# Memorandum

**Date** March 7, 2025

**From** Ashley Nazario Toole (Innovative Foods Staff (IFS))

Through

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**Subject** Cell Culture Consultation (CCC) 000008, Cultured *Sus scrofa domesticus* cell material

**To** Administrative File, CCC 000008

**Submission Received Date:** May 25, 2022, Disclosable Safety Narrative; March 16, 2022, Supplemental, Confidential Material

**Amendments Received Date:** March 6, 2023; June 5, 2023; August 23, 2023; September 26, 2023; October 26, 2023; October 31, 2023; November 6, 2023; December 11, 2023; January 31, 2024; June 3, 2024; July 8, 2024; September 25, 2024; October 24, 2024; November 13, 2024; December 24, 2024; February 11, 2025; February 18, 2025; February 19, 2025

**Sponsor:** Mission Barns (Mission Barns, the firm)

## Summary


- The Food and Drug Administration (FDA, we) evaluated the food that is the subject of CCC 000008 submitted by Mission Barns.
- This food is defined as the harvested cell material, comprised of cultured *Sus scrofa domesticus cells, with characteristics of adipocytes, in the form of cell biomass, as produced by the method of manufacture described in CCC 000008.*

- The cells used to establish the cell lines are originally isolated from subcutaneous belly fat tissue biopsied from domestic Yorkshire pigs. The isolated cells are phenotypically characterized using standard methods validated for their intended purpose, including microscopy.
- The cell lines are established by selective culture of adherent cells from growth in a serum-containing medium to a serum-free medium over several generations (passages). Species identity was verified using a porcine-specific polymerase chain reaction (PCR) assay and genetic stability was assessed by karyotyping (normal chromosomal spreads).
- The cells are cultured by first increasing total cell numbers in an adherent culture proliferation phase, followed by a subsequent cell fattening phase in which the cells are induced by specific medium factors to form intracellular lipid droplets.
- The cells are harvested by the addition of a harvest solution to dissociate the cells, centrifuged, washed, and stored in sterile containers within a temperature-controlled environment.
- The harvested material, following washing, is described as cultured pork (*Sus scrofa domesticus*) fat cells, similar in fatty acid content to conventional pork fat products. Microbial, toxic heavy metal, and trace metal specifications are provided.
- We evaluated information about the cell lines, the production process (including cell bank establishment), substances used in the production process, and properties of the harvested cell material, including information available in both the disclosable safety narrative as well as supporting, corroborative information in the supplemental, confidential material.
- Based on the data and information presented in CCC 000008, we have no questions at this time about Mission Barns' conclusion that foods comprised of or containing cultured pork fat cell material resulting from the production process defined in CCC 000008 are as safe as comparable foods[1] produced by other methods. Furthermore, we have not identified any information indicating that the production process as described

[1] FDA notes that there is no single conventional comparator, such as conventional pork lard, for Mission Barns' cultured pork fat cells, as the firm's harvested cell material only contains an average of 5.85% total fat. Mission Barns provided information for the following conventional comparators during the consultation: conventional pork fat (e.g., back fat, belly) from peer-reviewed literature in the March 6, 2023, amendments for the disclosable safety narrative and the supplemental, confidential material; U.S. Department of Agriculture (USDA) FoodData Central "Pork, fresh, belly, raw" (NDB Number 10005), "Pork, fresh, backfat, raw" (NDB Number 10004), "Pork, fresh, variety meats and by-products, leaf fat, raw" (NDB Number:10109), and "Pork, fresh, separable fat, cooked" (NDB Number 10007) in the June 5, 2023, amendment; and USDA FoodData Central "pork, fresh, separable fat, raw" (NDB Number:10006), "pork, fresh, composite of separable fat, with added solution raw" (NDB Number:10942) in the June 5, 2023, August 23, 2023, September 26, 2023, and October 26, 2023, amendments, and pork from peer-reviewed literature and "lard" (NDB Number:4002) in the November 13, 2024, amendment. Mission Barns reports that the fatty acid content in the harvested cell material is consistent with ranges of fatty acids reported for conventional pork nutrition data from in the USDA FoodData Central for "lard" (NDB Number:4002), "Pork, fresh, backfat, raw" (NDB Number:10004), "Pork, fresh, belly, raw" (NDB Number:10005), and "Pork, fresh, separable fat, raw" (NDB Number:10006). Mission Barns compares the levels of fatty acids not reported for conventional pork nutrition data in the USDA FoodData Central (i.e., arachidonic acid (20:4), eicosapentaenoic acid (20:5), and nervonic acid (24:1)), in the harvested cell material to ranges reported for conventional pork in publicly available scientific literature. Mission Barns' conclusions regarding the safety of the harvested cell material is not based on the establishment of exact equivalence of all nutrients.

