).

to the disclosable safety narrative, information about the expected fat content in these products resulting from inclusion of your cultivated pork cells in these products.

Mission Barns' Response:

To clarify, Mission Barns' estimated daily consumption for cultivated pork fat cells for the purpose of its safety assessment is based on the conservative assumption that Mission Barns cultivated pork fat cells will replace 100% of all conventional pork fat consumed by US consumers, not the pork fat in any specific product. Cultivated pork fat cells are intended to be included in each product up to its respective amount previously referenced (i.e., 20% for ground meat, 30% for formed products such as burgers and meatballs, and 40% for encased products such as hot dogs and sausages). If the cultivated pork fat cells were 100% fat, the products would contain up to 20% fat for ground meat, 30% fat for formed products, and 40% fat for encased products; in practice, products may contain less fat than these upper limits.[19]

Specification for trans fat

7. Information Requested

Batch analysis of three lots of the harvested cell material in your August 23, 2023, submission showed elaidic acid (18:1 trans) levels of 0.7%, 0.6%, and 0.7%, and total trans fat levels of 0.7%, 0.7%, and 0.7%. These levels appear to be comparable to levels of elaidic acid and total trans fat found in conventional pork fat. FDA has concerns if the levels of elaidic acid or total trans fat were higher than those found in conventional pork fat. One way to address this concern would be to add a specification for total trans fat in the fat derived from the harvested cell material. We request that you propose a specification for trans fat in your harvested cell material. Please provide a justification for the level chosen for the trans fat specification.

Mission Barns' Response:

Mission Barns hereby proposes a specification of: ≤ 0.7 g total trans fat / 100 g harvested cultivated pork fat cells. This specification is appropriate because it ensures that the total trans fat levels in harvested cultivated pork fat cells are comparable to or lower than the levels found in conventional pork fat, even in the "worst-case" scenario where cultivated pork fat cells were composed of 100% fat. Given that the cultivated pork fat cells are not 100% fat, it is expected that Mission Barns' harvested cultivated pork fat cells will not exceed this specification.

Adventitious Agent Hazard Analysis and Testing

8. Information Requested

On page 17 of the June 5, 2023, amendment to the disclosable safety narrative, you clarify that you consider *Staphylococcus* spp. as a potential biological hazard during the production process. You state the risk of *Staphylococcus* spp. contamination from human-sources is controlled through measures such as hygiene training, the use of PPE, and aseptic techniques. You also state that aerobic plate count (APC) testing is conducted under the environmental monitoring program (EMP) and as part of the batch release testing. You also state, "While APC

[19] Please note plant-based fats and oils commonly used as food ingredients may also supplement cultivated pork fat cells, but Mission Barns expects the overall fat levels to still be within these specified limits.

testing does not specifically target *Staphylococcus*, it covers a broad range of microorganisms including *Staphylococcus*. Following any presumptive positive test result for APC from EMP testing, Mission Barns sterilizes the affected area following standard operating procedures (SOPs) and resamples the area following sterilization. Following any presumptively positive test result from APC testing of spent media, Mission Barns performs further analysis to identify the species of the microbe(s), using methods such as gene sequencing (e.g., QA-0095-3000 GeneSeq) and/or mass spectroscopy (e.g., MALDI-TOF)".

For addition to the disclosable narrative, please provide the following:

a. Your rational for using a non-specific microbial test (i.e., APC) to test for a specific microbial hazard you have identified (i.e., *Staphylococcus* spp.) as a risk in your production process, in lieu of direct testing and specifications for the identified hazard.
b. A discussion of whether you consider the entire genus of *Staphylococcus* to be a hazard, or whether you have identified any specific species (e.g., *S. aureus*) as a hazard in your production process.
c. Clarification as to whether the additional testing regiment for APC plates is triggered by a "presumptive positive", as stated on page 7 of the June 5, 2023, amendment, or by "non-conforming batches that fail to pass the microbial testing plan acceptance criteria", as stated on 18 of the same amendment (i.e., if the result fails to meet the APC specification (negative (<10 CFU/mL)) provided on page 19 of the same amendment). We note that "presumptive positive" is not an appropriate term for a non-specific APC test, as "presumptive positive" is used when a microbial test detects an organism that may be the target of interest.

Mission Barns' Response:

a. The APC test can detect *Staphylococcus* organisms because the growth conditions, including the use of suitable growth media, incubation temperature, and observation of colony morphology, create an environment where *Staphylococcus* species can thrive and form colonies. As such, while APC testing does not specifically target *Staphylococcus*, APC testing effectively serves as an indirect screen for the presence of *Staphylococcus* organisms. In the event that any APC test result reports a value > 10 CFU/mL, a secondary microbial identification test is conducted to identify the presence of any potential pathogens of concern in the sample, including *Staphylococcus aureus*.

b. Mission Barns would like to clarify that the species *Staphylococcus aureus* is the only species in the genus identified in its hazard assessment to be a potential hazard in the manufacturing process. While a number of species of Staphylococcus can originate from the environment and human sources, *S. aureus* is widely considered the most significant pathogenic species within the genus due to its frequent association with foodborne illness and various types of human infections. For accuracy and clarity, Mission Barns has updated its hazard analysis and risk-based controls (HARPC) plan to specify the *Staphylococcus aureus* species as the microbial hazard of concern.

c. Mission Barns agrees with the agency that "presumptive positive" is not an appropriate term for a non-specific microbial detection test such as APC. The company would like to clarify that additional testing following a positive APC result (i.e., for species identification) is triggered whenever the result fails to meet the APC specification (negative (<10 CFU/mL)), as described on page 19 of the June 5, 2023, amendment to the disclosable safety narrative.

Points of Clarification

Additional Information about Cell Growth Conditions

9. Information Requested

The March 16, 2022, supplementary confidential material describes the cell line selection process and lists two adaptations selected for during the transition to serum-free media, the ability to grow in serum-free media and a second adaptation. For addition to the disclosable safety narrative, please describe the second adaptation of the cell line for *in vitro* culture conditions.

Mission Barns' Response:

Mission Barns respectfully submits that the only "adaptation" that occurs during the cell banking process is the transition to serum-free media. At all points in cell culture during and after the transition to serum-free media, food-grade coating reagents are introduced to cell culture vessels to support cell adhesion. However, prior to the transition to serum-free media, no coating reagents are required because serum supplies the functional biomolecules necessary for cell adhesion to culture vessels.

Substantive Information Requests

Substances Used During Cell Culture

Lipid Concentrate Identity

Product Safety Assessment

Fatty Acid Profile

2. Information Requested

In the September 26, 2023, amendment, you proposed a specification of ≤ 0.7 g total trans fat/100 g

harvested cell material. You indicated that you selected this level because it would be comparable to or lower than the levels found in conventional pork fat.

It is our opinion that to be comparable to or lower than the levels found in conventional pork fat, the specification should be based on the level of total trans fat on a fat content basis, and not on the harvested cell material. Thus, we would interpret the specification as ≤ 0.7 g total trans fat/100 g fat. Please comment on the appropriateness of a specification for total trans fat of ≤ 0.7 g total trans fat/100 g fat.

In addition, we note that the USDA Food Data Central entry NDB 10006 (Pork, fresh, separable fat, raw) indicates a level of 0.6 g total trans fatty acids/100 g. This corresponds to a level of 0.9 g total trans fatty acids/100 g fat when taking into account the presence of 65.7 g fat/100 g "Pork, fresh, separable fat, raw. The level of total trans fatty acids expressed on a total fat basis may inform your decision regarding a specification for total trans fatty acids on a total fat basis.

Mission Barns' Response:

Mission Barns believes that a specification of ≤1 g total trans fat / 100 g fat, which is based on the fat content, is appropriate to ensure the total trans fat in cultivated pork fat cells is comparable to the levels found in conventional pork.

As noted by the Agency, USDA Food Data Central entry NDB 10006 (Pork, fresh, separable fat, raw) indicates a level 0.9 g trans fatty acids / 100 g fat when taking into account the presence of 65.7 g fat/100 g "Pork, fresh, separable fat, raw". Similarly, USDA Food Data Central entry NDB 10942 (Pork, fresh, composite of separable fat, with added solution, raw) indicates a level of 0.579 g total trans fatty acids / 100 g, which corresponds to 1.1 g trans fatty acids / 100 g fat when taking into account the presence of 52.3 g fat/100 g "Pork, fresh, composite of separable fat, with added solution, raw". A specification of ≤1 g total trans fat / 100 g fat ensures that the levels of trans fat in the fat content of cultivated pork fat cells will be comparable to the range of total trans fat levels present in conventional raw pork fat (i.e., 0.9 g / 100 g fat - 1.1 g / 100 g fat) as reported by USDA Food Data Central data.

Product Characterization

Adventitious Agent Hazard Analysis and Testing

3. Information Requested

In response to question 8a in the September 26, 2023, amendment, you state "The APC test can detect *Staphylococcus* organisms because the growth conditions, including the use of suitable growth media, incubation temperature, and observation of colony morphology, create an environment where *Staphylococcus* species can thrive and form colonies." While the growth parameters (e.g., time, temperature, media) of an APC test may allow for the growth of *Staphylococcus aureus*, it may be difficult to distinguish colonies from those of other microorganisms that may be present. Further, it is inaccurate to characterize a non-specific APC test as being appropriate as the sole test used for the detection of a specific organism (i.e., *S. aureus*).

You explain that, if the results of the APC testing do not conform to the set specification for APC (<10 CFU/mL), you perform further analyses to identify the species of the microorganism(s) (using methods

such as gene sequencing (e.g., QA-0095-3000 GeneSeq) and/or mass spectroscopy (e.g., MALDI-TOF), as noted in the June 5, 2023, amendment).

That said, in your response to question 8b you state "Mission Barns has updated its hazard analysis and risk-based controls (HARPC) plan to specify the *Staphylococcus aureus* species as the microbial hazard of concern." Therefore, further analyses to identify the species of the microorganism(s) captured using the APC test is only performed if the APC test does not conform to the set specifications, meaning that specific testing for the presence of *S. aureus* may not be performed despite it being identified as an adventitious agent of concern in your production process.

a. As you have identified *S. aureus* as an adventitious agent of concern in your production process, we request a specification for *S. aureus* that utilizes a test specifically designed to detect this microorganism, such as the method describe in the FDA's *Bacteriological Analytical Manual* accessible at https://www.fda.gov/food/laboratory-methods-food/bam-chapter-12- staphylococcus-aureus.

Mission Barns' Response:

Mission Barns hereby sets the following *Staphylococcus aureus* testing specification to its harvested cultivated pork fat cell batch release criteria.

Mission Barns' Cultivated Pork Fat Cells Batch Release Criteria		
Test	**Method**	**Specification**
Staphylococcus aureus	FDA BAM, 8th ed., AOAC 2003.07-2006, or equivalent	< 10 CFU/g

Substantive Information Requests

Product Characterization

Adventitious Agent Hazard Analysis and Testing

Information Requested

In your October 26, 2023, amendment you provide a specification for *Staphylococcus aureus*. Please provide results from a minimum of three batches (preferably non-consecutive) to demonstrate that your product can be manufactured to meet the provided *S. aureus* specification listed in your October 26, 2023, amendment.

Mission Barns' Response:

The results from Mission Barns' new *Staphylococcus aureus* testing from the three non-consecutive batches are provided in the table below:

Mission Barns' Cultivated Pork Fat Cells Batch Release Criteria				
Test	**Specification**	**Batch 1**	**Batch 2**	**Batch 3**
Staphylococcus aureus	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g

Request for Additional Information re: CCC 000008

Substantive Information Requests

Product Characterization

Adventitious Agent Hazard Analysis and Testing

1. Information Requested

In your October 26, 2023, amendment you stated that your testing method for *Staphylococcus aureus* is "FDA BAM, 8th ed., AOAC 2003.07-2006, or equivalent". We request clarification on a few items related to this test:

a. Please name the method used to generate the batch analysis data on *S. aureus* provided in your October 31, 2023, amendment.
b. Please provide the Certificates of Analysis (COAs) for the batch data.
c. Regarding your statement that you may use "equivalent" testing methods, please provide additional detail about the source of these methods. Do you plan to only select from validated methods and, if so, please state the methods. If these other methods are in house methods, we request that you confirm they have been validated and are fit-for-purpose.

Mission Barns' Response:

a. The method used to generate the batch analysis data on *S. aureus* provided in our October 31, 2023 amendment was AOAC 2003.07-2006.
b. Certificates of analysis for the batch data presented in our October 31, 2023 amendment are included as Attachment 1.[1]
c. Mission Barns clarifies that as of today it has not identified any validated methods other than FDA BAM, 8th ed. and AOAC 2003.07-2006 to test for *S. aureus*. The inclusion of "or equivalent" testing methods in our October 31, 2023 amendment was intended to capture future validated methods that may be developed (e.g., newer editions of FDA BAM or AOAC method). Mission Barns confirms that any method used to test for *S. aureus* will be validated and fit-for-purpose. Unless any future validated method is identified, Mission Barns intends to only use FDA BM 8th ed. or AOAC 2003.07-2006 for the testing of *S. aureus.*

2. Information Requested

In your October 31, 2023, amendment you provide results from three non-consecutive batches of your product. Please confirm if these tests were performed on freshly produced batches or on batches that had been frozen. If these batch analyses were performed on frozen batches, please indicate if all batches will be frozen in a similar manner prior to sale. If not, please provide a brief discussion on the impact that testing these frozen batches, in contrast to testing fresh batches, may have on your safety assessment.

Mission Barns' Response:

To clarify, the *S. aureus* results that were provided in our October 31, 2023 amendment were produced by testing the spent media from each of the three non-consecutive batches. All spent media samples tested were not frozen at any time. As discussed in response #13 of our June 5, 2023 amendment, Mission Barns utilizes spent media in contact with cells immediately prior to harvest for all of its fungal and bacterial batch release testing, including for *S. aureus*.

3. Information Requested

Staphylococcus aureus may produce heat resistant toxins and is identified as a potential pathogen of concern in your production process. Further, there are reports in the literature that growth of *S. aureus* and production of staphylococcal enterotoxins may be decoupled (i.e., active growth of *S. aureus* may not be necessary for enterotoxin production) and staphylococcal enterotoxins are extremely heat

[1] Attachment 1 includes certificates of analysis for 12 batches Mission Barns has tested for *S. aureus* to date. We note that the third party laboratory lists the unit of measure as "/g", which is intended to signify "CFUs/g".

stable.[2,3]

Please describe whether the production of staphylococcal enterotoxins is a safety concern in your production process, providing citations where appropriate. If the production of staphylococcal enterotoxins is identified as a safety concern in your process, please describe the steps used to control for this hazard and provide a data-based narrative discussing why these control measures are appropriate. If you intended to use a specification to monitor this hazard, please provide the test you will use and data showing you can meet the specification, including COAs and test method.

Mission Barns' Response:

Mission Barns does not believe that staphylococcal enterotoxins are a safety concern for our production process. We note that even in studies that find that active growth of *S. aureus* and production of staphylococcal enterotoxins may be decoupled, a viable population of *S. aureus* is required for the production of enterotoxins.[4] Mission Barns has collected more than a year of environmental monitoring program (EMP) data testing for aerobic plate count (APC) and four months of EMP data testing specifically for *S. aureus*.[5] To date, Mission Barns has not identified a single appearance of *S. aureus* in its production environment. In addition, as noted in response #3 of our October 25, 2023 amendment, Mission Barns has incorporated an *S. aureus* testing specification to its cultivated pork fat cells batch release criteria. Mission Barns has subsequently tested 12 batches, each of which has resulted in negative test results for *S. aureus* (see Attachment 1).

Further, as noted in Schelin, J. et al. (2011), *S. aureus* optimal growth conditions are 35-41°C and a pH of 6-7, conditions that are present in Mission Barns' cell culture environment. Given the nutrient-rich environment and the absence of any antimicrobial agents in the cell culture media, any *S. aureus* contamination, if present, is expected to overtake animal cell cultures and be detected in Mission Barns' batch release criteria. Because Mission Barns has taken appropriate measures to assure the absence of *S. aureus* in cell cultures and the production environment, Mission Barns does not believe that staphylococcal enterotoxins produced by *S. aureus* are a food safety concern in our production process.[6]

Fatty Acid Profile

4. Information Requested

In your August 23, 2023, amendment, you provide test result data from three batches for elaidic acid (18:1 trans) in your product. Please provide the COAs.

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[2] Schelin, J. et al. (2011) The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Virulence, 2(6), p. 580-592. doi: 10.4161/viru.2.6.18122

[3] Rall V.L.M., Vieira F.P., Rall R., Vieitis R.L., Fernandes A. Jr, Candeias J.M.G. et al. (2008) PCR detection of staphylococcal enterotoxin genes in *S. aureus* strains isolated from raw and pasteurized milk. Vet. Microbiol. 132, 408–413 10.1016/j.vetmic.2008.05.011

[4] For example, Wallin-Carlquist, Nina et al. (2010) report that detectable levels of *sea* mRNA were observed in the Serrano ham with a decreasing but viable *S. aureus* count, but no *sea* expression or SEA could be detected in a salami where no viable *S. aureus* cells were found after inoculation with *S. aureus* cells. Wallin-Carlquist, Nina et al. "Prolonged expression and production of Staphylococcus aureus enterotoxin A in processed pork meat." International journal of food microbiology vol. 141 Suppl 1 (2010): S69-74.

[5] Mission Barns has collected APC EMP data from September 2022 to present and *S. aureus* EMP data from July 2023 to present.

[6] As detailed in response #11 of our June 5, 2023 amendment, Mission Barns included *S. aureus* as a potential microorganism of concern out of an abundance of caution due to the presence of human technicians during the production process. As discussed, Mission Barns has incorporated extensive preventive controls throughout the production process to mitigate risks of microbial contamination, including risks associated with *S. aureus*. All operations are conducted by technicians utilizing aseptic techniques, wearing appropriate personal protective equipment (e.g., gowns, hairnets, masks and gloves) in a positive air pressure filtration environment and under a Class II BSC or equivalent environment.

Mission Barns' Response:

Certificates of analysis for the fatty acid profile for the batch data presented in our August 23, 2023 amendment are included as Attachment 2.[7]

[7] Mission Barns notes that the third party laboratory reports total trans fats rounded up to the nearest whole percent. Consequently, Attachment 2 reports a total trans fat of 1% for the three batches, even though elaidic acid is the sole trans fat detected and is present at below 1%.

Mérieux NutriSciences

CERTIFICATE OF ANALYSIS

SILLIKER, Inc.

Salida, CA Laboratory

5262 Pirrone Court, Salida, CA 95368

Tel. 1-844-277-1680 Fax. 209-545-0245

Email: getresults6@mxns.com

COA No:	CCA-47610252-0
Supersedes:	None
COA Date	10/28/23
Page 1 of 4	

TO:

Mr. Richard Kwon

Director of Food Safety

Mission Barns

1155 Bryant Street

San Francisco, CA 94103

Received From:	San Francisco, CA
Received Date:	10/26/23

Location of Test: (except where noted)
Salida, CA

Analytical Results

Laboratory ID: 431099574 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 22A315- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099595 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 22A386- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099598 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 22A479- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099601 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 22A480- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Mérieux NutriSciences

CERTIFICATE OF ANALYSIS

SILLIKER, Inc.

Salida, CA Laboratory

5262 Pirrone Court, Salida, CA 95368

Tel. 1-844-277-1680 Fax. 209-545-0245

Email: getresults6@mxns.com

COA No:	CCA-47610252-0
Supersedes:	None
COA Date	10/28/23
Page 2 of 4	

TO:

Mr. Richard Kwon

Director of Food Safety

Mission Barns

1155 Bryant Street

San Francisco, CA 94103

Received From:	San Francisco, CA
Received Date:	10/26/23

Location of Test: (except where noted)
Salida, CA

Analytical Results

Laboratory ID: 431099603 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 22A520- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099604 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 22A533- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099609 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 23A006- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099612 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2

Sample Name: 23A059- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

SILLIKER, Inc.

Salida, CA Laboratory
5262 Pirrone Court, Salida, CA 95368
Tel. 1-844-277-1680 Fax. 209-545-0245
Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-47610252-0
Supersedes:	None
COA Date	10/28/23
Page 3 of 4	

TO:
Mr. Richard Kwon
Director of Food Safety
Mission Barns
1155 Bryant Street
San Francisco, CA 94103

Received From:	San Francisco, CA
Received Date:	10/26/23

Location of Test: (except where noted)
Salida, CA

Analytical Results

Laboratory ID: 431099614 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2
Sample Name: 23A136- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099626 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2
Sample Name: 23A228- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099633 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2
Sample Name: 23A253- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

Laboratory ID: 431099641 **Condition Rec'd:** NORMAL **Temp Rec'd (°C):** 3.2
Sample Name: 23A782- SA-26OCT2023- 25 mL

Analyte	Result	Units	Method Reference	Test Date	Loc.
* Staphylococcus aureus - Petrifilm	<10	/g	AOAC 2003.07	10/28/23	

SILLIKER, Inc.

Salida, CA Laboratory
5262 Pirrone Court, Salida, CA 95368
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Email: getresults6@mxns.com

CERTIFICATE OF ANALYSIS

COA No:	CCA-47610252-0
Supersedes:	None
COA Date	10/28/23
Page 4 of 4	

TO:
Mr. Richard Kwon
Director of Food Safety
Mission Barns
1155 Bryant Street
San Francisco, CA 94103

Received From:	San Francisco, CA
Received Date:	10/26/23

Location of Test: (except where noted)
Salida, CA

Analytical Results

Julienne Mørtensen Laboratory Director

I Customer supplied information * ISO17025 Accredited Analysis † Indicates reason for COA amendent when applicable

Certificate of Analysis

REPORT #: 1474040
PROJECT ID: NY14216-2308-002
REPORT DATE: 8/18/23
PRINT DATE: 8/18/23

*MISSION BARNS
2618 EIGHTH ST.
BERKELEY, CA 94710
*ATT: RICHARD KWON

LAB #: 4641797

DATE RECEIVED:8/4/23

***PRODUCT:** 22A429
***PACKAGE:** IN SEALED PACKAGE
ARRIVAL TEMPERATURE: -1.0ºC

ANALYTE	RESULT	UNITS	METHOD REFERENCE
FATTY ACID PROFILE			AOCS CE 1J-07
MYRISTIC (14:0)	0.8	% of fat	
PALMITIC (16:0)	12.6	% of fat	
PALMITOLEIC (16:1 CIS)	2.8	% of fat	
STEARIC (18:0)	9.8	% of fat	
OLEIC (18:1 CIS)	58.5	% of fat	
ELAIDIC (18:1 TRANS)	0.7	% of fat	
LINOLENIC (18:3 CIS)	1.5	% of fat	
TOTAL SATURATED	23	% of fat	
TOTAL MONOUNSATURATED	64	% of fat	
TOTAL POLYUNSATURATED	12	% of fat	
TOTAL TRANS	1	% of fat	
METHYL ARACHIDONATE (20:4)	1.7	% of fat	
METHYL EICOSAPENTAENOATE (20:5)	3.7	% of fat	
METHYL NERVONATE (24:1)	1.9	% of fat	

Patrick Christy
Lab Director

*The indicated information has been provided by the client to Certified Laboratories

65 Marcus Drive • Melville • NY • 11747 • 516-576-1400 • www.certified-laboratories.com

Certificate of Analysis

REPORT #: 1474040
PROJECT ID: NY14216-2308-002
REPORT DATE: 8/18/23
PRINT DATE: 8/18/23

*MISSION BARNS
2618 EIGHTH ST.
BERKELEY, CA 94710
*ATT: RICHARD KWON

LAB #: 4641798

DATE RECEIVED:8/4/23

***PRODUCT:** 22A431
***PACKAGE:** IN SEALED PACKAGE
ARRIVAL TEMPERATURE: -1.0ºC

ANALYTE	RESULT	UNITS	METHOD REFERENCE
FATTY ACID PROFILE			AOCS CE 1J-07
MYRISTIC (14:0)	0.8	% of fat	
PALMITIC (16:0)	13.4	% of fat	
PALMITOLEIC (16:1 CIS)	3.3	% of fat	
STEARIC (18:0)	9.6	% of fat	
OLEIC (18:1 CIS)	58.7	% of fat	
ELAIDIC (18:1 TRANS)	0.6	% of fat	
LINOLENIC (18:3 CIS)	1.5	% of fat	
TOTAL SATURATED	24	% of fat	
TOTAL MONOUNSATURATED	64	% of fat	
TOTAL POLYUNSATURATED	11	% of fat	
TOTAL TRANS	1	% of fat	
METHYL ARACHIDONATE (20:4)	1.4	% of fat	
METHYL EICOSAPENTAENOATE (20:5)	3.1	% of fat	
METHYL NERVONATE (24:1)	1.7	% of fat	

Patrick Christy
Lab Director

*The indicated information has been provided by the client to Certified Laboratories

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Certified Laboratories

Full Service Laboratory • Established 1926

Certificate of Analysis

REPORT #: 1474040
PROJECT ID: NY14216-2308-002
REPORT DATE: 8/18/23
PRINT DATE: 8/18/23

*MISSION BARNS
2618 EIGHTH ST.
BERKELEY, CA 94710
*ATT: RICHARD KWON

LAB #: 4641799

DATE RECEIVED:8/4/23

***PRODUCT:** 23A006
***PACKAGE:** IN SEALED PACKAGE
ARRIVAL TEMPERATURE: -1.0ºC

ANALYTE	RESULT	UNITS	METHOD REFERENCE
FATTY ACID PROFILE			AOCS CE 1J-07
MYRISTIC (14:0)	0.8	% of fat	
PALMITIC (16:0)	12.1	% of fat	
PALMITOLEIC (16:1 CIS)	3.3	% of fat	
STEARIC (18:0)	9.0	% of fat	
OLEIC (18:1 CIS)	58.2	% of fat	
ELAIDIC (18:1 TRANS)	0.7	% of fat	
LINOLENIC (18:3 CIS)	1.8	% of fat	
TOTAL SATURATED	23	% of fat	
TOTAL MONOUNSATURATED	65	% of fat	
TOTAL POLYUNSATURATED	11	% of fat	
TOTAL TRANS	1	% of fat	
METHYL ARACHIDONATE (20:4)	1.4	% of fat	
METHYL EICOSAPENTAENOATE (20:5)	3.2	% of fat	
METHYL NERVONATE (24:1)	1.9	% of fat	

Patrick Christy
Lab Director

*The indicated information has been provided by the client to Certified Laboratories

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Request for Additional Information re: CCC 000008

Cell Line Establishment

1) On page 2 of the March 6, 2023, amendment to the disclosable safety narrative, you list tests performed on the donor animals for Mission Barns cell lines, including viral and bacterial screening for Porcine Reproductive & Respiratory Syndrome Virus (PRRS), Transmissible Gastroenteritis Virus (TGV), Influenza A, *Brucella* spp., *Leptospira* spp., Pseudorabies Virus (PSR), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* (APP), and Porcine Epidemic Diarrhea Virus (PEDV) and note that "… the results of these screens are compiled in a test report." You then state, "Source animal health documents are stored as records as part of the company's safety and quality system." For addition to the disclosable safety narrative, please provide copies of the compiled adventitious agent test report as well as the health documents from the donor animal.

Mission Barns' Response:

For addition to the disclosable safety narrative, copies of an adventitious agent test report and health records for a donor animal are provided in Attachment 1, which is representative of the records Mission Barns collects under its current quality management system for tissue isolations conducted after October 27, 2022. Mission Barns notes that for tissue isolations conducted before this date, complete animal health records are not available; however, Mission Barns performed the same adventitious agents testing described on page 2 of the March 6, 2023 amendment to the disclosable safety narrative on cell cultures derived from those donor animals, the results of which are provided in Attachment 2. Mission Barns notes that viral tests performed on donor animals are intended to mitigate adventitious agent risks to cells derived from the tissue isolation. Therefore, directly testing the cells is a suitable alternative to verify the absence of porcine related viruses in Mission Barns cell banks.

2) Table 1 of the May 25, 2022, disclosable safety narrative lists "PCR (Porcine DNA)" as the test method for species verification of the master cell bank. For addition to the disclosable safety narrative, please provide additional information about the test method, including positive and negative controls, primers/gene targets, and data demonstrating that the tested cell lines are *Sus scrofa domestica*. Further, please include a narrative summary discussing whether the species verification test method can identify DNA from other species and, if not, describe controls that are in place to ensure that only *Sus scrofa* derived cells are used to manufacture the cell culture food. This should include whether the cells are manufactured in a single or multi species facility. Please discuss process controls that are in place to track cell lines derived from species other than *Sus scrofa*.

Mission Barns' Response:

For species verification, Mission Barns utilizes a GeneScan DNAnimal Ident Pork IPC (Cat. nos. 5422211910) kit, a test developed by Gold Standard Diagnostics Corp and conducted by Eurofins. This method is designed for general detection of a porcine sequence in DNA extracted from food and feed matrices. As part of this method, two no template controls (NTCs) serve as negative controls, and two positive controls (genomic DNA from pig) are utilized. Any potential false-negative or inhibited results are mitigated by the use of an internal positive control (IPC) contained in the MasterMix reagent which is amplified in parallel in each plate well. Regarding the specific primers/gene targets used in this test method, Mission Barns is unable to provide this information since the vendor considers it proprietary, protected information.[1] Copies of the test kit application manual and a complete method validation information sheet from Eurofins, however, are provided in Attachment 3.

While the GeneScan DNAnimal Ident Pork IPC kit does not differentiate between domesticated pig and wild

[1] Gold Standard Diagnostics Corp's confirmation of the proprietary nature of its specific primers/gene targets is included in Attachment 3.

boar subspecies (*Sus scrofa domestica* and *Sus scrofa scrofa*, respectively), further process controls provide sufficient confidence that Mission Barns' porcine cell lines are derived from the *Sus scrofa domestica* subspecies. Specifically, (1) Mission Barns collects donor animal supplier information which verifies the donor animal identity as a domestic breed of pig (Yorkshire or Yorkshire/Landrace hybrid); and, (2) Mission Barns has never utilized wild boar as a source for tissue isolation, eliminating cross-subspecies contamination risks during culturing.

For clarification, Mission Barns corrects Table 1 of the March 16, 2022 disclosable safety narrative. In the table, the specification for species verification testing should state "*Sus scrofa* (pig) DNA confirmed" rather than "*Sus scrofa domesticus* (pig) DNA confirmed."

The GeneScan DNAnimal Ident Pork IPC kit is designed to only detect the presence of porcine DNA (*Sus scrofa*) and does not detect any other animal species. However, Mission Barns implements the following controls to ensure only *Sus scrofa* derived cells are used in Mission Barn's manufacturing process:

1. Mission Barns' manufacturing facility is a single species (*Sus scrofa*) dedicated area. Although Mission Barns stores previously established research cell lines from other species (i.e., chicken, duck, cow) in a cryofreezer on site, it does not perform any on site non-porcine culturing activities at its manufacturing facility.
2. Cell line development is conducted in an R&D lab environment physically segregated from our manufacturing facility under stringent cross-contamination controls including:
    a. *Temporal Controls*. Species cell line development work is temporally separated. At no time is cell line development work conducted simultaneously for multiple species.
    b. *Physical Controls*. Cell line development work and testing is conducted in physically separated and dedicated areas and equipment.
    c. *Material Controls*. Cell line development is conducted predominantly using sterile, single use consumables and equipment. Equipment and surfaces that are not single use are cleaned and decontaminated after each use.
    d. *Record Keeping Controls*. All cell line development operations, including cell culture isolation, passaging, and banking, are recorded in lab notebooks and maintained in Mission Barns' quality system.
    e. *Training and Procedural Controls*. Personnel is trained on appropriate protocols for cell culture handling.
3. Mission Barns has implemented cell bank inventory controls, including vial labeling, material and lot coding, and periodic inventory audits.
4. Research cell banks are physically segregated into separate, species-dedicated racks within cryogenic (-150 deg C) storage freezers.

As further confirmation that only *Sus scrofa* cells are used to manufacture its cultivated fat cells, Mission Barns has tested its porcine manufacturing cell banks for cross-contamination with other animal species. This testing was performed for informational purposes and is not intended to be a part of Mission Barns' regular cell bank release testing. Results from cross-species testing have confirmed that no other animal species currently in Mission Barns' research cell banks (i.e., chicken, duck, cow) are present in its porcine cell lines/banks. A certificate of analysis for these test results are included as Attachment 4.[2]

Substances Used During Cell Culture

[2] The testing service provider Mission Barns' employed for its multi-species (meat) PCR analysis bundles testing for the DNA of multiple species that have never been used in Mission Barns' research or manufacturing facility and are not considered as cross-contamination risks. Therefore, the certificate of analysis includes cross-contamination testing for horse (Equus caballus), sheep (Ovis aries), goat (Capra hircus), and turkey (Meleagris gallopavo) DNA, even though Mission Barns has never isolated or cultured cells from any of these species.

3) Table 8 in the March 16, 2022, supplemental, confidential material lists several recombinant growth factors that are used to supplement the cell culture serum-free media. Table 10 of the same document summarizes analytical test results for levels of these growth factors in the cell harvest wash solution, with additional details provided in Attachment 8.

For addition to the disclosable safety narrative, please provide the species of origin of each recombinant growth factor, and the source organism used to produce the recombinant growth factor (e.g., pathogenicity, toxigenicity, allergenicity).

Mission Barns' Response:

All recombinant growth factors (rGFs) used in Mission Barns' production process have amino acid sequences which originate from agriculturally relevant species, specifically porcine and bovine species. The source organisms used to produce the recombinant growth factors are well-studied and widely-used strains of bacteria (e.g., E. coli K-12, E. coli BL21-DE3), common agricultural varieties of plants (e.g., barley (*Hordeum vulgare*), rice (*Oryza sativa*)), common yeast (e.g., *Saccharomyces cerevisiae*, *Pichia pastoris*), or mammalian protein expression systems (e.g., CHO cells). All organisms used possess a history of safe and suitable use for their respective intended uses.[3,4]

We do not identify any significant sources of potential pathogenicity, toxicity, nor allergenicity in any of the source organisms for the production of purified recombinant growth factors.[5] Barley, rice, and common yeasts are ubiquitously consumed as a part of the human diet and therefore are unlikely to present any human health hazard. Laboratory strains of bacteria and yeast, such as *E. coli* K-12 and BL21-DE3, are well-studied organisms that have been routinely used by humans since they were first isolated and characterized many decades ago, with no history of allergenicity concerns in humans.[6] Both are the most commonly used E. coli strains for recombinant protein therapeutics production, in part, for their consistency in production.[7] They are also used, in part, because they do not demonstrate any known pathogenicity in humans.[8]

CHO cells, like the prokaryotic fermentation systems, are used for their well-studied consistency and predictability.[9] CHO cells are a highly favorable protein production platform due to their ability to be adapted for chemically-defined, serum-free culture conditions, their stable expression of recombinant proteins, and long-standing safety profile for use in humans. The recombinant proteins produced by these expression systems are collected and purified either from a cellular lysate (e.g., E. coli) or from a conditioned medium (e.g. CHO cells), both of which are further subject to the company's supplier qualification program prior to use. We therefore do not believe the source organisms present a significant hazard when the rGFs are introduced into culture as a result of potential contamination from the source organism.

4) On page 39-40 of the March 16, 2022, supplemental, confidential material, you present exposure

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[3] See, e.g., FDA. UPSIDE Foods, Inc., Premarket Notice for Integral Tissue Cultured Poultry Meat (Oct. 1, 2021), available at: https://www.fda.gov/media/163262/download (accessed 25 April 2024).

[4] FDA. Microorganisms & Microbial-Derived Ingredients Used in Food (Partial List). 1 April 2018 https://www.fda.gov/food/generally-recognized-safe-gras/microorganisms-microbial-derived-ingredients-used-food-partial-list (accessed 2024 May 31).

[5] As part of Mission Barns quality management system, growth factors are procured with certificates of analysis certifying appropriate purity considering the source organism and the intended use.

[6] Daegelen P., Studier F. W., Lenski R. E., Cure S., Kim J. F. (2009). Tracing ancestors and relatives of Escherichia coli B, and the derivation of B strains REL606 and BL21(DE3). J. Mol. Biol. 394 634–643 10.1016/j.jmb.2009.09.022

[7] Selas Castiñeiras, T., Williams, S. G., Hitchcock, A. G., & Smith, D. C. (2018). E. coli strain engineering for the production of advanced biopharmaceutical products. FEMS Microbiology Letters, 365(15). doi:10.1093/femsle/fny162

[8] Examples of FDA GRAS notices where E coli. K-12 and BL21(DE3) are used as fermentation hosts for food production include GRN 624, available at https://www.fda.gov/food/gras-notice-inventory/agency-response-letter-gras-notice-no-grn-000624 (accessed May 31, 2024) (K-12 used for the fermentation of a fructose enzyme) and **GRN 977** available at: https://www.fda.gov/media/155443/download) (accessed 31 May 2024) (BL21(DE3) used for the fermentation of maltodextrin enzymes).

[9] Butler M, Spearman M. The choice of mammalian cell host and possibilities for glycosylation engineering. Curr Opin Biotechnol. 2014;30C:107–12.

estimates for HEPES, a media buffer used in cell culture. Your exposure estimates assume that, due to multiple washing steps, the concentration of all residuals (including processing aids present in the cell culture media) would be substantially reduced in the final product. In support of this argument, you tested for the presence of a surrogate protein (see Attachment 7 of the March 16, 2022, supplemental, confidential materials) in spent media from both the end of the culturing process and in the final wash solution using an ELISA assay. You note a dilution factor of 1×10^{-5} for the surrogate protein between the two tested solutions. Further, you presume that this same level of dilution will be present for all substances present in the cell culture media. FDA notes that the dilution factor of $1x10^{-5}$, which is based on a surrogate protein analytical test, may or may not be relevant for HEPES. For example, there is evidence that HEPES may be taken up by cells during the cell culture process.[10] Further, HEPES has not been previously evaluated by FDA for food applications and is new to the food supply. Please provide, for addition to the disclosable safety narrative, the following information regarding the levels of HEPES in the harvested cell material:

a. Analytical data for the presence of HEPES in the harvested cell material. b. Estimated daily intake (EDI) based on analytical data.

c. Please identify the best representative toxicology study for HEPES, explain why you select that study (rationale behind study selection), and calculate a margin of safety (MOS) between the safe intake level established in this study and the analytical EDI. Please discuss whether the MOS is adequate and conclude whether the intake of HEPES is safe at the analytical EDI.

Mission Barns' Response:

Mission Barns has removed HEPES from its manufacturing process.[11] However, Mission Barns' still intends to use HEPES in its premanufacturing cell banking process in small amounts (less than 10 g/L) in cell culture media. Below, Mission Barns has provided an assessment of the residual concentration of HEPES in its cultivated fat cells that derives a > 10^9 margin of exposure, supporting the conclusion its use in Mission Barns' premanufacturing cell banking process does not present a safety concern.

The duration between the thaw of a manufacturing cell bank and the harvest of cultivated fat cells is at least 30 days. In the study cited in information request #4, the half life of HEPES in cells is determined to be 25 hours.[12] Given that half life, it is expected that over the course of a manufacturing run, intracellular HEPES concentrations would be reduced by a factor of > 10^9, as calculated below, where $[HEPES]_f$ is the final HEPES concentration in culture and $[HEPES]_0$ is the initial HEPES concentration in culture.

$$[HEPES]_f = [HEPES]_0 * (1/2)^{(30\ days * 24\ hours/day)/\ 25\ hour\ HEPES\ half\ life}$$

$$\frac{[HEPES]_f}{[HEPES]_0} = (1/2)^{(30\ days * 24\ hours/day)/\ 25\ hour\ HEPES\ half\ life}$$

[10] Depping R, Seeger K. 1H-NMR spectroscopy shows cellular uptake of HEPES buffer by human cell lines-an effect to be considered in cell culture experiments. Anal Bioanal Chem. 2019 Feb;411(4):797-802. doi: 10.1007/s00216- 018-1518-4. Epub 2018 Dec 1. PMID: 30506504.

[11] HEPES is one of several buffering agents in Mission Barns cell culture media and does not play an essential role in the proliferation of fattening of Mission Barns cultivated pork fat cells. Mission Barns does not expect that the removal of HEPES from its manufacturing process will meaningfully affect the phenotype of its cell cultures or composition of its cultivated fat cells. Likewise, no evidence suggests that the removal of HEPES would negatively affect the safety profile of Mission Barns' cultivated pork fat cells in any material manner.

[12] Depping, et. al (2019).

$$\frac{[HEPES]_f}{[HEPES]_0} = 2.1 * 10^{-9}$$

Additionally, during a manufacturing run, the mass of an initial culture is expanded at least 1,000X prior to harvest, meaning that any intracellular HEPES would be diluted by a factor of 1,000X, since it would be spread over 1,000X more cell mass.

Combining these two factors, HEPES is expected to be present at a ~10^{12} times lower intracellular concentration in cultures after our manufacturing process as compared to cultures that are newly thawed from a manufacturing cell bank.

Making an extremely conservative assumption that HEPES makes up 10% of the mass in cells thawed from manufacturing cell banks,[13] HEPES concentration in cultivated fat cells after manufacturing can be calculated as follow:

$$0.1 \frac{g\ HEPES}{g\ cell\ bank} * 10^{-12} = 10^{-13} \frac{g\ HEPES}{g\ cultivated\ pork\ fat\ cells}$$

Mission Barns believes the study by Park et. al[14] is the best representative toxicological study of HEPES relevant to Mission Barns' intended use. In this study, HEPES was used as a vehicle control in a 90-day subchronic oral toxicity study of zinc oxide nanoparticles in Sprague Dawley rats. While the study was not designed to evaluate the toxicity of orally administered HEPES, the duration of HEPES exposure was the longest amongst the identified studies that involved oral administration, giving the greatest likelihood that subchronic effects would manifest in the test subjects.[15] Given that the estimated concentration of HEPES in our cultivated fat cells is very low, chronic toxicity not acute toxicity is deemed to be more relevant for safety. The study results indicate that subchronic oral administration of HEPES does not adversely affect Sprague Dawley rats at the vehicle control doses (4.77 mg/kg bw/day), given by the 10 mL oral administration of 20 mM HEPES solution, and HEPES' 238.3 g/mol molar mass.

Using the historical default 100-fold uncertainty factor used in regulatory toxicology, accounting for a 10-fold factor for interspecies differences and a 10-fold factor for intraspecies differences, and the oral dose of the HEPES vehicle control from the Park et al. study of 4.77 mg/kg bw/day, the equivalent human daily intake of 47.7 µg/kg bw/day can be established as a NOAEL.

A margin of exposure (MOE) of 100-fold or greater between the NOAEL/NOEL and EDI from food exposures is typically considered adequate to support safety. Given an EDI of 0.4 g / kg bw / day of cultivated fat cells, and the estimated amount of HEPES in harvested cultivated fat cells calculated above, a margin of exposure (MoE) of ~10^9 can be calculated, supporting the conclusion that Mission Barns' use of HEPES in its premanufacturing cell banking process does not present a safety concern:

$$MoE = \frac{HEPES\ NOAEL}{cultivated\ fat\ cell\ EDI\ *\ assumed\ HEPES\ mass\ fraction\ *\ manufacturing\ process\ dilution}$$

[13] HEPES concentration in cell banking media is less than 10 g/L, or 1% of the media's mass.

[14] Park, H.-S., Kim, S.-J., Lee, T.-J., Kim, G.-Y., Meang, E., Hong, J.-S., Kim, S.-H., Koh, S.-B., Hong, S.-G. and Sun, Y.-S. (2014) A" 90-day study of sub-chronic oral toxicity of 20 nm positively charged zinc oxide nanoparticles in Sprague Dawley rats," *International journal of nanomedicine*, 9(sup2):93-107.

[15] Mission Barns notes longer toxicological studies involving intravenous administration of HEPES are contained in the scientific literature and support a higher NOAEL than the study selected by Mission Barns. See Theodore, T. R., Van Zandt, R. L. and Carpenter, R. H. (1997a) "Pilot ascending dose tolerance study of parenterally administered 4-(2 hydroxyethyl)-1-piperazine ethane sulfonic acid (TVZ-7) in dogs," *Cancer Biother Radiopharm* 12(5):345-9 (finding no significant adverse events were observed at intravenous doses up to 520 mg/kg bw/day of HEPES for a period of 148 days).

$$MoE = \frac{47.7 * 10^{-6} g / kg\ bw / day}{0.4\ g / kg\ bw / day * 10^{-13}} = 1.19 * 10^{9}$$

5) On pages 42-43 of the March 16, 2022, supplemental, confidential material you provide analytical data, exposure estimates, and a safety assessment for folic acid added to the cell culture media. Page 43 states, "nutritional concentrations of folic acid in pork range from, on the low end, 10 ng/g of pork to as high as 120 ng/g of pork[73]" and lists the following as the reference in footnote 73: "Muller, H. (1993) '[The determination of the folic acid content of foods of animal origin using high performance liquid chromatography (HPLC)]', Z Lebensm Unters Forsch, 196(6), pp. 518-521 [article in German]." It is unclear if the levels of folic acid in the reference in footnote 73 are for conventional pork fat, or from another pork tissue type.

FDA discussed the safety concerns associated with folic acid in 2016 (81 FR 22176) when authorizing the use of folic acid in corn masa flour. As noted, exposure for certain populations, exceeds the Upper Limit (UL). We would have significant safety concerns with any new source of folic acid in the diet. Any increase in use would require a food additive petition for the intended use, and given current exposures, providing sufficient information to support safety may not be possible.

For addition to the disclosable safety narrative, please provide the following information regarding the safety assessment for folic acid:

a. Information on the levels of folic acid in conventional pork fat.

b. If the levels of folic acid from the harvested cell material exceed those in conventional pork fat, please reach out to the Division of Food Ingredients to discuss the potential for a new use to be authorized. They may be reached by sending an email to premarkt@fda.hhs.gov. Alternatively, you may take steps to reduce the levels of folic acid in your harvested cell material. If you take such steps, please provide new batch data, including COAs, and include a narrative discussing the changes that you have made to the process and whether the changes may impact other characteristics of the harvested cell material.

Mission Barns' Response:

Folic acid is a form of folate (vitamin B9) that is widely present in food. Folate concentrations of between 20 - 270 ng / g of conventional pork fat are reported in the USDA database[16] and by Greenfield, H. *et al*.[17] Additionally, Mission Barns clarifies that the study by Muller, H. *et al* found levels of folate ranging from 10 - 40 ng per gram of pork meat (schweinefleisch), 1,360 ng per gram of pork liver (schweineleber), and 930 ng per gram of pork kidneys (schweinenieren), shown in Table 1 of the paper.[18]

To assess levels of folic acid in Mission Barns' cultivated pork fat cells, Mission Barns performed an ELISA assay to quantify folic acid levels in harvested cultivated pork fat cells. The results from three non-consecutive batches are summarized below:

[16] USDA FoodData Central, NDB Number:10167. Available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167878/nutrients (accessed April 19, 2024).

[17] H. Greenfield, J. Arcot, J.A. Barnes, J. Cunningham, P. Adorno, T. Stobaus, R.K. Tume, S.L. Beilken, W.J. Muller, Nutrient composition of Australian retail pork cuts 2005/2006, Food Chemistry, Volume 117, Issue 4, 2009, Pages 721-730, ISSN 0308-8146, https://doi.org/10.1016/j.foodchem.2009.04.048.

[18] "Gesamtfolat" in Table 1 of the paper translates to "Total folate". Muller, H. (1993) '[The determination of the folic acid content of foods of animal origin using high performance liquid chromatography (HPLC)]', Z Lebensm Unters Forsch, 196(6), pp. 518-521 [article in German].

Test item	Batch #1	Batch #2	Batch #3
ng Folic Acid per g cultivated fat cells	13.3 ± 1.3	5.7 ± 0.4	21 ± 4

Our measured concentrations of folic acid in three non-consecutive batches of cultivated pork fat cells (average of 13.33 ng folic acid / g cultivated pork fat cells) are consistent with those of conventional pork products. Since Mission Barns' cultivated pork fat is intended to be a 1:1 replacement for conventional pork fat in the market today, it is not considered as an additional source of folic acid in the diet.

6) On page 45-47 of the March 16, 2022, supplemental, confidential material you provide analytical data, exposure estimates, and safety assessments for hormones added to the cell culture media. In the March 6, 2022, amendment to the supplemental, confidential material you provide additional details for the safety assessment of each hormone. For addition to the disclosable safety narrative, please provide as much of the information as possible regarding your safety evaluation of the use of hormones during cell culture from the March 16, 2022, supplemental, confidential material and the March 6, 2023, amendment to the supplemental, confidential material. FDA notes that we do not accept tolerance levels as safety arguments in place of a proper safety evaluation based on relevant safety data (i.e., safe reference levels, and margin of exposure or safety calculations).

Mission Barns' Response:

Hormones are chemicals that are produced naturally in the bodies of all animals, including humans. They are chemical messenger molecules released into the blood by hormone-producing organs, and travel to and affect different parts of the body.[19] Mission Barns notes that the three hormones added to the cell culture media are proprietary, confidential commercial information and a trade secret, and are identified herein as "hormone A", "hormone B", and "hormone C".[20]

Mission Barns notes our safety rationale for residual hormones is based on a weight-of-evidence approach, considering actual test data showing low levels of residual hormones; the comparison to naturally occurring levels of these hormones from common food intake; safety limits established by regulatory or scientific bodies such as JECFA; and any additional processing or cooking that may further mitigate potential chemical risk or activity of the residual hormones used during the manufacturing process.

When establishing the appropriate safety threshold levels or safe limits for hormones, Mission Barns takes into consideration established safe levels (e.g., acceptable daily intake) derived from a relevant authoritative body (e.g., U.S. FDA, Joint FAO/WHO Expert Committee on Food Additives). If comparisons of anticipated dietary intakes relative to an authoritative reference intake value is not readily available, Mission Barns takes into consideration the published no-observed-adverse-effect levels (NOAELs) or no-observed-effect levels (NOELs) from animal toxicology studies to evaluate food safety risks. For a given hormone, a margin of exposure (MOE) of 100-fold or greater between the hormone's NOAEL/NOEL and its estimated dietary intake is typically considered adequate for food safety. For all hormones of concern with established NOAELs/NOELs, the MOEs are well over 100-fold based on Mission Barns' testing data from three non-consecutive batches.

In instances where established safety levels or NOAELs/NOELs are not available, Mission Barns compared calculated EDIs with published reports on the natural-occurrence and concentration of the specific

[19] Gandhi, Renu, and Suzanne M. Snedeker. "Consumer Concerns About Hormones in Food, BCERF Fact Sheet No. 37." (2000).
[20] The identity of hormone A, hormone B, and hormone C are on file with FDA in Mission Barns' supplementary, confidential material.

hormones in commonly consumed foods such as cow's milk or fruit.

For the three hormones Mission Barns adds as part of the cell culture media, we have analyzed their levels in cultivated pork fat cells for three non-consecutive batches.

Table 9. Analytical Testing Results with Cultivated Pork Fat Cells from Three Non consecutive Batches (ng/g)			
Item	**Batch #1**	**Batch #2**	**Batch #3**
Hormone A	0.3	0.34	0.33
Hormone B	0.76	0.86	1.79
Hormone C	0.677	0.656	3.473

The estimated daily intake based on the test data and the EDI for Mission Barns cultivated pork fat cells can be calculated as follows:

Hormone A = 16.7 g/day x (0.3 + 0.34 + 0.33)/3 ng/g= 0.005 µg/day

Hormone B = 16.7 g/day x (0.76 + 0.86 + 1.79)/3 ng/g= 0.019 µg/day

Hormone C = 16.7 g/day x (0.677 + 0.656 + 3.473)/3 ng/g = 0.026 µg/day

Hormone A

Hormone A is a steroid hormone that is important for reproductive function. It acts at the genomic level by binding to the nuclear receptors and modulating the expression of some target-genes.

No conventional studies of oral toxicity of hormone A in animals were identified in the published literature after a comprehensive literature search. JECFA reviewed the available safety data on hormone A to evaluate safe residual levels of veterinary drugs, including hormone A in meats and established the ADI of hormone A with an upper range of more than 20 µg/kg bw/day.

The analytically tested EDI for hormone A in Mission Barns cultivated pork fat cells is 0.4 g/kg bw/day x (0.3 + 0.34 + 0.33)/3 ng/g = 0.000129 µg/kg bw/day, which is orders of magnitude (*i.e.*, > 10^5 fold) lower than the JECFA established ADI.

Additionally, hormone A naturally occurs in common foods at levels comparable to or higher than levels observed in Mission Barns cultivated pork fat cells. For example, hormone A is present in many common foods at levels higher than Mission Barns' cultivated pork fat cells, including butter, eggs, ham, beef, milk, potatoes, turkey, and yogurt. As such, Mission Barns concludes that levels of hormone A in cultivated pork fat cells does not pose a safety concern.

Hormone B

Hormone B is a naturally occurring steroid hormone and is commonly used in veterinary medicine. Literature discloses that Hormone B was not mutagenic in either the presence or absence of metabolic activation. Carcinogenicity studies with related steroid hormones gave no indication of carcinogenic potential. No NOAELs were established for hormone B in the pharmacology and toxicity studies that could be used as the basis for an ADI calculation.

Hormone B is naturally found in foods, including conventional pork, at levels up to 40 times the average amount found in Mission Barns' cultivated pork fat cells. As such, Mission Barns concludes any

residual level of hormone B in the cultivated fat cells does not pose a safety concern.

Hormone C

Hormone C is a hormone that's commonly found in food. The hormone C levels were determined in 38 fruits and vegetables consumed in Japan. Hormone C is present in many common foods at levels higher than Mission Barns cultivated pork fat cells, including cherry tomatoes, plantains, pineapples, bananas, kiwi fruit; plums, tomatoes, and English walnuts. As such, Mission Barns concludes any residual level of hormone C in cultivated pork fat cells does not pose a safety concern.

7) The March 16, 2022, supplemental, confidential material discusses the use of three trace metal salts during the cell culture production process. Specifications for batch release testing for these trace metals are presented in the March 6, 2023, amendments to the disclosable safety narrative (page 19) and supplemental, confidential material (page 24) and on page 15 of the June 5, 2023, amendment. Further, pages 19-21 of the March 6, 2023, amendment to the disclosable safety narrative presents a safety assessment, based on analytical data, for the intake of these metals from the harvested cell material, including comparisons to the levels found in conventional food. However, the information, including the identity of the metal salts and specification for batch release testing, are confidential commercial information/trade secret (CCI/TS). For addition to the disclosable safety narrative, please include as much of the following information as possible regarding the safety assessment for metal salts:

a. The batch release specifications and analytical results for these metal salts.

Mission Barns' Response:

Mission Barns includes three trace metal salts in its cell culture media that are common nutrients in foods. The three trace metal salts used during the cell culture production process are proprietary, confidential commercial information and a trade secret, and are identified herein as "Trace Metal A", "Trace Metal B", and "Trace Metal C".[21]

The batch release specifications and analytical results from 3 representative batches of cultivated fat cells that were described in the supplementary, confidential material for the 3 trace metals are provided in the table below:

Trace metals testing results from three non-consecutive representative batches				
Trace Metal	**Specification**	**Batch 1**	**Batch 2**	**Batch 3**
Trace metal A	*< 0.2 ppm*	0.029 ppm	0.029 ppm	0.033 ppm
Trace Metal B	*< 0.03 ppm*	< 0.01 ppm*	< 0.01 ppm*	< 0.01 ppm*
Trace Metal C	*< 0.1 ppm*	0.011 ppm	0.011 ppm	0.012 ppm
* limit of quantification of assay				

b. Any information available to support the safe use of the metal salts during the production process (e.g., pages 19-21 of the March 6, 2023, amendment to the disclosable safety narrative).

[21] The identites of Trace Metal A, Trace Metal B, and Trace Metal C have been shared with FDA in Mission Barns' supplementary, confidential material.

Mission Barns' Response:

Mission Barns notes all three trace metals are either reported to be present in conventional US pork belly fat at the same or higher levels than the specifications above, or are considered common nutrients in food and the estimated daily intake from their intended use will constitute an insignificant percentage of the daily intake of the nutrients as reported in public literature. As such, the consumption of the cultivated pork fat cells is not expected to lead to a significant increase in consumers' cumulative exposures of these trace metals.

Trace Metal A

Trace Metal A is a known cofactor for certain enzymatic reactions and it has been suggested that it is required for protein synthesis in animals. It is approved for use as a food processing aid per 21 CFR § 172, § 176, and § 184, and is commonly present in animal foods including pork. For example, Trace Metal A concentrations in chicken, pork, and beef are reported to be between 1-2 ppm. Evaluations of the elemental composition of pork belly fat from pork samples of various geographical origins including the US by inductively coupled plasma-mass spectrometry (ICP-MS) show that the naturally-occurring level of Trace Metal A in pork belly fat from the US is ~0.20 ppm. As such, a specification for the cultivated pork fat cells of < 0.2 ppm ensures the dietary intake from Trace Metal A, if any, would be lower or comparable to the naturally-occurring Trace Metal A level in conventional pork belly fat. Accordingly, there is no expected increase to the cumulative Trace Metal A intake from the cultivated pork fat cells, which are intended to replace conventional pork fat in the US. Mission Barns concludes that any Trace Metal A exposure from our intended use would be comparable to the Trace Metal A exposure from conventional pork fat.

Trace Metal B

Trace Metal B can be found at concentrations that are >10X higher than Mission Barns specification for Trace Metal B in cultivated fat cells in many common foods, including shellfish, mushrooms, chicken meat, and rice. Evaluations of the elemental composition of pork belly fat from pork samples of various geographical origin by ICP-MS, reported levels of Trace Metal B in US pork belly fat at ~0.03 ppm. A specification for the cultivated pork fat cells at < 0.03 ppm ensures the dietary intake from Trace Metal B, if any, would be lower than or comparable to the naturally-occurring Trace Metal B levels in conventional pork belly fat. As such, there is no expected increase to the cumulative Trace Metal B intake from the cultivated pork fat cells, which are intended to replace conventional pork fat in the US.

Trace Metal C

Trace Metal C is an essential trace element for microorganisms, plants, and animals. FDA developed daily values or DVs to help consumers compare the nutrient contents of foods and dietary supplements within the context of a total diet. Under FDA regulation 21 CFR 101.9(c), the DV for Trace Metal C is established as 45 µg for adults and children aged 4 years and older. We note the 90th percentile EDI for Mission Barns' cultivated pork fat cells is 16.7 g/person/day. With a specification of 0.1 ppm for Trace Metal C in the cultivated pork fat cells, the estimated theoretical consumer daily intake of Trace Metal C from the consumption of cultivated pork fat cells is up to 1.67 µg/day, which constitutes < 5% of the Trace Metal C DV at 45 µg. We also note that 1.67 µg/day intake from our intended use is much smaller in comparison with the Trace Metal C exposure from one serving of the following common animal foods:

- Beef, liver, pan fried (3 ounces), 104 µg Trace Metal C
- Chicken, light meat, roasted (3 ounces), 9 µg Trace Metal C
- Beef, ground, regular, pan-fried (3 ounces), 8 µg Trace Metal C

Intake recommendations for Trace Metal C and other nutrients are also provided by the Food and Nutrition

Board (FNB) at the National Academies of Sciences, Engineering, and Medicine.[22] The FNB established ULs for Trace Metal C for healthy individuals based on levels associated with impaired reproduction and fetal development in rats and mice.[23] The UL for the general adult population (19+ years) is 2,000 µg/day, and the estimated daily intake of 1.67 µg/day from cultivated pork fat cells is less than 0.1% of the UL.

With a specification of Trace Metal C at 0.1 ppm, the daily intake of Trace Metal C from the cultivated pork fat cells consumption will be less than 5% of the Trace Metal C DV established by FDA, and less than 0.1% of the UL established by the FNB. Mission Barns concludes that levels of Trace Metal C in cultivated pork fat cells do not pose a safety concern.

8) In the December 11, 2023, amendment, you reported the addition of new processing aids and chemicals into the manufacturing process since the submission of the March 16, 2022, supplemental, confidential material. The December 11, 2023, amendment was designated as CCI in its entirety. The absence of any information on identity, decision criteria, or grounds for safety conclusions in the amendment does not provide a sufficient basis for FDA to document in our evaluation. For addition to the disclosable safety narrative, please provide as much as possible of the information presented in the December 11, 2023, amendment including:

a. Information on the identity or, if you believe the identity is CCI/TS, the classes (e.g., protein, surfactant) and characteristics of new substances added to the culture medium, including the species of origin of all animal-derived substances.

b. Information about the basis upon which you concluded that the use of the new substances does not pose a food safety concern, including prior exposure or presence in food as an ingredient or constituent, estimated exposure or margin of safety, prior authorization or evaluation, or other information that would provide insight into Mission Barns' assessment process and decision criteria.

Mission Barns' Response:

The components identified in the December 11, 2023 amendment fall into the following categories: a sterol, alcohol, vitamins, fatty acids, an antioxidant, a carbamide, a coenzyme, and an emulsifier. All of the components are commonly found in foods and are either considered GRAS or permitted for use through FDA's food additive regulations. With the exception of the emulsifier component, these components are present in Mission Barns' cell culture media at less than 0.1 g/L. The emulsifier component is a food safe surfactant. Its use concentration in the cell culture media is limited such that the resulting daily intake is below the acceptable allowance specified in 21 CFR 172.

As described in Section 3.2.2.2 of the May 25, 2022, disclosable safety narrative, the EDIs for these food safe components are well within the appropriate safety thresholds.[24]

Given cultivated fat cell EDIs of 16.7 g/day and 0.4 g/kg bw/day, the theoretical EDIs for any of these components in cell culture media can be calculated as:

$16.7 \text{ g/day} * 10^{-6} \text{ g/kg} = 0.0167 \text{ µg/day}$

$0.4 \text{ g/kg bw/day} * 10^{-6} \text{ g/kg} = 0.0004 \text{ µg/kg bw/day}$

Based on the above worst-case estimation, the EDIs for all these components are well within appropriate margins of safety.

12) For addition to the disclosable safety narrative, please provide analytical data from the harvested

[22] See Trumbo, P., Yates, A. A., Schlicker, S., & Poos, M. (2001), "Dietary reference intakes," Journal of the American Dietetic Association, 101(3): 294-301.
[23] *See id.*
[24] Mission Barns refers to our response in information request #6 regarding our approach to establishing appropriate safety thresholds.

cellular material demonstrating a sufficient MOS between the EDI and an established safe level (e.g., no observed adverse effect level (NOAEL), ADI, reference does (RfD), or UL) for the following substances for which no authorization for use in conventional food exists: Tris-HCl, HEPES, sodium selenite, and Pluronic F-68. Please provide a robust discussion summarizing the information upon which you concluded that the use of the new substances does not pose a food safety concern, including publicly available literature and toxicological studies supporting your safety conclusions.

Mission Barns' Response:

Effective as of the date of this Amendment, Mission Barns has removed Tris-HCl, HEPES, and Pluronic F-68 from its manufacturing process.[25] However, as noted in our response to information request #4, Mission Barns' still intends to use HEPES and Pluronic F-68 in its premanufacturing cell banking process.[26] Pluronic F-68 (poloxamer 188) is used in Mission Barns cell culture media at concentrations not exceeding 0.1 g/L as a surfactant that functions as anti-clumping agent in cell culture. A scientific literature search by Mission Barns has not found evidence that Pluronic F-68 is taken up by cells during culturing.[27] As discussed in Section 3.2.2 of our May 25, 2022 disclosable safety narrative, Mission Barns has established a theoretical EDI for cell culture media residuals of 0.0167 µg/day or 0.0004 µg/kg bw/day.[28] A non-clinical review conducted by the Center for Drug Evaluation and Research (CDER) reports that in rats and dogs, a NOEL of 2,500 and 100 mg/kg/day, respectively, was established for poloxamer 188.[29] Accounting for a 10-fold safety factor for interspecies differences and a 10-fold factor for intraspecies differences to the most sensitive species (dog, 100 mg/kg/day), results in an equivalent NOEL for humans at 1 mg/kg bw/day. A margin of exposure (MOE) of 100-fold or greater between the NOAEL/NOEL and EDI from food exposures is typically considered adequate to support safety. For Pluronic F-68 the MOE can be calculated as:

$$MoE = \frac{1 * 10^{-3} g / kg\ bw / day}{0.0004 * 10^{-6} g / kg\ bw / day} = 2.5 * 10^{6}$$

Sodium Selenite is an inorganic salt widely distributed in soil, water, air, vegetation and food that is highly soluble in water and other organic solvents.[30] Selenium is an essential trace element and necessary for a variety of biological functions. Considering its importance for humans, the recommended dietary intake for selenium is 55 µg/d for healthy adults in the US.[31] In the EU, sodium selenite is an authorized food additive to fortify selenium content.[32] Although selenium is an essential trace element, it can be toxic in high doses. The European Food Safety Authority (EFSA) recently published a scientific opinion on the tolerable upper intake level (UL) for selenium.[33] Following a systematic review of available literature, EFSA determined a UL for

[25] Tris-HCl was previously used as a solvent for coating reagents used to coat cell culture vessel surfaces. Mission Barns has replaced Tris-HCl with a saline solution.

[26] Mission Barns refers to its response to information requests #4 regarding the removal of HEPES from its manufacturing process and the accompanying safety narrative with respect to its use in cell banking activities.

[27] See, e.g., Dossier In Support of the Safety of GOOD Meat Cultured Chicken as a Human Food Ingredient (Mar. 4, 2022), available at: https://www.fda.gov/media/166346/download (accessed 25 April 2024) (reporting Pluronic F-68 levels below the assay limit of detection in three batches of chicken cells cultured in Pluronic F-68 containing media).

[28] Mission Barns notes that because Pluronic F-68 is only present in cell culture media during the cell banking phase and that dozens of fluid exchanges occur between cell banking and final cell harvest, the theoretical EDI for cell culture media residuals is expected to significantly overestimate exposure to Pluronic F-68 from cultivated pork fat cells.

[29] FDA, Center for Drug Evaluation and Research, Non-clinical Reviews for Application Number: 209139Orig1s000, available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209139Orig1s000PharmR.pdf (accessed May 30, 2024).

[30] Jacevic, Vesna, et al. "Acute toxicity of sodium selenite in rodents: Pathomorphological study." Military Medical Science Letters 80.3 (2011): 90-96.

[31] Selenium Fact Sheet for Consumers, National Institute of Health, available at https://ods.od.nih.gov/factsheets/Selenium-Consumer/ (accessed May 3, 2024).

[32] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, pp. 26–38.

[33] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), et al. "Scientific opinion on the tolerable upper intake level for selenium." EFSA Journal 21.1 (2023): e07704.

selenium in healthy adults of 255 µg/day from all sources, including sodium selenite.

We refer to our response to information request #7 of the June 5, 2023 amendment. Selenium levels in Mission Barns' cultivated pork fat cells were below the limit of detection of the applicable assay, which demonstrated selenium levels to be comparable to levels found in conventional pork fat, as reported in the USDA database:

Mineral[34]	Unit	Mission Barns' Cultivated Pork Fat Cells			Conventional pork fat
		Batch #1	Batch #2	Batch #3	
Selenium	µg / 100g	< 25	< 25	< 25	8 - 16[35]

As such, there is no expected increase to the cumulative selenium intake from the cultivated pork fat cells, which are intended as a replacement of conventional pork fat in the US. Further, assuming selenium is present in Mission Barns cultivated pork fat cells at the limit of detection of 25 µg / 100g, the estimated exposure to selenium can be calculated as 4.175 µg/day as follows:

$$Selenium\ Estimated\ Exposure = \frac{16.7\ g\ cultivated\ pork\ fat\ cells}{day} * \frac{25\ \mu g\ selenium}{100\ g\ cultivated\ pork\ fat\ cells} = 4.175\ \frac{\mu g\ selenium}{day}$$

The estimated selenium exposure represents less than 2% of the UL established by EFSA, providing further support to the conclusion that Mission Barns' intended use of sodium selenite does not pose a potential hazard.

Adventitious Agent Hazard Analysis and Testing

13) On page 17 of the June 5, 2023, amendment you state, "Mission Barns has updated its assessment to remove *Listeria monocytogenes* as a microorganism of concern" and cite the fact that "Regular EMP test data for Mission Barns' GMP manufacturing facility collected over more than a year-long period has resulted in zero occurrences of *Listeria*" as part of your rationale for removing *L. monocytogenes* from the environmental monitoring tests. In footnote 46 on the same page, you state, "Mission Barns no longer conducts regular testing for *Listeria* as part of its EMP as it is currently not considered a microorganism of concern." Please provide, for addition to the disclosable safety narrative:

   a. The identity of any additional indicator organisms tested for as part of the environmental monitoring program, results of analytical testing, and mitigation strategies that are in place to control for potential biological hazards, including discussion of other pathogens of concern from the environment, such as *Bacillus cereus*.

   b. Further discussion of why you no longer conduct regular monitoring of *L. monocytogenes*. We note that the absence of the organism from environmental monitoring in one year is not sufficient to demonstrate that *L. monocytogenes* may not pose a safety concern in your

[34] The mineral was analyzed by a third-party laboratory according to AOAC 2015.01 Mod<2232>.

[35] Reported ranges are the lowest and highest reported value from USDA FoodData Central for NDB Number 10004 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167811/nutrients, accessed 11/29/22), NDB Number 10006 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167813/nutrients, accessed 11/30/22), NDB Number 10942 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169179/nutrients, accessed 11/30/22), NDB Number 10109 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167861/nutrients, accessed 11/30/22), NDB Number 10007 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/168221/nutrients, accessed 5/29/23), NDB Number 10167 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/167878/nutrients, accessed 4/24/2024), NDB Number 10894 (available at https://fdc.nal.usda.gov/fdc-app.html#/food-details/169155/nutrients, accessed 4/24/2024) .

process. This discussion could include other indicator tests that are performed, other controls in place to identify environmental contamination, as well as a data-based discussion of the processing environment that justifies removing *L. monocytogenes* from regular monitoring.

Mission Barns' Response:

For addition to the disclosable safety narrative:

a. Mission Barns has implemented an environmental monitoring program (EMP) to ensure product safety and quality which entails the systematic sampling and testing of the production environment for potential sources of contamination, such as pathogens. Mission Barns regularly evaluates its EMP to identify any trends, patterns and opportunities for improvement. Adjustments to the EMP may include changes in the frequency of samplings, sample site locations, and/or the addition or elimination of organisms tested for based on a combination of test results, food industry practices, regulatory guidelines, and/or scientific justifications. The following indicator organisms are currently monitored at the Mission Barns' cultivated cell manufacturing facility: *Bacillus cereus, Staphylococcus aureus, coliforms, and Ralstonia insidiosa*. The table below summarizes EMP test results for these organisms.

EMP Testing Period: JUN 2023 - MAR 2024			
Organism	**Total of EMP Samples**	**Number of Positive IDs**	**Method Reference**
Bacillus cereus	146	14	AOAC 980.31
Coliforms	74	1	AOAC 991.14
Staphylococcus aureus	226	0	AOAC 2003.07
Ralstonia insidiosa	254	17	AOAC 966.23, AOAC 990.12, QA-0095-3000 GeneSeq CMMEF, 4th ed., AOAC 2012.02

In the event of any out-of-specification (OOS) EMP test result, mitigation strategies are in place to control microbiological hazards which include equipment and facility cleaning/decontamination procedures, quarantining of impacted equipment until successful cleaning verification sampling and testing is completed, and deviation and corrective and preventive action (CAPA) programs to investigate contaminations and implement corrective and preventive actions to address the OOS events.

As noted above, as of the date of this Amendment there have been a total of fourteen (14) *Bacillus cereus*, one (1) coliform, zero (0) *Staphylococcus aureus*, and seventeen (17) *Ralstonia insidiosa* OOS EMP results for the manufacturing facility.

B. cereus is a Gram-positive aerobic or facultatively anaerobic, spore-forming, bacterium that is widely distributed environmentally and is associated with foodborne illnesses in humans. An investigation to determine the root cause of the *B. cereus* contaminations in the manufacturing environment did not result in any conclusive findings. As of the date of this Amendment, there have been zero (0) bioreactor or cell harvest product contaminations from *B. cereus*. Out of an abundance of caution, *B. cereus* was added as a monitored organism to Mission Barns' positive release program.

Coliforms are characterized as Gram-negative, non-spore-forming anaerobic bacteria commonly found in soil, water and the intestinal tract of animals. Coliform counts serve as an indicator of hygienic/sanitary conditions. An investigation into the single coliforms OOS EMP result that occurred in the manufacturing area (floor) determined the most probable source of the

contamination was dirt/debris tracked into the manufacturing area from an improperly sanitized cart. Following implementation of an improved cart sanitation procedure, there have been zero (0) OOS EMP test results related to coliforms.

R.insidiosa is a Gram-negative, bacterium found naturally in ponds, rivers, soils, contaminated water and sludge, and has been known to be pathogenic to immunocompromised patients in hospital settings. An investigation determined that contaminated water recirculators used inside the facility were the source of *R.insidiosa*. As a corrective action, these recirculators were removed from the manufacturing space and decontamination cleanings were performed. While not a typical foodborne pathogen, out of an abundance of caution, *R.insidiosa* was added to the EMP sampling plan as a monitored organism.

b. In Q1 2023, *Listeria spp.* was removed from the EMP program as a monitored organism on the following bases:

   i. EMP results which demonstrated four consecutive quarters of monitoring (representing a total of 96 samples of Mission Barns' production environment) where no Listeria spp. organisms were detected (see table below);

EMP Testing Period: JUL 2022 - MAR 2023			
Organism	**Total of EMP Samples**	**Number of Positive IDs**	**Method Reference**
Listeria spp.	96	0	AOAC 2013.10

   ii. A reevaluation of the monitoring program in Q1 2023 as part of Mission Barns' continuous improvement efforts which concluded that Listeria (*L. monocytogenes*, specifically) was not a contamination risk it was initially believed it could be when the program was first established in Q1 2022. Mission Barns' cleanroom production environment does not have any of the common sources of Listeria contamination found in traditional food manufacturing facilities, such as wet processing environments, raw produce, live or decaying animal materials, or fecal matter. Mission Barns holds that its increased emphasis on stringent cleaning and sanitation protocols, combined with a deeper assessment of Listeria's common transmission vectors, supports the removal of Listeria spp. from the list of organisms of concern for its operations. Mission Barns strives to follow a risk-based, science-driven approach for its environmental monitoring program and is committed to ensuring its products are safe and of high quality.

Composition

15) In Table 2 of the March 16, 2022, amendment you provide specifications for cadmium (< 100 ppb), lead (< 100 ppb), arsenic (< 100 ppb), and mercury (< 50 ppb), as well as results for these toxic heavy metals from three batches of harvested cell material. We note that the reported levels from the three batches for all elements are reported as < 10 ppb, indicating levels below the limit of detection. For inclusion in the disclosable safety narrative, please consider lowering the specification limits for the four toxic heavy metals to a limit that is more representative of the results of the batch analyses (e.g., 50 ppb or lower).

Mission Barns' Response:

Mission Barns refers to our response to information request #22 of the March 6, 2023, amendment to the disclosable safety narrative. We confirm that we have already lowered the specification limits for the four heavy metals to the suggested levels.

Mission Barns' Cultivated Pork Fat Cells Batch Release Criteria - Heavy Metals				
Heavy Metal	Cadmium	Lead	Arsenic	Mercury
Specification	< 50 ppb	< 50 ppb	< 50 ppb	< 25 ppb

16) We request that you further describe at what point in the harvest process analytical testing is performed (e.g., for proximates, heavy metals, residue of media components). As an example, the March 16, 2022, amendment indicates that harvested cells are removed from the cultivator, washed and then pelleted. Are all analytical tests performed on the pelleted material? Are any tests performed on the harvested material before the pelleting process?

Mission Barns' Response:

Mission Barns confirms that all analytical tests performed on harvested cells are always performed after the cells are washed and pelleted. No tests are performed on the harvested cells before the pelleting process.

Mission Barns clarifies that certain analytical tests were not performed on harvested cells, but are instead performed on spent media or final wash solution. This is the case for:

- **Spent media and final wash solution**
    - Cell culture media and harvest reagent residual testing via surrogate protein was performed on spent media and final wash solution
- **Spent media**
    - Microbiological testing was performed on final spent media
    - Antimicrobials residuals were analyzed in the final wash solution
    - Growth factors residuals were analyzed in the final wash solution. Two representative growth factors (one that has the highest use concentration and one that is the most thermally stable) were also tested in harvested cell material.

Mission Barns also clarifies that the following tests were performed on pelleted cell material that was then diluted to reach the minimum quantity required for the applicable test. The analytical results were then multiplied by their respective dilution factors to calculate the actual concentration of each analyte (which were reported in the June 5, 2023 amendment to the disclosable safety narrative):

Test	Dilution factor	Diluent
Amino acids	2.5x	DPBS -/-
Vitamins	1.25x	DPBS -/-

Minerals	2.5x	Cell culture grade water

17) Table 2 of the March 16, 2022, amendment provides results for heavy metals in the harvested cell material. COAs for the toxic heavy metal testing is provided in Attachment 4 of the March 16, 2022, amendment. Please clarify if the tests were performed on pelleted harvested cell material. If not, please provide results based on levels present in the pelleted harvested cell material. On page 10 of the September 26, 2023, amendment, you provide information on moisture, fat, protein, ash, and carbohydrates of the harvested cell material. Please clarify whether these tests were performed on pelleted harvested cell material.

Mission Barns' Response:

Mission Barns confirms that all heavy metals testing and proximates testing (moisture, fat, protein, ash, and carbohydrates) is performed on undiluted pelleted harvested cell material.

Food Safety Management System

18) On page 5 of the June 5, 2023, amendment you state, "All cell culture vessels used by Mission Barns are either single use vessels that are purchased sterile from a third party with an accompanying COA or are stainless steel and are cleaned and sterilized using high temperature steam (>121°C). Environmental monitoring is used to evaluate the overall hygienic status of the manufacturing environment." For addition to the disclosable safety narrative, please provide a discussion of the process to clean/sterilize the bioreactors between production runs and daily sanitation protocol; e.g., the process you will use to clean/sterilize, the frequency of cleaning or sterilizing, and discussion on whether and how you plan to validate the cleaning process, as well as any monitoring activities.

Mission Barns' Response:

After the completion of a manufacturing run and prior to the next run, bioreactors used in Mission Barns' cell manufacturing process are cleaned and sterilized by trained personnel inside a dedicated room within the manufacturing facility. The cleaning process entails disassembling the bioreactor, soaking the parts in a dilute detergent solution for no less than 30 minutes, manually scrubbing the parts to remove process soils and then rinsing the parts with municipal water and then with high purity water (reverse osmosis deionized water or cell culture grade water) as a final rinse. To verify the effectiveness of the cleaning, each part is visually inspected for cleanliness and conductivity samples of the final rinse water are tested to verify the removal of the residual cleaning solution. Following cleaning, each bioreactor is reassembled with its associated parts and sterilized via high pressure steam using a validated sterilization cycle. All bioreactor cleanings and sterilizations are performed using approved standard operating procedures (SOPs) and documented on controlled forms that allow for the traceability of each bioreactor cleaning and sterilization event.

While Mission Barns has validated its sterilization process, Mission Barns has not and does not intend to formally validate its manual cleaning process. Mission Barns does not believe validation of its manual cleaning process is necessary, based on the combination of the following: 1) Two years of extensive bioreactor culture performance experience which supports the efficacy of the current cleaning process; 2) Use of a thorough post-cleaning visual inspection protocol to verify equipment surfaces are clean and residue-free, along with the common pharmaceutical method of performing conductivity testing of final rinse water to verify the removal of cleaning agents (i.e., verification that final rinse water conductivity level is comparable to the conductivity of source high purity water/control used in the final rinse step); 3) Implementation of manual cleaning and visual inspection training requirements for all personnel

performing bioreactor cleanings to ensure cleanings are performed properly; and 4) Utilization of a validated steam sterilization cycle as a terminal sterilization step of the bioreactor prior to its use in a production run which provides confidence that a sterility assurance level (SAL) of 10^{-6} is achieved.

Monitoring of the performance and completeness of bioreactor cleaning and sterilization processes is performed through the regular review of all associated documentation by both Manufacturing management and Quality.

19) For addition to the disclosable safety narrative, please provide a summary narrative of traceability program in your facility, including a discussion on the inventory controls for cell lines and substances used during the cell culture process.

Mission Barns' Response:

As part of its food safety and quality management system, Mission Barns has implemented a comprehensive traceability program that works in conjunction with an inventory management system to track the movement and status of raw materials, intermediates and products from procurement through the production and distribution chain. Traceability begins with inventory controls that include the labeling of every material from the company's cell banks, cell culture media and supplements, processing aids, to harvested cell material with unique identifiers (material ID and lot numbers) and linking those materials with relevant information such as the supplier/manufacturer, quantities, location, expiries, COAs, and QA release status. All materials used in Mission Barns' cell manufacturing process undergo material onboarding and supplier approval processes to ensure they meet pre-established safety and quality requirements. Only materials or final products that have successfully passed an internal Quality Control review that includes meeting positive release specifications are released into manufacturing or are distributed. As materials move through the supply chain, they are tracked using controlled documentation such as batch production records that are reviewed for accuracy and completeness. Traceability exercises and mock recalls are performed periodically to ensure the accuracy and effectiveness of the traceability program in the event of a material or product recall.

20) For addition to the disclosable safety narrative, provide a summary or discussion of your supplier control program.

Mission Barns' Response:

Mission Barns' Supplier Control Program establishes criteria for the selection, risk-based evaluation, and ongoing monitoring of its suppliers to ensure that all supplier-sourced materials are safe and compliant with regulations. Suppliers are qualified by Mission Barns only after an extensive review of the supplier's quality documentation (e.g., Certificates of Analysis or Compliance, Letters of Guarantee certifications) and quality management system is completed. Verifications are performed to ensure supplier products that Mission Barns utilizes in its process meet established physical, chemical, biological and regulatory compliance specifications.

21) For addition to the disclosable safety narrative, please provide a summary of other programs in your food safety plan which are important to Mission Barns' food safety management strategies but have not been discussed in disclosable safety narrative. A thorough discussion in the disclosable safety narrative regarding the food safety plan implemented by Mission Barn to address food safety risks during production will provide additional support for the overall public safety conclusion.

Mission Barns' Response:

Mission Barns is committed to producing safe, high-quality cultivated pork fat cells for use in human food products. A Food Safety Plan (FSP) has been established by the company as a risk-based approach to ensure the safety and quality of our products while adhering to applicable regulatory requirements.

Mission Barns' FSP integrates both prerequisite programs and preventive controls as part of its overall Food Safety and Quality Management System (FSQMS), to manage food safety risks effectively. The table below summarizes the prerequisite programs/procedures and preventive controls implemented by Mission Barns to address safety risks associated with its cultivated cell manufacturing process.

Prerequisite Programs (PRPs) and Procedures	Description
1. **Document Control**	Mission Barns' document control program establishes processes for the creation, revision, approval, distribution, and management of documents including document identification and classification, version control, controlled access, archiving/retention, audits, and change control procedures.
2. **Employee Training**	This program ensures that all employees involved in cultivated cell manufacturing processes receive adequate training on GMP, hygiene standards, and job-specific responsibilities.
3. **Personnel Hygiene & Gowning**	This procedure describes the personal hygiene requirements and protective equipment controls in place for all personnel working in manufacturing areas in order to minimize the risk of potential cross-contamination during cell cultivation activities.
4. **Cleaning and Sanitation**	This program establishes the procedures for cleaning and sanitizing equipment, utensils, surfaces, and the production area. It includes the selection of appropriate cleaning agents, cleaning schedules, cleaning methods, and verification of cleanliness to ensure the removal of contaminants and maintain a hygienic environment.
5. **Supplier Approval & Control**	This program ensures that all raw materials and processing aid suppliers meet specific quality standards. It establishes criteria for supplier selection, evaluation, and ongoing monitoring to ensure that the sourced materials are safe and compliant with regulations.
6. **Materials Management**	This program ensures the proper receiving, inspecting, and storing and release of raw materials, ingredients, and finished products. It includes temperature control during storage and distribution to prevent spoilage and contamination. The program establishes procedures for inventory control, first-in-first-out (FIFO) practices, and prevention of cross-contamination to maintain the integrity of materials used in production.
7. **Water Monitoring**	Process water quality is monitored to ensure that water utilized in cultivated cell production activities and food processing and preparation meets established specifications for purity or potability, and potential contaminants.
8. **Transportation and Distribution**	This procedure describes the requirements and controls implemented to ensure the safe, secure and traceable transport and delivery of cell and finished good products.
9. **Preventive Maintenance and Calibration**	This program establishes the schedule and procedures for maintaining production equipment, including calibration status, to ensure its proper functioning and prevent potential hazards caused by equipment malfunctions.
10. **Facility & Grounds Maintenance**	This program describes the requirements and practices for the regular maintenance and inspection of the manufacturing facility and surrounding grounds to ensure a safe and hygienic environment. It includes procedures for repairing and preventing physical hazards that may compromise food safety.
11. **Cell Manufacturing Validation and Qualification Policy**	This policy describes the requirements and approach for the validation of critical manufacturing equipment to ensure that equipment used in various processes meets predetermined standards, performs reliably, and consistently produces results within specified criteria.
12. **Pest Control**	This program establishes a procedure for preventing and controlling pests within the production facility. It includes regular inspections, preventive measures, and appropriate pest management methods to ensure that pests do not contaminate food products.
13. **Chemical Handling & Disposal**	This procedure defines the controls to safely and responsibly manage chemicals used in cell cultivation, food processing, cleaning, and sanitation, including the proper storage, handling, and disposal of chemicals to prevent contamination of cell/food products, equipment, and the environment.

14. Waste Management	This program outlines procedures for the proper handling, storage, and disposal of waste materials generated during the production process. It ensures that waste does not contribute to contamination or create potential food safety risks.
15. Foreign Materials Control	This procedure is established to prevent the presence of foreign materials, such as glass, metal, or plastic, being introduced into the cell culture manufacturing process.
16. Allergen Control	This program is established to prevent any major food allergens from contaminating cultivated cells or food products. Milk, eggs, fish, crustaceans, shellfish, tree nuts, peanuts, wheat, sesame and soybeans are not received, stored, nor utilized at any point in the cultivated cell manufacturing process at this facility.
17. Product Recall, Withdrawal and Stock Recovery Events **18. Mock Recall and Traceability Plan**	These procedures outline the process for tracing products through the supply chain and implementing recalls if necessary. It ensures the ability to identify and remove potentially unsafe products from the market promptly.
19. Consumer Complaints	The procedure establishes a process for managing and investigating consumer complaints related to product quality, safety, or labeling. It includes a process to address consumer complaints and taking corrective actions to prevent reoccurrence.
20. Food Defense & Security	This program addresses the protection of cell and food products from intentional contamination or tampering, whether by internal or external threats and is designed to safeguard the integrity and safety of cell and food products.
21. Audit Program	This program describes Mission Barns' approach to assessing and evaluating various aspects of the company's operations and supply chain related to food safety, quality, and regulatory compliance.
Preventive Controls (PCs)	**Description**
1. Use of steam sterilized product-contacting equipment and pre-sterilized consumables	This preventive control involves autoclaving cell cultivator (bioreactor) systems using a validated steam sterilization cycle prior to manufacturing use. Additionally, pre-sterilized consumables, such as single-use containers, filters, and tubing, are used to prevent potential contamination and ensure the integrity and safety of the final product.
2. Filtration sterilization of process fluids/reagents	As a crucial preventive control measure, filtration sterilization is implemented to purify process fluids and reagents utilized during the cultivation of cells. The process involves passing these substances through specialized filters (e.g., 0.2 micron filters) designed to remove microorganisms.
3. Use of antimicrobials (Cell Banking only)	In cell banking processes, where cell lines are maintained and preserved for future use, the use of antimicrobials serves as an essential preventive control. Antimicrobials, such as antibiotics or antifungal agents, are carefully applied to prevent the growth and proliferation of potentially harmful microorganisms within the cell cultures. This measure ensures the viability and stability of the cell lines, safeguarding the integrity and consistency of the final product.
4. Use of aseptic cell culture techniques and HEPA-supplied production environments (cleanrooms, biological safety cabinets, laminar flow hoods)	To maintain the sterility and purity of cell cultures during production, a combination of aseptic techniques and controlled environments is employed. Aseptic cell culture techniques involve handling cells and biological materials in a manner that prevents contamination, while HEPA-supplied production environments, such as cleanrooms, biological safety cabinets, and laminar flow hoods, provide highly filtered air to maintain aseptic working conditions. This preventive control ensures the protection of cell cultures from external contaminants.
5. Washing of harvested cells	The washing of harvested cells is a critical process step designed to remove processing aid residuals. After cells are harvested from culture systems, they are carefully washed with sterile solutions to remove residual media, by-products, or impurities. This washing step helps to eliminate potential contaminants and ensures that the harvested cells are of high quality and ready for further processing.

California Department of Food and Agriculture
1220 N St A 107
Sacramento, CA 95814
Phone 916 900 5002
Fax: 916-900-5333
http://www.cdfa.ca.gov//ahfss/Animal_Health/Entry_Requirements.html

CERTIFICATE OF VETERINARY INSPECTION

Contact State of Destination for Movement Requirements and Certificate Validity
FOR FOREIGN SHIPMENTS (Outside United States or Leaving United States) USE FEDERAL FORM
For Interstate Travel - Certificate Valid for 30 days from Inspection

CERTIFICATE NUMBER
22-CA-19307545

INSPECTION DATE	ISSUE DATE	ENTRY PERMIT NUMBER	BRAND INSPECTION NUMBER & ISSUE DATE
2022-10-24	2022-10-24		|

ORIGIN OF SHIPMENT	CONSIGNOR, PRESENT OWNER OF SHIPMENT	DESTINATION OF SHIPMENT	CONSIGNEE, NEW OWNER OF SHIPMENT	CARRIER, TRANSPORTER
Mission Barns 1155 Bryant St San Francisco, CA 94103 Phone: [redacted] PIN/LID: /	**Mission Barns** 1155 Bryant St San Francisco, CA 94103 Phone: [redacted] PIN/LID: /	**Sweet Farm** 210 Hall Road Himrod, NY 14842 Phone: [redacted] PIN/LID: /	**Sweet Farm** 210 Hall Road Himrod, NY 14842 Phone: [redacted] PIN/LID: /	**Mission Barns** 1155 Bryant St San Francisco, CA 94103 Phone: [redacted] PIN/LID: /

SPECIES - NUMBER IN SHIPMENT	PURPOSE(S) OF MOVEMENT	CARRIER TYPE	HERD STATUS NUMBER	HERD FREE FOR	CURRENT STATE/AREA STATUS
Swine (Market) - 2 animals	Adoption	Truck/Trailer		freeForTb | freeForJohnes | freeForScrapie | freeForBruc | freeForPrv | freeForNpip	Tuberculosis-Free | Brucellosis-Free | Pseudorabies-Free

REMARKS/ADDITIONAL CERTIFICATION STATEMENTS
I have examined all animals listed on this certificate of veterinarian inspection and included within this shipment and found them to be free from clinical signs of infections or contagious diseases. | All animals identified on this health certificate have been examined and found to be free from vesicular stomatitis and, to the best of my knowledge and belief, during the past 30 days these animals have not been exposed to said disease, nor held at a location within ten miles of any place in which said disease has been found to exist. | At the time of examination, the animal/s listed on this certificate did not have evidence of live ticks or was/were successfully treated for ticks if ticks were present.
Shipping Date: 2022-10-25

[redacted] **Color:** White | **Gender:** Female | **Breed:** American Yorkshire | **Head Count:** 2

Official ID Types: Brand,Brand | **IDs:**
1657, 1658

Remarks: White ear tags with visual ID number
Brand Description: Allflex

OWNER / AGENT STATEMENT
The animals in this shipment are those certified to and listed on this certificate.
Signature **Date**

OFFICIAL USE ONLY
The Veterinarian issuing this certificate is accredited and has been authorized to inspect animals and issue certificates

VETERINARIAN'S SIGNATURE:
This is a legally binding equivalent of a handwritten signature
[redacted]

VETERINARIAN CERTIFICATION - I certify, as an accredited Veterinarian, that the above animals have been inspected by me and that they are not showing signs of infectious contagious and/or communicable disease (except where noted) The vaccinations and results of tests are indicated on the certificate. To the best of my knowledge, the animals listed on this certificate meet the state of destination and federal interstate requirements No further warranty is made or implied

[redacted]
License Number and State
National Accreditation Number
[redacted]

General Workup

Thursday, October 27, 2022

Patient Name: 1657 Mission Barns, [redacted], Yorkshire, [redacted]
[redacted] Technician: [redacted]

History (Subjective):

1) No active health concerns on presentation.
2) Eating and drinking normally, normal activity level, normal BMs and urination.
3) Free of Tb, Johnes, Scrapie, Brucellosis, Prv, Npip, and Pseudorabies.

Exam (Objective):

Nose and Throat ☑ Normal ☐ Did Not Exam ☐ Abnormal Remarks: __	**Mouth/Teeth/Gum** ☑ Normal ☐ Did Not Exam ☐ Abnormal Remarks: __ Periodontal Stage:
Eyes and Ears ☑ Normal ☐ Did Not Exam ☐ Abnormal Remarks: __	**Coat and Skin** ☑ Normal ☐ Did Not Exam ☐ Abnormal Remarks: __
Lymph Nodes ☑ Normal ☐ Did Not Examine ☐ Enlarged Remarks: __ ☐ Abnormal Remarks: __	**Legs/Paws/Back** ☑ Normal ☐ Did Not Exam ☐ Nail Trim ☐ Abnormal Remarks: __
Nervous System ☑ Normal ☐ Did Not Examine ☐ Abnormal Remarks: __	**Heart and Lungs** ☑ Normal ☐ Did Not Examine ☐ Heart Murmur Grade __/VI Murmur Comments: __ ☐ Abnormal Remarks: __
GI Tract/Abdominals ☑ Normal ☐ Did Not Examine ☐ Abnormal Remarks: __	**Urinary And Genitals** ☑ Normal ☐ Did Not Examine ☐ Expressed Anal Glands ☐ Abnormal Remarks: __

Assessment & Plan

Assessment	**Healthy animal __**
Plan	**_1) Surgical biopsy under general anesthesia and transfer to animal sanctuary after 3 day supervision. _**

[redacted]

Accession: **2022100985**

Final Report
Accessed Date: 11/07/2022 7:14 am

[redacted]

Site : SWEET FARM
210 HALL RD
HIMROD, NY 14842

Premises ID# :

Lot/Group ID :
Source/Flow ID :
Reference :
Case Tags :

Owner : MISSION BARNS
Diagnostician : [redacted]

Client Phone: [redacted]
Client Fax: N/A
Client Account#: 000406840
Date Received: 10/28/2022
Sample Taken: 10/25/2022
Accompanying Cases:

Species: Porcine
Breed: Unknown
Sex: Female
Previous Case:
Farm Type: Non-Commercial ...
Animal ID(s): 1657 ..., 1657 ..., 1657 ..., 1658 ..., 1658 ...

[redacted]
Weight:
Received:
5 Sera, 2 Oral Fluids
Reason: General Diagnostics

Final Report(s): 11/07/2022 07:14 am

Molecular:
Test results listed below. (11/04/22 kw/mf)

[redacted]
Katie Woodard, DVM, MS
Veterinary Specialist
Molecular Diagnostic Case Coordinator
Client Outreach and Education

Serology:
*Please see table below for result interpretation for PRRS OF IgA/IgM.

PRRSV ELISA antibody target	Cutoff (S/P)	Diagnostic sensitivity (95% CI)	Diagnostic specificity (95% CI)
IgA	0.4	0.66 (0.61, 0.71)	0.98 (0.97, 0.99)
	0.5	0.63 (0.58, 0.68)	0.99 (0.98, 0.99)
	0.6	0.60 (0.55, 0.65)	0.99 (0.98, 0.99)
IgM	0.4	0.50 (0.44, 0.56)	1.00 (0.99, 1.00)
	0.5	0.45 (0.39, 0.51)	1.00 (0.99, 1.00)
	0.6	0.42 (0.36, 0.48)	1.00 (0.99, 1.00)
Combined IgM-IgA	0.40	0.74 (0.69, 0.78)	0.99 (0.98, 0.99)
	0.50	0.72 (0.67, 0.77)	0.99 (0.99, 1.00)
	0.60	0.69 (0.64, 0.74)	1.00 (0.99, 1.00)

Rotolo et al (2018).Veterinary Microbiology. Vol 214. pp. 13-20 (11/04/22 lgl/rml)

Virology:
ANTIBODY TITER RESULT INTERPRETATION

Samples that generate titers with a numerical value are considered positive for antibody detection to the stated agent at the reported dilution of the tested sample.
The (>) symbol would indicate the sample is positive for antibody detection at the highest sample dilution tested.
The (<) symbol would indicate the sample antibody level is below the detection sensitivity of this assay at the beginning sample dilution, therefore negative at this dilution. (11/07/22 jq/mf)

[redacted]

Jianqiang Zhang, MD, MS, PhD
Professor
Virologist

KEY: Tests: FA = Fluorescent Antibody, IHC = Immunohistochemistry, ISH = *in situ* hybridization, MALDI = Matrix-assisted laser desorption/ionization, MLV = Modified Live Virus, ORF = Open Reading Frame, PCR = Polymerase Chain Reaction, RFLP = Restriction Fragment Length Polymorphism, VI = Virus Isolation. Agents: BCV = Bovine Coronavirus, BHV = Bovine Herpesvirus, BRSV = Bovine Respiratory Syncytial Virus, BVDV = Bovine Viral Diarrhea Virus, CSF = Classical Swine Fever, GPS = *Glaesserella* (*Haemophilus*) *parasuis*, IAV = Influenza A Virus, MHP = *Mycoplasma hyopneumoniae*, MHR = *Mycoplasma hyorhinis*, MHS = *Mycoplasma hyosynoviae*, PCV = Porcine Circovirus, PDCV = Porcine Deltacoronavirus, PEDV = Porcine Epidemic Diarrhea Virus, PPV = Porcine Parvovirus, PRCV = Porcine Respiratory Coronavirus, PRRSV = Porcine Reproductive & Respiratory Syndrome Virus, PRV = Pseudorabies Virus, SVA = Senecavirus A, TGEV = Transmissible Gastroenteritis Virus.

Laboratory Result(s)

Test Ordered	Order Date	Current Status	Complete Date
APP CF screen (no serotyping)	10/28/2022	Result Released	11/03/2022
Brucella BAPA SCREEN	10/28/2022	Result Released	10/28/2022
Influenza A Virus NP Elisa	10/28/2022	Result Released	10/28/2022
LEPTO 5 MAT	10/28/2022	Result Released	11/01/2022
PCR - M. hyopneumoniae	10/28/2022	Result Released	10/28/2022
PCR - PEDV Applied Biosystems	10/28/2022	Result Released	10/28/2022
PRRSV OF Ab	10/28/2022	Result Released	10/28/2022
PRRSV OF IgM/IgA ELISA - R&D	10/28/2022	Result Released	10/31/2022
PRRSV X3 ELISA	10/28/2022	Result Released	10/28/2022
PRV gB - ELISA SCREEN	10/28/2022	Result Released	10/28/2022
Transmissible Gastroenteritis Virus VN	10/28/2022	Result Released	11/02/2022

Molecular Diagnostic

PCR - M. hyopneumoniae

Animal ID	Specimen	Ct / Result	Comment
1658 [redacted], SID #6	Oral fluid	>=37 / Negative	
says 1657 [redacted], SID #7	Oral fluid	>=37 / Negative	

PCR - PEDV Applied Biosystems

Animal ID	Specimen	PEDV / Result	Comment
1658 [redacted], SID #6	Oral fluid	>=36 / Negative	
says 1657 [redacted] SID #7	Oral fluid	>=36 / Negative	

Serology

TITER RESULT INTERPRETATION
Samples that generate titers with a numerical value are considered positive for antibody detection to the stated agent at the reported dilution of the tested sample.
The (>) symbol would indicate the sample is positive for antibody detection at the highest sample dilution tested.
The (<) symbol would indicate the sample antibody level is below the detection sensitivity of this assay at the beginning sample dilution, therefore negative at this dilution.

Brucella BAPA SCREEN

Animal ID	Specimen	Result
1657 , SID #1	Serum	Neg
1657 , SID #2	Serum	Neg
1657 SID #3	Serum	Neg
1658 , SID #4	Serum	Neg
1658 , SID #5	Serum	Neg

LEPTO 5 MAT

Serology Grid

Animal ID	SID #	Seq	Lepto Canicola Titer / Result	Lepto Grippo Titer / Result	Lepto Hardjo Titer / Result
1657	SID #1		<100 /	<100 /	<100 /
1657	SID #2		<100 /	<100 /	<100 /
1657	SID #3		<100 /	<100 /	<100 /
1658	SID #4		<100 /	<100 /	<100 /
1658	SID #5		<100 /	<100 /	<100 /

Serology Grid

Animal ID	SID #	Seq	Lepto Ictero Titer / Result	Lepto Pomona Titer / Result
1657	SID #1		<100 /	<100 /
1657	SID #2		<100 /	<100 /
1657	SID #3		<100 /	<100 /
1658	SID #4		<100 /	<100 /
1658	SID #5		<100 /	<100 /

PRRSV OF Ab

Caution: The PRRS OF Ab Assay may detect antibodies against PRRSV in samples collected from pigs consuming diets containing spray dried plasma of porcine origin.

Animal ID	SID	S/P / Result
1658	SID #6	0.036 / Neg
says 1657	SID #7	0.028 / Neg

PRRSV OF IgM/IgA ELISA - R&D

Caution: The PRRS OF Ab Assay may detect antibodies against PRRSV in samples collected from pigs consuming diets containing spray dried plasma of porcine origin.

Animal ID	SID	S/P / Result
1658	SID #6	0.109 / *
says 1657	SID #7	0.000 / Qns

Serology Grid

Animal ID	SID #	Seq	APP CF SCREEN Titer / Result	IAV NP S/N / Result	PRRSV X3 S/P / Result
1657	SID #1		<4 /	0.870 / Neg	0.002 / Neg
1657	SID #2		<4 /	0.857 / Neg	0.000 / Neg
1657	SID #3		<4 /	0.874 / Neg	0.002 / Neg
1658	SID #4		<4 /	0.900 / Neg	-0.002 / Neg

1658 [redacted]	SID #5	<4 /		1.015 / Neg	0.000 / Neg

Serology Grid

Animal ID	SID #	Seq	PRV gB S/N / Result
1657 [redacted]	SID #1		0.905 / Neg
1657 [redacted]	SID #2		0.939 / Neg
1657 [redacted]	SID #3		0.895 / Neg
[redacted]			
1658 [redacted]	SID #4		0.943 / Neg
1658 [redacted]	SID #5		0.934 / Neg

Virology

Transmissible Gastroenteritis Virus VN

Animal ID	Specimen	Titer	Comment
1657 [redacted]	Serum	<4	
1657 [redacted]	Serum	<4	
1657 [redacted]	Serum	<4	
[redacted]			
1658 [redacted]	Serum	<4	
1658 [redacted]	Serum	<4	

Animal ID Information

SID #	Animal ID	Age	Gender	Location	Parity
SID #1	1657 [redacted]	[redacted]	Female		
SID #2	1657 [redacted]		Female		
SID #3	1657 [redacted]		Female		
SID #4	1658 [redacted]		Female		
SID #5	1658 [redacted]		Female		
SID #6	1658 [redacted]		Female		
SID #7	[redacted] 1657 [redacted]				

Vaccination History

Company:	Grand Total	Delivery Date
Mission Barns	2	10/24/22

Tag #	Ear Notch #	Sex	Weight	Date of Birth	Vaccination 1 Date	Vaccination 2 Date	Vaccination 3 Date	Vaccination 4 Date	Pen #
1657	65 - 4	Fe	[redacted]	[redacted]	09/22/22	09/29/22			27N
1658	70 - 2	Fe	[redacted]	[redacted]	09/22/22	09/29/22			27N

Breed: Domestic Invoice #: 15326 Doctor:

Vacc1

Rhini Shield TX4
PCV MH

Vacc2

H+S

Vacc3

Enterisol Ileitis

Customer Info	Delivered By:
Delivery Address	[redacted]
	Driver
Special Directions	[redacted]
	Vehicle
	[redacted]

Vaccine Info

Ivermax (or equivalent): 1 ml intramuscularly to piglets upon weaning
Rhini Shield TX4 (or equivalent): 1 ml intramuscularly at 14+ days of age and 2ml at 28+ days of age (Bordetella Bronchiseptica, Erysipelothrix Rhusiopathiae, Pasteurella Multocida Bacterin, Toxoid)
PCV MH (or equivalent): 2 mL intramuscularlly at 21+ days old (Porcine Circovirus Type 1 and Type 2 Chimera, Mycoplasma Hyopneumoniae Bacterin)
H+S (or equivalent): 1 ml intramuscularly at 14+ days old and 2 ml at 28+ days of age (Haempophilus parasuis-Streptococcus suis)
Enterisol Ileitis (or equivalent): 2 ml orally to piglets at 35+ days old (Lawsonia Intracellularis)

[redacted]

☒ E-mail to: [redacted]

☐ Hard copy to:

Date results transmitted: 08may24

Assay Results Report **24043002b**

Client: Mission Barns	Client #: [redacted]
[redacted]	PO #: [redacted]
Date samples received: 30apr24	

Initials [redacted]

Client sample ID	[redacted] accession ID	Sample type	Assay	Assay result
[redacted]	2404300001	Frozen cell pellet	B0002	**Negative**
[redacted]	2404300001	Frozen cell pellet	B0051	**Negative**
[redacted]	2404300001	Frozen cell pellet	B0075	**Negative**
[redacted]	2404300001	Frozen cell pellet	B0099	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0064	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0066	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0071	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0077	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0121	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0165	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0167	**Negative**
[redacted]	2404300001	Frozen cell pellet	S0213	**Negative**

Assay descriptions and notes

Assay B0002: Ultrasensitive qualitative detection of *Mycoplasma pneumoniae* by real time PCR

Assay B0051: Ultrasensitive qualitative detection of *Leptospira* by real time PCR

Assay B0075: Ultrasensitive qualitative detection of *Brucella* by real time PCR. Assay detects but does not differentiate *Brucella abortus, B. microti, B. melitensis, B. pinnipedialis, B. suis, B. canis, B. ovis* and *B. neotomae*

Assay B0099: Ultrasensitive qualitative detection of *Actinobacillus pleuropneumoniae* by real time PCR

Assay S0064: Ultrasensitive qualitative detection of equine infectious anemia by real time PCR

Assay S0066: Ultrasensitive qualitative detection of equine arteritis virus by reverse transcription coupled real time PCR

Assay S0071: Ultrasensitive qualitative detection of equine herpesvirus type I by real time PCR

Assay S0077: Ultrasensitive qualitative detection of influenza virus by reverse transcription coupled real time PCR. This assay detects but does not differentiate most known strains of influenza A viruses, including H5N1, H5N2, H1N1, H2N2, H3N8, H4N6, H7N7, H8N4 and H9N2.

Assay S0121: Ultrasensitive qualitative detection of pseudorabies by real time PCR

[redacted]

Initials [redacted]

Assay Results Report **24043002b**

Client sample ID	[redacted] accession ID	Sample type	Assay	Assay result

Assay S0165: Ultrasensitive qualitative detection of porcine reproductive & respiratory syndrome virus (PRRSV) by reverse transcription coupled real time PCR

Assay S0167: Ultrasensitive qualitative detection of porcine transmissible gastroenteritis virus (TGEV) by reverse transcription coupled real time PCR

Assay S0213: Ultrasensitive qualitative detection of porcine epidemic diarrhea virus (PED, PEDV) targeting M gene by reverse transcription coupled real time PCR

[redacted] has verified the performance characteristics of these tests. However, diagnosis and management of the animal patient should not rely solely upon the results of these tests, as unusual genetic variations of the pathogen can affect results. Correlation with other clinical data is recommended. Specimens will be held for six months by [redacted] to facilitate followup testing, after which time specimens will be disposed of at the discretion of [redacted] unless otherwise directed by client.

REPORT OF ANALYSIS

Customer: **Mission Barns**
1155 Bryant St
San Francisco, CA 94103
[redacted]

Date Received: 04/18/24
Report Date: 05/06/24

‡Description: [redacted]
Lab Number: CK54163
Commodity: Cell Pellet

Analysis	Result	Unit	Analyzed
Multi-species (meat) PCR semi-qnt			
Bos taurus	Not detected	NA	05/06/24
Sus scrofa	major part	NA	05/06/24
Equus caballus	Not detected	NA	05/06/24
Ovis aries	Not detected	NA	05/06/24
Capra hircus	Not detected	NA	05/06/24
Gallus gallus	Not detected	NA	05/06/24
Meleagris gallopavo	Not detected	NA	05/06/24

Test: *Meat Species Determination† (heterogeneous mixtures – BJ00X semi-quantitative assay)*

Species DNA	
Bos tarus	Cow
Sus scrofa	Pig
Equus caballus	Horse
Ovis aries	Sheep
Capra hircus	Goat
Gallus gallus	Chicken
Melagris gallopavo	Turkey

Key

Major part		60-100%
Medium part		30-60%
Minor part		5-30%
Diminutive part		1-5%
Very minute part	<1%	
Not Detected		<LOD in minced meat: *Bos taurus, Equus caballus, Ovis aries, & Gallus gallus LOD ~0.01%* *Sus scrofa, Capra hircus, & Meleagris gallopavo LOD ~0.1%)*
Not Quantifiable		Due to very low or degraded DNA levels, it is not possible to quantify the animal species present in the sample

** A signal for this species was detected.
***No amplifiable DNA could be recoved from the samples, therefore, we cannot identify the species

‡The results shown in this report relate solely to the item submitted for analysis.

†Eurofins Medigenomix, Ebersberg, Germany

Eurofins GeneScan

[redacted]

Dr. Frank Spiegelhalter
Executive Vice President

eurofins | GeneScan

REPORT OF ANALYSIS

Customer: **Mission Barns**
1155 Bryant St
San Francisco, CA 94103
[redacted]

Date Received: 04/18/24
Report Date: 05/02/24

‡Description: [redacted]
Lab Number: CK54163
Commodity: Cell Pellet

Analysis	Result	Unit	Analyzed
Anasine (duck) DNA by PCR	Negative	NA	04/25/24

‡The results shown in this report relate solely to the item submitted for analysis.

ISO/IEC 17025
Testing Cert. 1940.01

Eurofins GeneScan
[redacted]
Dr. Frank Spiegelhalter
Executive Vice President

CCC 000008 Amendment to the disclosable safety narrative

Request for Clarification

Please provide, for addition to the disclosable safety narrative, a table summarizing the classes (e.g., protein, surfactant) and characteristics of all substances added to the culture medium.

The following table contains the complete list of classes of the components added to Mission Barns' manufacturing cell culture media, with a brief description of their characteristics and/or function in cell culture.

Class	Characteristics / function
Alcohol	A class of non-polar organic compounds used to dissolve other non-polar media components in stock solutions that are subsequently added to cell culture media
Amino acid	Nutrient molecules that are the monomers of proteins, used as reactants in energy metabolism, and serve various other functions
Amino acid derivative	Molecules derived from amino acids that serve various cellular functions
Antioxidant	Compounds that inhibit oxidation either in media or used by cells to do so intracellularly
Buffer	Compounds added to cell culture media to make media insensitive to pH shifts from the addition of acids or bases.
Carrier protein	Proteins that carry various compounds into and out of cells
Coenzyme	Compounds used by enzymes to catalyze enzymatic reactions
Common connective tissue constituent	Compounds used by cells to assemble extracellular matrices
DNA precursor	Compounds used by cells to synthesize DNA
Emulsifier	Compounds that help mix otherwise immiscible compounds into cell culture media as emulsions
Fatty acid	A class of organic compounds which are characterized by a hydrocarbon chain and carboxylic acid end that constitute the majority of lipids, are used as components in energy metabolism, and serve various other functions
Growth factor	Signaling compounds that instruct cells to grow, differentiate, and how to regulate metabolism, amongst various other functions.
Hormone	Hormones and growth factors are mainly differentiated by what organs they're produced by and the localness of their signaling *in vivo*.
Metabolic precursors	Compounds that are used as the initial reactants in a chain of reactions involved in energy metabolism
Metabolic intermediates	Compounds that support metabolism through various ways (e.g. serving as components in synthesis, catalyzing or inhibiting certain metabolic reactions)
Metabolites	
Mineral	A nutritional class of chemical elements used to support biochemical processes in cells

Nucleobase	Nitrogenous molecules that are components of nucleotides and nucleosides. These molecules can be methylated to serve epigenetic purposes in cells.
Nucleoside	Biomolecules that have nitrogenous bases and are involved in a large variety of cellular processes including nucleotide synthesis and energy metabolism
Nucleotide	Monomers of DNA and RNA
Proteoglycan precursor	Compounds that are used by cells to synthesize proteoglycans which are heavily glycosylated proteins that serve various functions in biological systems including extracellular matrix formation and cell signaling
RNA precursor	Compounds used by cells to synthesize RNA
Signaling molecule precursor	Compounds used by cells to synthesize hormones or other types of signaling molecules
Sterol	A class of biomolecules that are important in various cellular activities including signaling and metabolism
Sugar	Nutrient molecules used as monomers for carbohydrate synthesis and energy metabolism
Vitamin	A class of organic compounds that are characterized by being produced in insufficient amounts by the organism for which they are essential to assure the function of critical biochemical processes.
Vitamin precursor	Compounds used by cells to synthesize vitamins

Request for Additional Information re: CCC 000008

Requests for Information to be added to the DSN

Cell Line Establishment

1) Page 13 of the June 3, 2024, amendment to the DSN Attachment 3B (Att 3B) outlines the protocol and data analysis utilized for the GeneScan DNAnimal Ident Pork IPC (Cat. no. 5422211910) kit, a test developed by Gold Standard Diagnostics Corp, which Mission Barns uses for species verification of the master cell bank. The third table on page 13 of Att 3B, describes the criteria for interpreting the result of a test sample by combination of the preliminary target results (pig = FAM™ pig target 8041a listed on page 10 of Att 3B) and the internal positive control (IPC = VIC®/HEX™ IPC target 8041i listed on page 10 of Att 3B). The table indicates that if the preliminary target result for pig DNA is negative, and the IPC result is valid, the sample is negative for pig DNA. FDA notes that this test is not capable of identifying the species of origin of DNA other than *Sus scrofa,* as a negative result indicates the absence of pig DNA but does not provide information about the presence of DNA from cells of other species.

   For addition to the DSN, please explain if follow-up testing of the MCB is performed if the GeneScan DNAnimal Ident Pork IPC test yields a negative result.

In the event that a porcine MCB has a *negative* result for pig DNA using the GeneScan DNAnimal Ident Pork IPC (Cat. no. 5422211910), Mission Barns would retest the MCB to confirm the original result is not a false negative for pig DNA (e.g., caused by a sample handling error). Additionally, species identification testing would be conducted for other species that Mission Barns has cell lines of in order to verify the porcine cell bank in question is not cross-contaminated with cells from those other cell lines. Any cell bank that is confirmed to be negative for pig DNA or positive for non-pig species DNA will not be released into Manufacturing.

Adventitious Agent Hazard Assessment

2) FDA notes that you provided additional justification for removal of *Listeria* spp. from environmental monitoring on page 15 of the June 3, 2024, amendment to the DSN. This rationale was primarily based on the assumption that the current processing environment does not allow for introduction of *L. monocytogenes* contamination, including absence of wet processing.

   It is unclear what is meant by wet processing in this context, as the cell culture process does occur in liquid medium and listeria harborage sites may occur at various points in water infrastructure, including refrigerator cooling coils and drains, which we assume are present in the processing environment. Additionally, we note that environmental sources of listeria overlap with environmental sources of *Bacillus cereus*, while *Ralstonia insidiosa* (reportedly detected as part of the firm's environmental monitoring program, as described in response to question 13a) is a waterborne bacterium that is capable of survival in wet environments.

   In light of the identification and on-going testing for *B. cereus* and *R. insidiosa* in your processing environment and considering the severe health impact of *L. monocytogenes*, we request that you consider continuing to monitor for *Listeria* spp. in your environmental monitoring program.

Mission Barns confirms that it will add *Listeria* spp. to its environmental monitoring program.

Food Safety Management System

3) On page 2 of the June 3, 2024, amendment to the DSN, Mission Barns indicates that it stores previously established research cell lines from other species (i.e., chicken, duck, cow) in a cryofreezer on site and notes that the firm does not perform any on site non-porcine culturing

activities at its manufacturing facility. The firm also indicates that there are physically segregated, and separate, species-dedicated racks. In addition, Mission Barns states that it has implemented cell bank inventory controls, including vial labeling, material and lot coding, and periodic inventory audits. FDA notes that, based on the information provided in the June 3, 2024, amendment to the DSN, it appears that this cryofreezer is the same one to store the current working cell bank materials.

For addition to the DSN, please confirm whether Mission Barns has any plan to separate these different cell materials in different freezers.

Mission Barns confirms that we will store cell materials from non-porcine species either on-site in different cryofreezers from cryofreezers containing porcine cell materials or off-site.

4) Page 19 of the June 3rd, 2024, amendment presents a table that "summarizes the prerequisite programs/procedures and preventive controls implemented by Mission Barns to address safety risks associated with its cultivated cell manufacturing process." For addition to the DSN, please provide a detailed summary of the eleventh prerequisite program listed, "Cell Manufacturing Validation and Qualification Policy" in the table as it was not explained previously in the original submission and amendments. Additional discussion of this policy will help FDA and readers of the DSN understand how the policy supports your safety conclusion.

Mission Barns has implemented a *Cell Manufacturing Validation and Qualification* policy as a prerequisite program (PRP) in its food safety and quality management system. "Qualification" typically refers to the evaluation of equipment, systems, or facilities to ensure they meet specified requirements and function as intended (i.e., verifying that something is fit for its intended use). "Validation" is a broader concept that focuses on providing documented evidence that a process, when operated within established parameters, can perform effectively and reproducibly to produce a product meeting its predetermined specifications and quality attributes.

As a PRP, Mission Barns' *Cell Manufacturing Validation and Qualification* program ensures that critical manufacturing equipment such as autoclaves, incubators, freezers and refrigerators are qualified to ensure that they are properly installed and operate as expected. For example, all controlled temperature GMP storage equipment such as -20 deg C and -150 deg C freezers, and 2-8 deg C refrigerators undergo installation and operational qualification (IOQ) testing that includes verifying the equipment is installed properly per manufacturer's requirements as well as chamber temperature mapping to ensure that the equipment can achieve and maintain their respective temperature ranges as required by Mission Barns' manufacturing process. In the case of autoclaves used to sterilize manufacturing equipment, autoclaves are IOQ'd and individual sterilization cycles for specific loads undergo performance qualification (PQ) utilizing biological indicators and temperature sensors in order to ensure that each cycle is able to effectively and reproducibly achieve minimum lethality (sterilization) requirements.

Also, per the company's *Cell Manufacturing Validation and Qualification* program, critical processes (e.g., equipment clean-in-place [CIP] cycles) and analytical methods (e.g., microbiological detection methods) are required to validated prior to commercial application in order to ensure they meet predetermined specifications and quality attributes.

5) Please provide, for addition to the DSN, the hazard analysis of media preparation (Media Prep and Media Equilibration), as well as the control strategies for the hazards. Discussion of the relationship between specific microbiological, chemical, or physical hazards and the controls used to address any risks arising from these hazards provides additional support for your overall public safety conclusion.

Cell culture media used for cell manufacturing is prepared at Mission Barns by completing a custom-formulated basal medium with various supplements which support the proliferation and fattening of cells. Media preparation is performed under aseptic conditions in a controlled clean room environment.

Mission Barns' hazard identification and analysis of its media preparation process has identified microbial contamination as a potential biological hazard. Microbial contaminants, including bacteria, yeasts and molds, can originate from the environment, equipment used to prepare or store media, personnel, or contaminated media components. Appropriate controls have been implemented to mitigate this hazard and include the use of:

a) Aseptic Techniques, such as the use of laminar flow hoods or biosafety cabinets, coupled with proper personal hygiene, material handling and sanitization practices, and personal protective equipment (PPE), to significantly reduce the risk of introducing microbial contaminants from personnel and the environment;

b) Regular facility cleaning and environmental monitoring, such as periodic air and surface sampling, to identify and control environmental sources of microbial contamination and allow for prompt corrective actions;

c) Sterile filtration of prepared media supplements and buffers used to prepare powdered supplements prior to use via validated sterilizing-grade filters (0.1 - 0.2 µm) to reduce the risk of microbial contaminants in cell culture media;

d) Sterilization via pressurized steam for non-consumable media preparation equipment (e.g., carboys) using a validated autoclave cycle to reduce microbial contamination risk from contact surfaces; and

e) Supplier and Materials Control, including supplier qualification, CoA review, and incoming material positive release, to reduce the risk of microbial contamination from media components and consumables (e.g., single use flasks and containers).

Mission Barns has not identified any chemical (e.g., heavy metals, detergents) or physical (e.g., glass, metal or brittle plastic particulates) hazards that are present, or are likely to be present, in its media preparation process.

Substances Used During Cell Culture

6) Page 7 of the June 13, 2024, amendment to the SCM, the states "Mission Barns considers the use of ammonium metavanadate, ammonium molybdate tetrahydrate, and nickel chloride during the cell culture production process to be proprietary confidential commercial information and a trade secret. As such, Mission Barns notes it anonymized references to these metal salts contained in information request #7 in our amendment to the disclosable safety narrative, identifying nickel chloride as "trace metal A," ammonium metavanadate as "trace metal B," and ammonium molybdate tetrahydrate as "trace metal C." FDA notes that the three compounds these statements refer to have no applicable U.S. authorization for use in human food, nor do these substances naturally occur in food (Please note, the presence of a substance as a contaminant is not considered to be natural occurrence).

   For any compounds with no U.S. authorization for use in human food, and which are not naturally occurring in food, please name these compounds and provide a detailed safety evaluation with all references in the DSN. This information is important to provide evidence that your safety assessment process appropriately considers publicly available toxicological data and the properties of any substances you have evaluated in context. Further, the basis for your conclusion of safety regarding any residual presence of these substances is an important element of your disclosable safety narrative and further discussion provides useful additional context for FDA and readers of the disclosable safety narrative regarding your conclusion.

Mission Barns includes nickel (in the form of nickel chloride), vanadium (in the form of ammonium metavanadate) and molybdenum (in the form of ammonium molybdate tetrahydrate) in its cell culture media and has established specifications for the presence of such trace metals in its cultivated pork fat cells. All three trace metals are either reported to be present in conventional US pork belly fat at the same or higher levels than the specifications, or are considered common nutrients in food and the estimated daily intake from their intended use will constitute an insignificant percentage of the daily intake of the nutrients as reported in public literature. As such, the consumption of the cultivated pork fat cells is not expected to lead to a significant increase in consumers' cumulative exposures of these trace metals.

Nickel (nickel chloride)

Nickel is a known cofactor for certain enzymatic reactions and it has been suggested that it is required for protein synthesis in animals.[1] Nickel is approved for use as a food processing aid per 21 CFR §172.864, §176.180, and §184.1537, and is commonly present in meats including pork. For example, Onianwa et al. reported nickel concentrations in chicken, pork, and beef are 1.637 ppm, 1.4 ppm, and 1.2 ppm, respectively.[2] Nho et al. evaluated the elemental composition of pork belly fat from pork samples of various geographical origins including the US by inductively coupled plasma-mass spectrometry (ICP-MS).[3] For the US, the naturally-occurring level of nickel in pork belly fat is reported as 0.201 ppm.[4] As such, a specification for the cultivated pork fat cells of < 0.2 ppm ensures the dietary intake from nickel, if any, would be lower or comparable to the naturally-occurring nickel level in conventional pork belly fat. Accordingly, there is no expected increase to the cumulative nickel intake from the cultivated pork fat cells, which are intended as a replacement of conventional pork fat in the US. Mission Barns concludes that any nickel exposure from our intended use would be comparable to the nickel exposure from conventional pork fat.

Vanadium (ammonium metavanadate)

Vanadium can be found in many common foods, and according to Abrarin and Ahmed, vanadium-rich foods include shellfish (108 ppm vanadium concentration), mushrooms (2.08 ppm), chicken meat (2.61 ppm), and rice (1.52 ppm).[5] For vanadium, Nho et al. evaluated the elemental composition of pork belly fat from pork samples of various geographical origin by ICP-MS, with the vanadium level reported for US pork belly fat at 0.034 ppm.[6] A specification for the cultivated pork fat cells at < 0.03 ppm ensures the dietary intake from vanadium, if any, would be lower than or comparable to the naturally-occurring vanadium levels in conventional pork belly fat. As such, there is no expected increase to the cumulative vanadium intake from the cultivated pork fat cells, which are intended as a replacement of conventional pork fat in the US.

Molybdenum (ammonium molybdate tetrahydrate)

Molybdenum is an essential trace element for microorganisms, plants, and animals.[7] FDA developed daily values or DVs to help consumers compare the nutrient contents of foods and dietary supplements within the context of a total diet. Under FDA regulation 21 CFR 101.9(c), the DV for molybdenum is established as 45 µg for adults and children aged 4 years and older. We note the 90th percentile EDI for Mission Barns' cultivated

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[1] Das, K. K., Das, S. N., and Dhundasi, S. A. (2010) "Nickel: molecular diversity, application, essentiality and toxicity in human health. Biometals," *Molecular Structures, Binding Properties and Applications*. New York, USA, Nova Science Publishers Inc, 33-58.

[2] Onianwa, P.C., Lawal, J.A., Ogunkeye, A.A. and Orejimi, B.M. (2000) "Cadmium and Nickel Composition of Nigerian Foods," *Journal of Food Composition and Analysis*, 13(6):961-969.

[3] Nho, E. Y., Choi, J. Y., Lee, C. M., Dang, Y. M., Khan, N., Jamila, N. and Kim, K. S. (2019) "Origin authentication of pork fat via elemental composition, isotope ratios, and multivariate chemometric analyses," *Analytical Letters*, 52(9):1445-1461.

[4] *See* id.

[5] Abrarin, S. and Ahmed, M.J. (2020) "A highly sensitive and selective spectrophotometric method for the determination of vanadium at nanotrace levels in some environmental, biological, soil, food, and pharmaceutical samples using salicylaldehyde-benzoylhydrazone," *European Journal of Chemistry* 11(4):385-395.

[6] Nho et al. (2019).

[7] Novotny, J.A. and Peterson, C.A. (2018) "Molybdenum," *Advances in Nutrition* 9(3):272-273.

pork fat cells is 16.7 g/person/day. With a specification of 0.1 ppm for molybdenum in the cultivated pork fat cells, the estimated theoretical consumer daily intake of molybdenum from the consumption of cultivated pork fat cells is up to 1.67 µg/day, which only constitutes < 5% of the molybdenum DV at 45 µg. We also note that 1.67 µg/day intake from our intended use is much smaller in comparison with the molybdenum from one serving of the following common animal foods:

- Beef, liver, pan fried (3 ounces), 104 µg molybdenum
- Chicken, light meat, roasted (3 ounces), 9 µg molybdenum
- Beef, ground, regular, pan-fried (3 ounces), 8 µg molybdenum[8]

Intake recommendations for molybdenum and other nutrients are also provided by the Food and Nutrition Board (FNB) at the National Academies of Sciences, Engineering, and Medicine.[9] The FNB established ULs for molybdenum for healthy individuals based on levels associated with impaired reproduction and fetal development in rats and mice.[10] The UL for the general adult population (19+ years) is 2,000 µg/day, and the estimated daily intake of 1.67 µg/day from cultivated pork fat cells is less than 0.1% of the UL.

With a specification of molybdenum at 0.1 ppm, the daily intake of molybdenum from the cultivated pork fat cells consumption will be less than 5% of the molybdenum DV established by FDA, and less than 0.1% of the UL established by the FNB.

Product Characterization

7) Since CCC 000008 was filed on June 27, 2022, Mission Barns has provided the following analytical data for three non-consecutive batches of harvested cell material:

   a. Page 14 of the May 25, 2022, safety dossier = heavy metals (i.e., cadmium, lead, arsenic, mercury);

   b. Pages 6 – 15 of the June 5, 2023, amendment to the DSN = disclosable compositional data (i.e., fatty acid profile, proximates, amino acids, vitamins, and minerals) and confidential trace heavy metals; and,

   c. Page 2 of the August 23, 2023, amendment to the DSN = disclosable fatty acid profiles for harvested cell material produced using the "in house" lipid mixture.

   FDA met with Mission Barns on June 28, 2024, to discuss the firm's proposed responses to a request for information we sent the firm on May 6, 2024. During the meeting, Mission Barns informed FDA of several changes to substances used during the downstream stages of the production process (i.e., biomass accumulation and differentiation), including the replacement of recombinant proteins derived from human genome (rHPs) with recombinant proteins derived from the genomes of agriculturally relevant species (e.g., bovine, porcine), as well as the removal of Tris-HCl, HEPES, and Pluronic F-68. We stated that, before requesting new analytical data, we would consider the firm's written argument for why batch testing of the harvested cell material produced using the current, canonical manufacturing process is not warranted or should be limited to certain tests (e.g., fatty acid profile). In the June 3, 2024, and July 8, 2024, amendments to the SCM, Mission Barns provided the following rationale to support its conclusion that new batch data is not needed for harvested cell material produced using the current manufacturing process:

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[8] Hunt, C.D., and Meacham, S.L. (2001) "Aluminum, boron, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, and zinc: concentrations in common western foods and estimated daily intakes by infants; toddlers; and male and female adolescents, adults, and seniors in the United States" *Journal of the Academy of Nutrition and Dietetics* 101(9):1058-60.

[9] *See* Trumbo, P., Yates, A. A., Schlicker, S., & Poos, M. (2001), "Dietary reference intakes," *Journal of the American Dietetic Association*, 101(3): 294-301.

[10] *See id.*

- Data showing comparable proliferation rate for cells grown with rHPs versus cells grown with orthologous recombinant animal proteins;
- Regarding the replacement of rHPs with recombinant proteins derived from porcine or bovine genomes, on page 4 of the June 3, 2024, amendment to the SCM: “Mission Barns has implemented porcine recombinant growth factors at their same concentrations in cell culture media and found proliferation rates of cell cultures to be consistent with cells cultured in human sequence growth factor containing media …" and "… given that growth factor function is highly conserved across species, we expect the data reported in Section 3.2.2.4 (pages 47-52) and Attachment 8 of our March 16, 2022 supplemental, confidential materials to remain representative.”
- Regarding the removal of Tris-HCl, HEPES, and Pluronic F-68, on page 12 of the June 3, 2024, amendment to the SCM: “… these components were included as functional aids to maintain specific environmental conditions within the culture, such as pH stability and prevention of cell clumping. These environmental characteristics can be effectively maintained through alternative means that are equally efficient and well-established. Consequently, the analytical data previously generated from cultures prepared using Tris-HCl, HEPES, and Pluronic F-68 remain fully representative of cells produced from cultures lacking these components.”

FDA notes that currently there is not enough data or evidence to support the firm’s conclusion that the manufacturing changes do not alter the composition of the harvested cell material. For example, tris(hydroxymethyl)aminomethane is known to function as a metal chelating agent. The removal of tris HCl from the cell culture medium could have unintended effects on the levels of metal ions present in the harvested cell material, thus warranting an updated analytical assessment of inorganic minerals. Pluronic F-68, included as a surfactant for its function as an anti-clumping agent, was removed from the production phase medium and not replaced by an alternative substance. Though Mission Barns states in the June 3, 2024, amendment to the SCM, “... its cell culture system continues to function without Pluronic F-68 ...,” no evidence was provided to substantiate the claim.

Given the number of manufacturing changes that have been implemented throughout the evaluation of CCC 000008, please provide, for addition to the DSN, batch analysis data for proximates, vitamins, minerals, toxic heavy metals, trace heavy metals, and fatty acids for 3 non-consecutive batches of harvested cell material produced using the current, canonical method of production. If, based on the new batch analysis data, the estimated daily intake (EDI) of an analyte (e.g., vitamins, minerals, fatty acids, heavy metals) is higher than the safe reference level, please provide an updated safety discussion for addition to the DSN.

Mission Barns is in the process of generating batch analysis data for proximates, vitamins, minerals, toxic heavy metals, trace heavy metals, and fatty acids and will provide such results to the Agency promptly when available.

Request for Additional Information re: CCC 000008
To be added to the Disclosable Safety Narrative (DSN)

Request for Clarification

1. Ammonium metavanadate is classified as a Type 4 substance (i.e., a substance that is not naturally present in food and which has not been previously evaluated by FDA for use in human food in the U.S.). We note that vanadium, present in the conventional comparator, is a result of bioaccumulation of an environmental/chemical contaminant and is not an essential micronutrient. Therefore, using the argument that the levels of vanadium in the harvested material do not exceed those in the conventional comparator is insufficient as the sole safety rationale. Please provide a more comprehensive safety discussion for this substance, including expected exposure compared to a safe level based on results from relevant *in vivo* safety studies. A toxicological profile for vanadium from the Agency for Toxic Substances and Disease Registry may be helpful, which we have linked.

The Agency for Toxic Substances and Disease Registry performed a review of the available scientific literature for vanadium exposure and developed estimates of vanadium exposure levels that pose minimal risk to humans.[1] A minimal risk level (MRL) is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure.

For intermediate-duration oral exposure (15–364 days) to vanadium, the Agency determined an MRL of 0.01 mg vanadium / kg / day, based primarily on a human exposure study conducted by Fawcett et al.[2] In the study, participants in a year-long weight training program were given 0 or 0.12 mg vanadium / kg / day for 12 weeks. Blood samples and body measurements at weeks 4, 8, and 12 showed no significant changes in hematology, serum chemistry, body weight, or blood pressure. Using the NOAEL of 0.12 mg vanadium / kg bw / day and an uncertainty factor of 10 for human variability, the MRL was determined to be 0.01 mg vanadium / kg bw / day.

Although no human studies were identified on chronic-duration oral exposure to vanadium, the Agency reviewed several studies showing no adverse effects in rodents at low doses. No adverse effects were observed in rats and mice exposed to 0.7 or 4.1 mg vanadium / kg bw / day, respectively, for 2–2.5 years.[3] Despite these findings, the Agency declined to determine an MRL for chronic-duration oral

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[1] Agency for Toxic Substances and Disease Registry (ATSDR). (2012). Toxicological Profile for Vanadium. U.S. Department of Health and Human Services, Public Health Service. Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp58.pdf.

[2] Fawcett JP, Farquhar SJ, Thou T, Shand BI. "Oral vanadyl sulphate does not affect blood cells, viscosity or biochemistry in humans." *Pharmacology & toxicology vol. 80,4 (1997): 202-6*. The Agency also considered a second human study that determined a NOAEL of 0.19 mg vanadium/kg/day, but selected the Fawcett study to establish an MRL because it assessed more subjects and in greater detail. The Agency also discussed several animal toxicology studies establishing LOAELs ranging between 0.12 and 2.1 mg/vanadium/kg/day, but placed greater confidence in a reliable human study.

[3] Schroeder HA, Mitchener M, Nason AP "Zirconium, niobium, antimony, vanadium and lead in rats: Life term studies." *The Journal of nutrition* vol. 100,1 (1970): 59-68; Schroeder HA, Balassa JJ. "Arsenic, germanium, tin and vanadium in mice: Effects on growth, survival and tissue levels." *The Journal of nutrition* vol. 92,2 (1967): 245-52.

vanadium exposure, given the most sensitive target of vanadium toxicity following chronic-duration oral exposure had not been identified.

The estimated daily exposure to vanadium in Mission Barns' cultivated pork fat cells can be calculated by multiplying the vanadium specification (<0.03 ppm, i.e. 0.00003 mg vanadium / g cultivated pork fat cells) by the estimated daily intake (EDI) for cultivated pork fat calls (0.4 g cells / kg bw / day, as discussed in Section 3.1 of the disclosable safety narrative, dated May 25, 2022).

$$0.00003\ \frac{mg\ vanadium}{g\ cultivated\ pork\ fat\ cells} \times 0.4 \frac{g\ cultivated\ pork\ fat\ cells}{kg\ bw\ /\ day} = 0.000012\ \frac{mg\ vanadium}{kg\ bw\ /\ day}$$

The EDI of 0.000012 mg vanadium / kg bw / day is approximately three orders of magnitude lower than the intermediate-duration oral exposure MRL of 0.01 mg vanadium / kg bw / day, established by the Agency for Toxic Substances and Disease Registry.

Although the Agency did not determine an MRL for chronic oral exposure, using the lowest NOAEL established by studies the Agency reviewed of 0.7 mg / kg bw / day, a margin of exposure (NOAEL/EDI) to vanadium from cultivated pork fat cells can be calculated as follows:

$$0.7\ \frac{mg\ vanadium}{kg\ bw/day} \div 0.000012 \frac{mg\ vanadium}{kg\ bw/day} = 58{,}333$$

A margin of exposure of 100 or greater is typically considered adequate to support safety. Because the estimated exposure to vanadium from Mission Barns pork fat cells is several orders of magnitude below the MRL established for intermediate-duration chronic exposure and has a margin of exposure of over 50,000 using the lowest available NOAEL established in chronic duration animal toxicity studies, Mission Barns concludes that exposure to vanadium does not present a safety concern.

2. On Page 3 of the September 25, 2024, amendment to CCC 000008, you provide a general summary of the preventive controls for biological hazards and indicate that you have "not identified any chemical (e.g., heavy metals, detergents) or physical (e.g., glass, metal or brittle plastic particulates) hazards that are present, or are likely to be present, in its media preparation process."

   Please clarify why chemical hazards such as cleaning agent residues, incorrect measurement of media components, mis-formulation of media inputs, and chemical hazards in the supplier's materials are not known or reasonably foreseeable hazards in your process. Also, please clarify why physical hazards from the environment or employees will not be present at this step. If there are any chemical and physical hazards identified at this step, please provide, for addition to the DSN, a brief discussion of preventive controls implemented to control these hazards.

Mission Barns clarifies that the chemical hazards identified in FDA's information request (i.e., cleaning agent residues, incorrect measurement of media components, mis-formulation of media inputs, and chemical hazards in supplier's materials) have been thoroughly considered as part of Mission Barns' hazard analysis. These potential hazards are adequately addressed through our comprehensive Prerequisite Program (PRP) controls, which include:

1. **Cleaning and Sanitation Procedures**. Mission Barns has implemented procedures for cleaning and sterilizing reusable (non-consumable) media preparation equipment including a verification step where final rinse water conductivity testing is performed to ensure the effective removal of residual cleaning agents from media-contacting surfaces. This process effectively mitigates the risk of media being contaminated from residual cleaning agents.
2. **Document Control and Training Programs**. To address the risks of incorrect measurements and mis-formulation, Mission Barns has implemented the following controls:
    - All media preparation activities are documented using approved batch records, which record the identities and quantities of all components added to cell culture media.
    - Only trained personnel are authorized to prepare cell culture media and document such activities with batch records.
    - Prior to use of any cell culture media, all batch records undergo a dual review process by both Manufacturing and Quality personnel. This double-check system verifies that media is formulated according to specifications and all inputs have been accurately recorded.
3. **Supplier Control and Raw Material Receiving Programs**. To manage potential chemical hazards from supplier materials, Mission Barns has established robust Supplier Control and Raw Material Receiving Programs, which are described below:
    - Basal media and supplemental components used in our media preparation processes are sourced exclusively from approved and monitored suppliers. As part of the supplier approval process, the supplier's quality control systems are assessed to ensure they meet appropriate standards. Mission Barns conducts periodic audits of its suppliers to ensure they continue to meet required quality standards. These controls reduce the risk of receiving incorrectly formulated or labeled components from suppliers.
    - Mission Barns has performed risk assessments for each raw material used in its manufacturing process and has established appropriate documentation requirements (e.g., certificates of analysis) to address chemical contamination risks associated with such materials. Before any raw materials are used in manufacturing, quality professionals review material receipt documentation, including COAs, to ensure the material meets required quality specifications. These controls further reduce the risk of receiving materials containing chemical contaminants.

Mission Barns further clarifies that physical hazards (e.g., glass, metal or brittle plastic particulates) from the environment or employees are potentially present during media preparation activities. As such, Mission Barns has implemented the following PRPs to mitigate such risks:

1. **Facility Access and Inspection Controls**:
    - Access to manufacturing areas is limited to approved personnel that have been trained on appropriate gowning and GMP clothing restrictions.
    - Trained personnel perform a pre-operation visual inspection of all equipment to be used and all surrounding surfaces, including the floors, walls, ceilings, and general work areas, for any visible contamination such as spills, stains, debris, or dust. Manufacturing

operations are only performed if the equipment and environment pass this inspection, reducing the risk of physical debris contaminating the media from equipment and the environment.

2. **Personnel-Related Hazards Controls**:
    - All media preparation activities (like all other GMP manufacturing activities) are performed by trained personnel wearing appropriate personal protective equipment (PPE), including full body gowns, hairnets, masks, eye protection, gloves and shoe covers. Personnel are required to remove any jewelry or other loose items before entering the manufacturing environment. These programs reduce the risk of physical contaminants of the cell culture media from manufacturing personnel.
3. **Sterile Filtration of Media Supplements**: Cell culture media supplements are sterile filtered using a 0.2 micron filter prior to addition to basal media, reducing the risk of physical contaminants in cell culture media.
4. **Post-Preparation Inspection of Prepared Media and Equipment:** Following media preparation operations, manufacturing personnel visually inspect the media and all equipment used in the preparation process for irregularities (i.e. the absence of glass, plastic, or metal particulates). These steps reduce the risk of undetected physical contaminants in the cell culture media.

Preventive controls are integral to Mission Barns' food safety management system and are designed to effectively prevent, eliminate, or reduce to an acceptable level the chemical and physical hazards associated with our media preparation process.

Request for Additional Information re: CCC 000008
To be added to the Disclosable Safety Narrative (DSN)

Product Characterization

7) Since CCC 000008 was filed on June 27, 2022, Mission Barns has provided the following analytical data for three non-consecutive batches of harvested cell material:

   a. Page 14 of the May 25, 2022, safety dossier = heavy metals (i.e., cadmium, lead, arsenic, mercury);

   b. Pages 6 – 15 of the June 5, 2023, amendment to the DSN = disclosable compositional data (i.e., fatty acid profile, proximates, amino acids, vitamins, and minerals) and confidential trace heavy metals; and,

   c. Page 2 of the August 23, 2023, amendment to the DSN = disclosable fatty acid profiles for harvested cell material produced using the "in house" lipid mixture.

   FDA met with Mission Barns on June 28, 2024, to discuss the firm's proposed responses to a request for information we sent the firm on May 6, 2024. During the meeting, Mission Barns informed FDA of several changes to substances used during the downstream stages of the production process (i.e., biomass accumulation and differentiation), including the replacement of recombinant proteins derived from human genome (rHPs) with recombinant proteins derived from the genomes of agriculturally relevant species (e.g., bovine, porcine), as well as the removal of Tris-HCl, HEPES, and Pluronic F-68. We stated that, before requesting new analytical data, we would consider the firm's written argument for why batch testing of the harvested cell material produced using the current, canonical manufacturing process is not warranted or should be limited to certain tests (e.g., fatty acid profile). In the June 3, 2024, and July 8, 2024, amendments to the SCM, Mission Barns provided the following rationale to support its conclusion that new batch data is not needed for harvested cell material produced using the current manufacturing process:

   - Data showing comparable proliferation rate for cells grown with rHPs versus cells grown with orthologous recombinant animal proteins;
   - Regarding the replacement of rHPs with recombinant proteins derived from porcine or bovine genomes, on page 4 of the June 3, 2024, amendment to the SCM: "Mission Barns has implemented porcine recombinant growth factors at their same concentrations in cell culture media and found proliferation rates of cell cultures to be consistent with cells cultured in human sequence growth factor containing media …" and "… given that growth factor function is highly conserved across species, we expect the data reported in Section 3.2.2.4 (pages 47-52) and Attachment 8 of our March 16, 2022 supplemental, confidential materials to remain representative."
   - Regarding the removal of Tris-HCl, HEPES, and Pluronic F-68, on page 12 of the June 3, 2024, amendment to the SCM: "… these components were included as functional aids to maintain specific environmental conditions within the culture, such as pH stability and prevention of cell clumping. These environmental characteristics can be effectively maintained through alternative means that are equally efficient and well-established. Consequently, the analytical data previously generated from cultures prepared using Tris-HCl, HEPES, and Pluronic F-68 remain fully representative of cells produced from cultures lacking these components."

FDA notes that currently there is not enough data or evidence to support the firm's conclusion that the manufacturing changes do not alter the composition of the harvested cell material. For example, tris(hydroxymethyl)aminomethane is known to function as a metal chelating agent. The removal of tris HCl from the cell culture medium could have unintended effects on the levels of metal ions present in the harvested cell material, thus warranting an updated analytical assessment of inorganic minerals. Pluronic F-68, included as a surfactant for its function as an anti-clumping agent, was removed from the production phase medium and not replaced by an alternative substance. Though Mission Barns states in the June 3, 2024, amendment to the SCM, "... its cell culture system continues to function without Pluronic F-68 ...," no evidence was provided to substantiate the claim.

Given the number of manufacturing changes that have been implemented throughout the evaluation of CCC 000008, please provide, for addition to the DSN, batch analysis data for **proximates, vitamins, minerals, toxic heavy metals, trace heavy metals, and fatty acids** for 3 non-consecutive batches of harvested cell material produced using the current, canonical method of production. If, based on the new batch analysis data, the **estimated daily intake (EDI)** of an analyte (e.g., vitamins, minerals, fatty acids, heavy metals) is higher than the safe reference level, please provide an updated safety discussion for addition to the DSN.

Mission Barns met with the FDA on August 15, 2024 to discuss the above July 25, 2024 request for additional batch analysis data for 3 non-consecutive batches of harvested cell material produced using the current, canonical method of production. Mission Barns asked whether FDA would consider limited analytical batch data from a batch produced without HEPES, rHPs, Tris-HCl, and Pluronic-F68 in conjunction with data from three non-consecutive batches produced using media with HEPES, but without rHPs, Tris-HCl, and Pluronic-F68. FDA considered the request and informed Mission Barns that, if the firm plans to remove HEPES from the production process, FDA would request analytical data (i.e., proximates, vitamins, minerals, toxic heavy metals, trace heavy metals, and fatty acids) for three batches produced using basal media containing HEPES, but without rHPs, Tris-HCl, and Pluronic F-68 and limited batch data (i.e., proximates and toxic heavy metals) from one batch produced without HEPES, rHPs, Tris-HCl, and Pluronic F-68, as well as an accompanying narrative regarding why this data is sufficiently representative.

As noted in Information Request #7 above, Mission Barns has replaced all rHPs previously used in its manufacturing process with orthologous recombinant proteins derived from the genomes of agriculturally relevant species (e.g., bovine, porcine) at equivalent use concentrations. Further, Mission Barns has removed Tris-HCl and Pluronic-F68 from its manufacturing process. Although Mission Barns has altered the concentrations of certain media components to optimize cell culture performance, all components continue to be used at levels below the maximum use concentrations outlined in Section 3.2.2.2 of the May 25, 2022 Disclosable Safety Narrative (i.e. between 0.1 and 10 g/L for specified components either approved by FDA for various food applications or well characterized for their safety when used in foods, or below 0.1 g/L for all other components). Therefore, other than the requested analytical batch data discussed below, none of the post-submission manufacturing changes affect the safety rationales previously provided in the Disclosable Safety Narrative and the various amendments thereto.

With respect to HEPES, Mission Barns intends to remove this substance from its manufacturing process going forward. Mission Barns notes that the substance has been used commonly as a cell culture buffering agent since the early 1970s. Early studies of cell culture media containing HEPES demonstrated

that it did not have phenotypic effects on a variety of cell lines at common use concentrations.[1] Although some literature suggested that HEPES may alter the phenotype of certain cell types,[2] many publications report the use of HEPES in cell culture media or buffers for fat-tissue-derived cell cultures with no reported phenotypic abnormalities.[3] Consistent with these reported findings, Mission Barns has not observed any phenotypic abnormalities in cells cultured in HEPES-containing media or HEPES-free media. Even if the presence of HEPES were to affect Mission Barns' cultivated pork fat cells, Mission Barns expects that HEPES would make the cells increasingly abnormal relative to their conventional comparator. Therefore, the batch analysis data of cells cultured in media containing HEPES would deviate more from the conventional comparator than cells cultured in HEPES free media. As such, the batch analysis data from cells cultured in media containing HEPES represent a "worst-case-scenario" for purposes of safety analysis. Therefore, Mission Barns concludes that batches of cultivated pork fat cells produced with media containing HEPES are sufficiently representative of its canonical method of production to assess safety.

Below, Mission Barns presents batch analysis data (i.e., proximates, vitamins, minerals, toxic heavy metals, trace metals, and fatty acids) for three batches produced using basal media containing HEPES, but without rHPs, Tris-HCl, and Pluronic F-68 and limited batch analysis data (i.e., proximates, toxic heavy metals, and trace metals) from one batch produced without HEPES, rHPs, Tris-HCl, and Pluronic F-68.[4]

[1] Fisk, A, and S Pathak. "HEPES-buffered medium for organ culture." *Nature* vol. 224,5223 (1969): 1030-1.

[2] See, e.g., Bowman, C M et al. "HEPES may stimulate cultured endothelial cells to make growth-retarding oxygen metabolites." *In vitro cellular & developmental biology : journal of the Tissue Culture Association* vol. 21,3 Pt 1 (1985): 140-2; Tol, Marc J et al. "HEPES activates a MiT/TFE-dependent lysosomal-autophagic gene network in cultured cells: A call for caution." *Autophagy* vol. 14,3 (2018): 437-449; and Lleu, P L, and G Rebel. "Effect of HEPES on the taurine uptake by cultured glial cells." *Journal of neuroscience research* vol. 23,1 (1989): 78-86.

[3] See, e.g., Roh, Hyun Cheol et al. "Adipocytes fail to maintain cellular identity during obesity due to reduced PPARγ activity and elevated TGFβ-SMAD signaling." *Molecular metabolism* vol. 42 (2020): 101086; Lee, Mi-Jeong, and Susan K Fried. "Optimal protocol for the differentiation and metabolic analysis of human adipose stromal cells." *Methods in enzymology* vol. 538 (2014): 49-65; Hazen, S A et al. "Monolayer cell culture of freshly isolated adipocytes using extracellular basement membrane components." *Journal of lipid research* vol. 36,4 (1995): 868-75; Chu, Xiaoqing et al. "Suppression of adipogenesis program in cultured preadipocytes transfected stably with cyclooxygenase isoforms." *Biochimica et biophysica acta* vol. 1791,4 (2009): 273-80; Williams, Stuart K et al. "Encapsulation of adipose stromal vascular fraction cells in alginate hydrogel spheroids using a direct-write three-dimensional printing system." *BioResearch open access* vol. 2,6 (2013): 448-54; and Harms, Matthew J et al. "Mature Human White Adipocytes Cultured under Membranes Maintain Identity, Function, and Can Transdifferentiate into Brown-like Adipocytes." *Cell reports* vol. 27,1 (2019): 213-225.e5.

[4]

Proximates

Parameter[5]	Units	Mission Barns' Cultivated Pork Fat Cells				Range of previously reported batches[6]
		HEPES-containing media			HEPES-free media	
		Batch 1	Batch 2	Batch 3	Batch 4	
Moisture	%	89.02	89.80	87.78	86.16	86.82 - 89.81
Fat	%	4.99	5.77	6.78	5.58	4.56 - 5.85
Protein	%	6.24	5.51	7.44	7.64	4.27 - 5.59
Ash	%	0.93	0.96	1.06	0.93	0.88 - 1.01
Carbohydrates	%	<0.01	<0.01	<0.01	<0.01	0 - 1.03

The proximates data for the three batches produced using our canonical method of production, other than inclusion of HEPES in cell culture media, and our current, canonical method of production (i.e., HEPES free media) are generally consistent with proximates previously reported to the FDA.

[5] Mission Barns utilizes a third-party laboratory to conduct proximates analysis. Moisture content is determined using AOAC 950.46B methodology, fat is determined using AOAC 933.05 methodology, protein is determined using AOAC 981.10 methodology and ash is determined by AOAC 920.153 methodology. Carbohydrate content is determined by calculating the remainder of 100% following the subtraction of moisture, fat, protein and ash. In cases where moisture, fat, protein and ash sum to 100% or more, carbohydrates are reported as "<0.01". Mission Barns notes that our third-party laboratory previously used AOAC 990.20 and AOAC 991.20 methods to analyze moisture and protein, respectively. The updated methodologies are designed for assessment of meat-based matrices and are suitable for our sample matrix.

[6] Mission Barns refers to our response to information request #7 of the June 5, 2023 Amendment to the disclosable safety narrative.

Vitamins

Vitamin[7]	Unit	Mission Barns' Cultivated Pork Fat Cells			Range of previously reported batches[8]	Conventional pork fat[9]
		Batch 1	Batch 2	Batch 3		
Vitamin A	mcg RAE /100 g	< 10	< 10	< 10	< 10	0 - 26
Thiamin (B1)	mg/100g	0.40	0.28	0.40	0.275 - 0.45	0.084 - 0.21
Riboflavin (B2)	mg/100g	0.38	0.38	0.43	0.3 - 0.325	0.051 - 0.2
Niacin (B3)	mg/100g	2.75	4.00	3.50	3 - 3.625	0.985 - 3.23
Pantothenic Acid (B5)	mg/100g	0.38	0.35	0.35	0.55 - 0.6	0 - 0.611
Pyridoxine (B6)	mg/100g	0.58	0.73	0.78	0.475 - 0.5	0.03 - 0.275
Vitamin E	mg a-tocoph /100g	9.25	11.25	10.25	1.25	0 - 0.42

The vitamin results for three non-consecutive batches produced using our canonical method of production (other than inclusion of HEPES in cell culture media) generally fall within the range of previously reported results, with the exception of vitamin B2, vitamin B6 and vitamin E, which are measured at higher levels. Vitamins B2, B6, and E are widely present in food, including conventional pork fat. Mission Barns has conducted a dietary exposure assessment for vitamins B2, B6, and E using the EDI for cultivated pork fat cells (discussed in Section 3.1 of the May 25, 2022 disclosable safety narrative) of 16.7 g/day or 0.4 g/kg-bw/day. The daily intake of these vitamins can be calculated as follows:

[7] The vitamins were analyzed by a third-party laboratory according to the following methods: Vitamin A was assayed according to Analyst(1984) 109:489 (an accredited ISO method), Vitamin B1 was assayed according to AOAC 942.23, Vitamin B2 was assayed according to AOAC 970.65, Vitamin B3 was assayed according to AOAC 985.34 with associated VitaFast kits, Vitamins B5 and B6 were assayed according to AOAC 960.46 with associated VitaFast kits, and Vitamin E was assayed according to AOAC 992.03. Mission Barns clarifies that the tests were performed on pelleted cell material that was then diluted by a factor of 2.5 with DPBS (-/-) to reach the minimum quantity required for the applicable test. The analytical results were then multiplied by the dilution factor (2.5x) to calculate the actual concentration of each analyte.

[8] Mission Barns refers to our response to information request #7 of the June 5, 2023 Amendment to the disclosable safety narrative.

[9] Reported ranges are the lowest and highest reported value from USDA FoodData Central for NDB Number 10004 (Pork, fresh, backfat, raw) available at https://fdc.nal.usda.gov/food-details/167811/nutrients (accessed November 12, 2024), NDB Number 10006 (Pork, fresh, separable fat, raw) available at https://fdc.nal.usda.gov/food-details/167813/nutrients (accessed November 12, 2024), NDB Number 10942 (Pork, fresh, composite of separable fat, with added solution, raw) available at https://fdc.nal.usda.gov/food-details/169179/nutrients (accessed November 12, 2024), NDB Number 10109 (Pork, fresh, variety meats and by-products, leaf fat, raw) available at https://fdc.nal.usda.gov/food-details/167861/nutrients (accessed November 12, 2024), and NDB Number 10007 (Pork, fresh, separable fat, cooked) available at https://fdc.nal.usda.gov/food-details/168221/nutrients (accessed November 12, 2024).

Riboflavin (B2)

The average amount of vitamin B2 measured in the three HEPES-containing batches is 0.40 mg / 100 g.

EDI for vitamin B2 using 16.7 g/day cultivated pork fat cell EDI:

$$\frac{0.40\ mg\ vitamin\ B2}{100\ g\ cultivated\ fat\ cells} \times \frac{16.7\ g\ cultivated\ fat\ cells}{day} = \frac{0.067\ mg\ vitamin\ B2}{day}$$

EDI for vitamin B2 using 0.4 g/kg-bw/day cultivated pork fat cell EDI:

$$\frac{0.40\ mg\ vitamin\ B2}{100\ g\ cultivated\ fat\ cells} \times \frac{0.4\ g\ cultivated\ fat\ cells}{kg-bw\ /\ day} = \frac{0.0016\ mg\ vitamin\ B2}{kg-bw\ /\ day}$$

The FDA has established Daily Values (DVs) that represent the recommended amount of nutrients to consume or not to exceed each day. The DV for vitamin B2 is 1.3 mg for adults and children age 4 years and older.[10] The EDI of vitamin B2 from Mission Barns' cultivated pork fat cells represents ~5% of the Daily Value. Because adverse effects from high riboflavin intakes from foods or supplements have not been reported, the Food and Nutrition Board (FNB) has not established an Upper Limit (UL) – i.e., the maximum daily intake unlikely to cause adverse health effects – for riboflavin.[11] JECFA, however, has established an acceptable daily intake (ADI) – an estimate of the amount of a food additive that can be ingested daily over a lifetime without appreciable health risk – of 0 - 0.5 mg/kg-bw/day for vitamin B2.[12] The EDI of vitamin B2 from Mission Barns' cultivated pork fat cells represents less than 0.5% of the upper end of JECFA's ADI range. Mission Barns concludes that dietary exposure to vitamin B2 from cultivated pork fat cells does not present a safety concern.

Pyridoxine (B6)

The average amount of vitamin B6 measured in the three HEPES-containing batches is 0.70 mg / 100 g.

EDI for vitamin B6 using 16.7 g/day cultivated pork fat cell EDI:

$$\frac{0.70\ mg\ vitamin\ B6}{100\ g\ cultivated\ fat\ cells} \times \frac{16.7\ g\ cultivated\ fat\ cells}{day} = \frac{0.12\ mg\ vitamin\ B6}{day}$$

The DV for vitamin B6 is 1.7 mg for adults and children age 4 years and older.[13] The EDI of vitamin B6 from Mission Barns' cultivated pork fat cells represents ~7% of the Daily Value. High intakes of vitamin B6 from food sources have not been reported to cause adverse effects, however the FNB has established a UL for vitamin B6.[14] The UL varies by age, with the lowest established level of 30 mg/day

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[10] U.S. Food and Drug Administration. (2024). Daily Value on the Nutrition and Supplement Facts Labels, available at https://www.fda.gov/food/nutrition-facts-label/daily-value-nutrition-and-supplement-facts-labels (accessed November 4, 2024).

[11] National Institutes of Health (NIH), Office of Dietary Supplements (ODS). Vitamin B2 Fact Sheet for Health Professionals, available at: https://ods.od.nih.gov/factsheets/Riboflavin-HealthProfessional/ (accessed November 4, 2024).

[12] RIBOFLAVIN, Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), available at: https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/4091 (accessed November 4, 2024).

[13] U.S. Food and Drug Administration. (2024). Daily Value on the Nutrition and Supplement Facts Labels, available at https://www.fda.gov/food/nutrition-facts-label/daily-value-nutrition-and-supplement-facts-labels (accessed November 4, 2024).

[14] National Institutes of Health (NIH), Office of Dietary Supplements (ODS). Vitamin B6 Fact Sheet for Health Professionals, available at: https://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/ (accessed November 4, 2024).

for children aged 1-3 years.[15] The EDI of vitamin B6 from Mission Barns' cultivated pork fat cells represents less than 0.5% of this lowest established UL level. Furthermore, vitamin B6 is reported to be found at similar levels (0.535 - 0.726 mg / 100 g) in common cuts of pork, such as loin, bacon, and shoulder.[16] Mission Barns concludes that dietary exposure to vitamin B6 from cultivated pork fat cells does not present a safety concern.

Vitamin E

The average amount of vitamin E measured in the three HEPES-containing batches is 10.25 mg a-tocoph/100g.

EDI for vitamin E using 16.7 g/day cultivated pork fat cell EDI:

$$\frac{10.25\ mg\ vitamin\ E}{100\ g\ cultivated\ fat\ cells} \times \frac{16.7\ g\ cultivated\ fat\ cells}{day} = \frac{1.71\ mg\ vitamin\ E}{day}$$

The DV for vitamin E is 15 mg alpha-tocopherol for adults and children age 4 years and older.[17] The EDI of vitamin E from Mission Barns' cultivated pork fat cells represents ~11% of the Daily Value. Research has not found any adverse effects from consuming vitamin E in food, however the FNB has established ULs for vitamin E, with the lowest established level of 200 mg/day alpha-tocopherol for children aged 1-3 years.[18] The EDI of vitamin E from Mission Barns' cultivated pork fat cells represents less than 1% of this lowest established UL level. Mission Barns concludes that dietary exposure to vitamin E from cultivated pork fat cells does not present a safety concern.

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[15] *Id.*

[16] Reported range is the lowest and highest reported value from USDA FoodData Central for NDB Number 168322 (Pork, cured, bacon, pre-sliced, cooked, pan-fried), available at https://fdc.nal.usda.gov/food-details/168322/nutrients (accessed November 4, 2024), NDB Number 167839 (Pork, fresh, loin, top loin (chops), boneless, separable lean and fat, raw), available at https://fdc.nal.usda.gov/food-details/167839/nutrients (accessed November 4, 2024), NDB Number 169187 (Pork, Shoulder breast, boneless, separable lean and fat, raw), available at https://fdc.nal.usda.gov/food-details/169187/nutrients (accessed November 4, 2024), and NDB Number 167853 (Pork, fresh, spareribs, separable lean and fat, raw), available at https://fdc.nal.usda.gov/food-details/167853/nutrients (accessed November 4, 2024).

[17] U.S. Food and Drug Administration. (2024). Daily Value on the Nutrition and Supplement Facts Labels, available at https://www.fda.gov/food/nutrition-facts-label/daily-value-nutrition-and-supplement-facts-labels (accessed November 4, 2024).

[18] National Institutes of Health (NIH), Office of Dietary Supplements (ODS). Vitamin E Fact Sheet for Health Professionals, available at: https://ods.od.nih.gov/factsheets/VitaminE-HealthProfessional/ (accessed November 12, 2024).

Minerals

Mineral[19]	Unit	Mission Barns' Cultivated Pork Fat Cells			Range of previously reported batches[20]	Conventional pork fat[21]
		Batch 1	Batch 2	Batch 3		
Calcium	mg per 100g	1.50	2.50	2.75	< 0.25	1 - 22
Copper	mg per 100g	0.018	0.015	0.020	< 0.0025	0.009 - 0.09
Iron	mg per 100g	0.58	0.35	0.53	< 0.125 - 0.15	0.09 - 0.47
Magnesium	mg per 100g	8.05	4.85	8.98	4.3 - 7.1	1 - 9
Manganese	mg per 100g	0.02	0.02	0.03	0.0175 - 0.0225	0 - 0.006
Phosphorus	mg per 100g	146	125	159	116 - 154	19 - 121
Potassium	mg per 100g	90.50	46.75	114.75	42 - 79	31 - 333
Selenium	mg per 100g	< 0.025	< 0.025	< 0.025	< 0.025	0.008 - 0.0121
Sodium[22]	mg per 100g	302.5	347.5	252.5	507 - 530	5 - 81
Zinc	mg per 100g	0.80	0.62	0.83	0.49 - 0.78	0.18 - 0.9

The minerals data for the three batches produced using our canonical method of production (other than inclusion of HEPES in cell culture media) are generally consistent with or lower than either (1) mineral levels for Mission Barns cultivated pork fat cells previously reported to the FDA or (2) levels reported to be present in conventional pork fat.

[19] The minerals were analyzed by a third-party laboratory according to AOAC 2015.01 Mod<2232>. Mission Barns clarifies that the tests were performed on pelleted cell material that was then diluted by a factor of 2.5 with cell culture grade water to reach the minimum quantity required for the applicable test. The analytical results were then multiplied by the dilution factor (2.5x) to calculate the actual concentration of each analyte.

[20] Mission Barns refers to our response to information request #7 of the June 5, 2023 Amendment to the disclosable safety narrative.

[21] Reported ranges are the lowest and highest reported value from USDA FoodData Central for NDB Number 10004 (Pork, fresh, backfat, raw), available at https://fdc.nal.usda.gov/food-details/167811/nutrients (accessed November 12, 2024), NDB Number 10006 (Pork, fresh, separable fat, raw), available at https://fdc.nal.usda.gov/food-details/167813/nutrients (accessed November 12, 2024), NDB Number 10942 (Pork, fresh, composite of separable fat, with added solution, raw), available at https://fdc.nal.usda.gov/food-details/169179/nutrients (accessed November 12, 2024), NDB Number 10109 (Pork, fresh, variety meats and by-products, leaf fat, raw), available at https://fdc.nal.usda.gov/food-details/167861/nutrients (accessed November 12, 2024), and NDB Number 10007 (Pork, fresh, separable fat, cooked), available at https://fdc.nal.usda.gov//food-details/168221/nutrients (accessed November 12, 2024).

[22] Please note the cells are aseptically washed with a saline solution containing sodium after harvest.

Trace Metals

Trace Metal[23]	Unit	Specification	Mission Barns' Cultivated Pork Fat Cells			
			HEPES-containing media			HEPES-free media
			Batch 1	Batch 2	Batch 3	Batch 4
Nickel	ppm	< 0.2	< 0.02*	0.06	0.08	0.06
Vanadium	ppm	< 0.03	< 0.01*	<0.01*	0.01	0.02
Molybdenum	ppm	< 0.1	< 0.01*	0.01	0.01	0.01
* limit of quantification of assay.						

The trace metal data for the three batches produced using our canonical method of production (other than inclusion of HEPES in cell culture media) and one batch produced using our current, canonical method of production (i.e. HEPES free media) are all below the specifications established for such trace metals.

Heavy Metals

Heavy Metal[24]	Unit	Specification	Mission Barns' Cultivated Pork Fat Cells			
			HEPES-containing media			HEPES-free media
			Batch 1	Batch 2	Batch 3	Batch 4
Cadmium	ppb	< 50	< 1*	< 1*	< 1*	< 1*
Lead	ppb	< 50	< 10*	< 10*	< 10*	< 10*
Arsenic	ppb	< 50	< 10*	< 10*	< 10*	< 10*
Mercury	ppb	< 25	< 5*	< 5*	< 5*	< 5*
* limit of quantification of assay.						

The heavy metals data for the three batches produced using our canonical method of production (other than inclusion of HEPES in cell culture media) and one batch produced using our current, canonical method of production (i.e. HEPES free media) are all below the specifications established for such heavy metals.

[23] Trace metals were analyzed by a third-party laboratory according to AOAC2015.01Mod<2232>.
[24] Heavy metals were analyzed by a third-party laboratory according to AOAC2015.01Mod<2232>.

Fatty Acids

Fatty Acid[25]	Mission Barns' Cultivated Pork Fat Cells			Range of previously reported batches[26]	Conventional food comparator[27]
	Batch 1	Batch 2	Batch 3		
Myristic acid (14:0)	1.01	1.02	1.17	0.8	1.26 - 1.32%
Palmitic acid (16:0)	29.53	27.03	27.73	12.1 - 13.4	20.70 - 23.80%
Palmitoleic acid (16:1 CIS)	ND	ND	ND	2.8 - 3.3	1.95 - 3.10%
Stearic acid (18:0)	13.09	11.71	11.50	9.0 - 9.8	10.76 - 13.50%
Oleic acid (18:1 CIS)	43.18	43.06	42.06	58.2 - 58.7	40.03 - 43.40%
Elaidic acid (18:1 TRANS)	ND	ND	ND	0.6 - 0.7	0 - 0.71%
Linoleic acid (18:2 CIS)	10.59	13.37	13.20	ND	9.49 - 17.96%
Linolenic acid (18:3 CIS)	ND	ND	ND	1.5 - 1.8	0.83% - 1.00%
Eicosenoic acid (20:1)	1.03	1.07	0.90	ND	0.73 - 1%
Arachidonic acid (20:4)	1.58	2.74	2.40	1.4 - 1.7	0 - 4.7% (pork)[28]
Eicosapentaenoic acid (20:5)	ND	ND	ND	3.1 - 3.7	0 - 0.73% (pork)[29]
Nervonic acid (24:1)	ND	ND	ND	1.7 - 1.9	0 - 0.17% (pork)[30]
ND = Not Detected					

The fatty acid data for the three batches produced using our canonical method of production (other than inclusion of HEPES in cell culture media) are generally comparable to either (1) fatty acid levels for

[25] Fatty acids were analyzed by a third-party laboratory according to AOCS CE 1J-07 methodology.

[26] Mission Barns refers to our August 23, 2023 Amendment to the disclosable safety narrative.

[27] Unless otherwise indicated, conventional comparator is the highest and lowest reported value from USDA FoodData Central, NDB Number:4002 (lard), available at https://fdc.nal.usda.gov/food-details/171401/nutrients (accessed November 12, 2024), USDA FoodData Central, NDB Number:10004 (Pork, fresh, backfat, raw),available at https://fdc.nal.usda.gov/food-details/167811/nutrients (accessed November 12, 2024), USDA FoodData Central, NDB Number:10005 (Pork, fresh, belly, raw),available at https://fdc.nal.usda.gov/food-details/167812/nutrients (accessed November 12, 2024) NDB Number:10006 (Pork, fresh, separable fat, raw), available at https://fdc.nal.usda.gov/food-details/167813/nutrients (accessed November 12, 2024), in each case, calculated as a percentage of total lipid (fat).

[28] The upper end of the reported range is sourced from Matthews, K R et al. "Effect of whole linseed (Linum usitatissimum) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues." *The British journal of nutrition* vol. 83,6 (2000): 637-43.

[29] The upper end of the reported range is sourced from M. Enser, et al. "Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages." *Meat Science*, vol. 55, no. 2, Jun. 1999, p. 201.

[30] The upper end of the reported range is sourced from Kloareg, Maela et al. "Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs." The British journal of nutrition vol. 97,1 (2007): 35-44.

Mission Barns cultivated pork fat cells previously reported to the FDA or (2) levels reported to be present in the conventional food comparator.

Mission Barns notes that it now detects linoleic acid (18:2 CIS) and eicosenoic acid (20:1) in its cultivated pork fat cells, which were not detected in previous results. The average values of these fatty acids from three batches produced using our canonical method of production (other than inclusion of HEPES in cell culture media) are 12.39% and 1.00%, respectively. As noted in the above table, linoleic acid and eicosenoic acid are reported to be present at similar levels in pork backfat, pork belly, separable pork fat and lard. Additionally, eicosenoic acid is reported to be present in pork muscle (0.70 - 1.42%).[31] Because Mission Barns pork fat cells are intended as a replacement of conventional pork fat, the presence of linoleic acid and eicosenoic acid in Mission Barns' cultivated pork fat cells is not expected to increase dietary exposure to these fatty acids. Therefore, Mission Barns concludes the measured levels of linoleic acid and eicosenoic acid in its cultivated pork fat cells do not present a safety concern.

As noted in the September 26, 2023 amendment to the DSN, Mission Barns established a specification of ≤ 1 g total trans fat / 100 g fat content. No trans fats were detected in the three batches of cultivated pork fat cells produced using our current canonical method of production (other than inclusion of HEPES in cell culture media). As noted above, Mission Barns has removed all rHPs from its manufacturing process. Mission Barns has identified that one of the rHPs previously used (recombinant human serum albumin) was not fatty acid free, and could have been a source of previously detected trans fatty acids, such as elaidic acid. Mission Barns has replaced this rHP with a protein sourced from an agriculturally relevant species that has been specifically sourced and certified to be fatty acid free.[32] Mission Barns hypothesizes that the absence of trans fats in the above reported batches is due to this media component replacement.

[31] Li, Yongxiang et al. "Comparison of meat quality and glycolysis potential of two hybrid pigs in three-way hybrid model." *Frontiers in veterinary science* vol. 10 1136485. 17 Feb. 2023, doi:10.3389/fvets.2023.1136485.

[32]

Request for Clarification re: CCC 000008
To be added to the Disclosable Safety Narrative (DSN)

Request for Clarification

> Ferric nitrate nonahydrate is listed as a substance used in the cell culture media during the growth, proliferation, and differentiation stages of production. On page 40 of the March 16, 2022, final submission to the SCM, you provided a theoretical EDI of 0.0004 µg/kg-bw/d (based on a maximum use level of 0.1 g/L and the 10^{-5} washing factor) and an accompanying safety assessment for the use of ferric nitrate nonahydrate. On page 8 of the November 13, 2024, amendment to the DSN, you provided analytical data for the levels of iron in three batches of harvested cell material produced using your canonical method of production (other than the inclusion of HEPES in the cell culture media). You also provided a range for the levels of iron reported for conventional pork fat from the USDA FoodData Central database.
>
> Although ferric nitrate is listed in the completed consultation CCC 000001, we consider it to be a Type 4 substance. For Type 4 substances, we ask firms to disclose the identity and provide a full safety assessment, including safe reference levels and an EDI based on analytical measurements of the substance in the harvested cell material, in the DSN. We acknowledge that you have provided analytical data for the levels of iron in the harvested cell material, and as such, we are not asking for you to provide additional analytical data at this time.
>
> For addition to the DSN, please address the safety of this substance through a sufficient narrative argument (which may be partially supported by the use of this substance in CCC 000001, which has received a "no questions" letter from FDA) as well as analytical data for iron from the November 13, 2024, amendment to the DSN demonstrating the analytical EDI of the substance would be safe.
>
> As there are two substances in the manufacturing process that contain iron, we recognize measuring total iron levels within the harvested cellular material may compound the challenges of demonstrating the safety of ferric nitrate solely. Please consider an approach to delineate the concentration of ferric nitrate from measurements of total iron in the harvested cellular material based on a relative percentage of ferric nitrate to all iron containing components used in the manufacturing process. You should then provide an EDI for ferric nitrate based on the November 13, 2024, analytical data for iron which would be proportional to ferric nitrate solely and discuss whether exposure to ferric nitrate at that EDI is safe. We also suggest that you further support your safety narrative with appropriate, existing human food regulations for substances similar to ferric nitrate, such as other forms of nitrates and iron used in food or that are naturally present in foods.

Exposure Estimate for Ferric Nitrate

Mission Barns' cell cultivation media includes ferric nitrate nonahydrate, an inorganic compound formed from iron and nitrate. Ferric nitrate dissociates into ferric and nitrate ions in Mission Barns' cell cultivation media.

As disclosed in our November 13, 2024, amendment to the DSN, Mission Barns measured iron concentration in three non-consecutive batches of its cultivated pork fat cells:

Mineral	Unit	Mission Barns' Cultivated Pork Fat Cells			
		Batch 1	Batch 2	Batch 3	Average
Iron	mg per 100g	0.58	0.35	0.53	0.49

As noted in the request for clarification above, two substances are used in the manufacturing process that contain iron. To delineate the proportion of total iron in the harvested cellular material attributable to ferric nitrate, Mission Barns calculated the ratio of the concentration of ferric nitrate to all iron containing components used in the manufacturing process and estimates that ferric nitrate contributes approximately 4% of the total iron.[1]

Thus, using the average iron level from three representative batches, the estimated concentration of ferric nitrate in cultivated pork fat cells can be calculated as follows

$$[Fe(NO_3)_3] = 4\% \times \frac{0.00049\ g\ Fe}{100g\ pork\ fat\ cells} \times \frac{Mol\ Fe}{55.84\ g\ Fe} \times \frac{241.86g\ Ferric\ Nitrate}{Mol\ Ferric\ Nitrate} = 84.9\frac{\mu g\ Ferric\ Nitrate}{100g\ pork\ fat\ cells}$$

Iron and nitrates compose 23.1% and 76.9%, respectively, of the mass of ferric nitrate. Thus, the EDI for each of component can be calculated as follows:

$$EDI(Fe) = 23.1\% \times 84.9\frac{\mu g\ Ferric\ Nitrate}{100g\ pork\ fat\ cells} \times \frac{16.7\ g\ pork\ fat\ cells}{day} = 3.27\frac{\mu g\ Fe}{day}$$

$$EDI(Nitrates) = 76.9\% \times 84.9\frac{\mu g\ Ferric\ Nitrate}{100g\ pork\ fat\ cells} \times \frac{16.7\ g\ pork\ fat\ cells}{day} = 10.9\frac{\mu g\ nitrates}{day}$$

Dietary Exposure to Iron

As noted in our November 13, 2024 amendment to the DSN, iron levels in conventional pork fat cells are reported to range from 0.09 - 0.47 mg/100g. Mission Barns measured iron concentration in three non-consecutive batches of its cultivated pork fat cells, with an average of 0.49 mg/100g, which is comparable to levels in conventional pork fat. Because Mission Barns' pork fat cells are intended as a replacement of conventional pork fat, the level of iron in Mission Barns' cultivated pork fat cells is not expected to meaningfully increase dietary exposure to iron.

Mission Barns further notes that the Food and Nutrition Board (FNB) has established an Upper Limit (UL) for iron. The UL varies by age, with the lowest established level of 40 mg/day (persons 0-13 years olds) and the highest level of 45 mg/day (persons 14 years and older).[2] Mission Barns theoretical EDI of 81.8 µg/day of total iron (3.27µg/day from ferric nitrate) represents less than 0.25% of the lowest established UL level for iron. Mission Barns concludes the measured levels of iron in its cultivated pork fat cells (including levels contributed by ferric nitrate) do not present a safety concern.

[1] [redacted]

[2] National Institutes of Health (NIH), Office of Dietary Supplements (ODS). Iron Fact Sheet for Health Professionals, available at: https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/ (accessed December 20, 2024).

Dietary Exposure to Nitrates

The FNB has not established a UL or other reference value for nitrates. The Joint FAO/WHO Expert Committee on Food Additives (JECFA), however, has established an acceptable daily intake (ADI) – an estimate of the amount of a food additive that can be ingested daily over a lifetime without appreciable health risk – for nitrates as 3.7 mg/kg-bw/day, which is equivalent to 222 mg nitrate per day for a 60 kg adult.[3] The theoretical EDI for nitrates from ferric nitrate of 10.9 μg/day or 0.182 μg/kg-bw/day is several orders of magnitude below the ADI and is negligible in comparison. Mission Barns concludes that the exposure to nitrates from ferric nitrate in its cell culture media does not present a safety concern.

[3] JECFA (Joint (FAO/WHO) Expert Committee on Food Additives) (1995) Evaluation of certain food additives and contaminants: Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No. 859. World Health Organization, Geneva.

Request for Clarification re: CCC 000008

1. **Please provide, for addition to the DSN, the following:**
    a. **A statement clarifying whether Mission Barns uses microscopy, or a similar method validated for its intended use, to verify and/or characterize the cellular phenotype cell lines during the cell line establishment process.**

    Mission Barns confirms that we use light microscopy to verify and characterize the cellular phenotype of cell lines during the cell line establishment process.

    b. **A statement clarifying whether the recombinant proteins used during the production process have been modified to increase their stability in the cell culture media.**

    As mentioned in the March 6, 2022, submission to the SCM, and the June 3, 2024, supplement, Mission Barns clarifies that one of the recombinant proteins used during the production process has been modified to increase its stability in cell culture media.

    c. **The information contained in the last two sentences of the first paragraph of page 41 of the March 6, 2022, submission to the SCM and the reference provided in footnote 67 on the same page.**

    Based on the findings from this study, EPA established a RfD for nickel at 0.02 mg/kg bw/day or 20 µg/kg bw/day.[67] For the purpose of our assessment, we adopt 20 µg/kg bw/day as the ADI.

    [67]: *EPA, Chemical Assessment Summary for Nickel, available at: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0271_summary.pdf#nameddest=rfd (accessed on February 10, 2025).*

2. **FDA notes that the January 31, 2024, amendment, is not disclosable based on a statement included in the amendment. That said, in the amendment you explain, "We note for the hormones […], these are non-protein molecules that have a conserved structure across species (i.e. they do not have a specific species of origin)." As this explanation regarding the conserved structure of the hormones is relevant to your safety assessment for the use of hormones in your production process, please provide a statement authorizing the disclosure of the quoted text included in this question for addition to the DSN.**

    Mission Barns authorizes the following statement for addition to the DSN:
    "We note for the hormones […], these are non-protein molecules that have a conserved structure across species (i.e. they do not have a specific species of origin)."

3. **In response to question 10 of the September 25, 2024, amendment you state, "With a specification of molybdenum at 0.1 ppm, the daily intake of molybdenum from the cultivated pork fat cells consumption will be less than 5% of the molybdenum DV established by FDA, and less than 0.1% of the UL established by the FNB." However, the specification for molybdenum provided in the table listing trace metal specifications and analytical data in the November 13, 2024, amendment to the DSN is "< 0.1 ppm." For addition to the DSN, please clarify whether the specification for molybdenum in the harvested cell material is "at 0.1 ppm" (i.e., levels of molybdenum equal or less than 0.1 ppm in the harvested cell material) or "< 0.1 ppm" (i.e., levels of molybdenum less than 0.1 ppm in the harvested cell material).**

   Mission Barns clarifies that our specification for molybdenum is "< 0.1 ppm" (i.e., levels of molybdenum less than 0.1 ppm in the harvested cell material).

4. **In the November 13, 2024, amendment to the DSN, you provided analytical data for the level of nickel in a single batch of harvested cell material produced using HEPES-free medium and reported a value of 0.06 ppm nickel. Based on this analytical data and the provided serving size of 16.7 g, FDA calculated an estimated daily intake (EDI) of 1.7 x 10^{-5}mg/kg body weight (bw)/d, to enable us to compare the EDI based on analytical data to the acceptable daily intake (ADI) (i.e., the EPA established reference dose (RfD) of 0.02 mg nickel/kg bw/d you referenced). For addition to the DSN, please confirm whether you agree with the calculated EDI of 1.7 x 10^{-5} mg nickel/kg bw/d.**

   Mission Barns agrees with the EDI calculation of 1.7 x 10^{-5} mg nickel/kg bw/d.

5. **For addition to the DSN, please provide a statement confirming that no material prohibited under 21 CFR 189.5, prohibited cattle materials, is used in the production process, nor present in the harvested cell material. The requirements under 21 CFR 189.5 apply to both the animal cell line and any material inputs sourced from cattle.**

   Mission Barns confirms that we do not use materials prohibited under 21 CFR 189.5, prohibited cattle materials, in the production process, nor present in the harvested cell material.

From: Russ Neldam
To: HFP-OFCSDSI-Animal Cell Culture
Cc: [redacted] Hice, Stephanie; Eitan Fischer; [redacted]
Subject: [EXTERNAL] Re: Request for clarification - RE CCC 000008
Date: Tuesday, February 18, 2025 1:49:38 PM
Attachments: image003.png

Hi Ashley,

For addition to the DSN, Mission Barns confirms that the functional modification in the amino acid sequence of the recombinant porcine protein that has been modified to increase its stability in cell culture media is not expected to alter the allergenic potential of the protein compared to that of the native porcine growth factor.

Please let us know if you have any questions.

Best regards,

On Tue, Feb 18, 2025 at 10:16 AM HFP-OFCSDSI-Animal Cell Culture <HFP-OFCSDSI-AnimalCellCultureFoods@fda.hhs.gov> wrote:

> Hello Mr. Neldam –
>
> Below, please find a request for clarification regarding information in CCC 000008. Your response will be appended to the CCC 000008 disclosable safety narrative (DSN) as an amendment and will subsequently be treated as part of the disclosable safety narrative in the administrative record for CCC 000008 unless we specifically note otherwise. We are happy to discuss any individual point if you have questions about how to convey substantive safety information without disclosing other details that may contain confidential commercial information or trade secrets, or if you believe that we are directly requesting you to disclose such information.
>
> Request for Clarification
>
> 1. In the February 11, 2025, amendment to the DSN, you state, “As mentioned in the March 6, 2022, submission to the SCM, and the June 3, 2024, supplement, Mission Barns clarifies that one of the recombinant proteins used during the production process has been modified to increase its stability in cell culture media.” For addition to the DSN, please provide a statement confirming that the functional modification in the amino acid sequence of this recombinant porcine protein is not expected to alter the allergenic potential of the protein compared to

that of the native porcine growth factor.

Sincerely,

Ashley E. Nazario-Toole, Ph.D

Biologist & Regulatory Review Scientist

Innovative Foods Staff

Human Foods Program

U.S. Food and Drug Administration

Tel: 301-796-5839

Ashley.NazarioToole@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION

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Russ Neldam (he/him)
AVP, Legal & Regulatory
www.missionbarns.com
San Francisco, California

Request for Clarification re: CCC 000008

To be added to the Disclosable Safety Narrative (DSN)

Requests for Clarification

1. In the February 18, 2025, amendment to the DSN, you state, "Mission Barns confirms that the functional modification in the amino acid sequence of the recombinant porcine protein that has been modified to increase its stability in cell culture media is not expected to alter the allergenic potential of the protein compared to that of the native porcine growth factor." For addition to the DSN, please provide information regarding the modified growth factor as follows:

    a) Clarify how you confirmed that the amino acid modification of the porcine growth factor is "not expected to alter the allergenic potential" of the recombinant protein. FDA notes that allergenicity assessments for proteins present in or added to food typically include a comparison of the amino acid sequence of the modified protein to known allergens. Examples of databases typically used to evaluate the potential allergenicity of novel proteins are (1) the Food Allergy Research and Resource Program (FARRP) available at http://www.allergenonline.org/databasehelp.shtml) and (2) the Comprehensive Protein Allergen Resource (COMPARE) available at Compare Database – Allergen Database.

    b) Clarify whether the modification to the growth factor increases the recombinant protein's "stability" in cell culture (e.g., increased thermostability, decreased digestibility) or increases the activity of the protein in cell culture (e.g., increased affinity for the cognate receptor).

Mission Barns notes that the modified recombinant porcine protein was measured in both the final cell pellet and the final wash solution from three non-consecutive batches of cultivated pork fat cell production and was found not to be present at quantifiable levels, as disclosed in Section 3.2.2.4.b of the May 25, 2022 DSN.[1] Regarding the protein modification, Mission Barns notes that the core functional aspects of the recombinant porcine protein itself are not modified. Rather, the N-terminus of the protein contains an additional sequence appended onto the wild-type sequence, which increases its affinity for the cognate receptor in cell culture. While this modification enhances receptor engagement, it does not alter the fundamental function of the protein itself, which retains the same receptor activation properties as the wild-type form.

1. a) To confirm that amino acid modification of the porcine growth factor is not expected to alter the allergenic potential of the recombinant protein, Mission Barns compared the amino acid sequence of the modified protein to known allergens contained in the following peer-reviewed allergen bioinformatics databases: (1) the Food Allergy Research and Resource Program (FARRP) and (2) the Comprehensive Protein Allergen Resource (COMPARE).

    As suggested by the Food and Agricultural Organization/World Health Organization, Mission Barns used a minimum threshold of >35% identity over an 80 amino acid stretch (80-mer sliding-

[1] Mission Barns clarifies that modified recombinant porcine protein was one of the two "representative growth factors" which Mission Barns analyzed in final cell pellet.

window) in sequence comparisons to identify potential sequence similarity to known allergens in the novel food protein.[2] Additionally, Mission Barns searched for exact 8-amino-acid contiguous matches between the novel food protein and known allergens.[3]

The bioinformatic analyses found no matches with greater than 35% identity using a window of 80 amino acids, and no exact matches using a window of 8 amino acids. These results indicate that the modified protein does not share significant sequence similarity with known allergens and is not expected to increase its allergenic potential, compared to an unmodified protein.

See **Confidential Attachment A** for screenshots of the bioinformatics analysis.

1. b) As noted above, Mission Barns clarifies that the modification to the protein increases its affinity for the cognate receptor in cell culture.[4]

   While Mission Barns is not aware of scientific literature directly assessing the modification's effect on thermostability, studies of the modified protein indicate that its susceptibility to enzymatic digestion is similar to that of native form, with no significant increase in resistance to degradation.[5] Further, based on the structure and function of the modified protein, we do not expect it to exhibit increased thermal stability or reduced digestibility for the following reasons:

   - Growth factors, as a class of signaling molecules, are generally known to possess high thermal lability and be more susceptible to degradation than many other naturally occurring proteins. Proteins that exhibit increased resistance to acid hydrolysis are often associated with allergenicity. Since the modified protein does not exhibit sequence-homology with known allergens, there is no indication that the modification confers increased resistance to acid hydrolysis. Consequently, it is reasonable to expect this

[2] FAO/WHO, Evaluation of allergenicity of genetically modified foods. Report of Joint FAO/WHO Expert Consultation. Rome, Italy: Food and Agriculture Organization of the United Nations; 2001. (available at https://cdn.who.int/media/docs/default-source/documents/publications/evaluation-of-allergenicity.pdf, accessed on February 18, 2025).

[3] Song, P., Herman, R. A., & Kumpatla, S. (2014). Evaluation of global sequence comparison and one-to-one FASTA local alignment in regulatory allergenicity assessment of transgenic proteins in food crops. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *71*, 142–148. https://doi.org/10.1016/j.fct.2014.06.008

[4] [redacted]

[5] [redacted]

modified protein would degrade in a similar manner to its native counterpart under thermal stress or digestive conditions.

- Amino acid modifications that are known to affect thermal stability—such as proline substitutions, hydrophobic core modifications, or disulfide bond changes—are not present in the modified protein. Similarly, features known to reduce digestibility, such as proline-rich sequences, glycosylation, increased hydrophobicity, and enhanced folding stability, are absent in the modification.

Given these factors, Mission Barns does not reasonably expect the modified protein to exhibit significantly different thermal stability or digestibility compared to its native form.