in CCC 000008 would be expected to result in food that bears or contain any substance or microorganism that would adulterate the food.[2]

**Production Method**

Mission Barns describes an overall production process involving the establishment of a cell bank that provides a standardized source of cells for food production, and a production process including proliferation of the cells, fattening of the cells to acquire expected characteristics of fat cells[3], and harvest or collection of the cell material for subsequent conventional food processing.

The firm states that a food safety and quality system is in use during production, and provides information about the following programs and measures that will be used in its production facilities, including:

- A current good manufacturing practice (cGMP) program that includes all the items enumerated in 21 CFR part 117 subpart B;
- Development of a hazard analysis and risk-based preventive controls (HARPC) food safety plan, including preventive measures and corrective actions for prevention and mitigation of biological, chemical, and physical hazards;
- A supplier approval program;
- Validated sanitation processes and an environmental monitoring program;
- In-process checks and controls of key process parameters;
- Document and records control including material and product specifications;
- Controls for prevention of biological, chemical, and physical hazards;
- A product release system involving quality assurance review for incoming raw materials, intermediate products, and finished products;
- Allergen controls;
- Batch record review; and
- Traceability of raw materials and finished products.

Mission Barns also states that its production process follows internal standard operating procedures (SOPs) and is performed by authorized and trained personnel. The firm states that cell culture occurs in a controlled, dry cleanroom environment that utilizes high efficiency particulate air (HEPA) filters to maintain air quality. The firm also notes the use of supporting programs such as water monitoring, to ensure that water used during the production process meets specifications for purity, and internal auditing, as well as aseptic technique training, employee hygiene, and personal protective equipment (PPE) gowning.

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[2] Our review did not address other provisions of the Federal Food, Drug and Cosmetic Act (FD&C Act).

[3] FDA notes that Mission Barns' cell fattening phase results in cells with characteristics of fat cells (i.e., increased fat content) through intracellular accumulation of lipids from the cell culture medium. Unlike cellular differentiation, cell fattening does not induce gene expression changes or result in a specialized cell type (e.g., mesenchymal stem cell (MSC) differentiation into adipocytes).

An overview of the production process, potential hazards or quality issues at each process step, and management strategy is provided in Table 1 based on the information provided by Mission Barns. A more detailed version of this table is provided in the Appendix of this memorandum.

***Table 1: Overview of potential identity, quality, and safety issues***

| Process Step | Potential Issues | Management Strategies |
|---|---|---|
| Cell Isolation | Cell identity; contaminants from source, reagents, or environment | Antimicrobials, aseptic procedures, documentation, sterilization, supply-chain controls, testing program |
| Establishment of Cell Lines | Cell identity; contaminants from materials or environment; appropriate adaptation to culture | Allergen controls, aseptic procedures, cell stability testing, documentation, foreign materials management program, environmental monitoring, supply-chain controls, sterilization, testing program |
| Manufacturing Cell Bank Establishment | Cell identity; contaminants from materials, equipment, or environment; media components | Allergen controls, aseptic procedures, cGMP, documentation, environmental monitoring, foreign materials management program, material handling and positive release program, materials risk assessment sterilization, supply-chain controls, testing program |
| Proliferation Phase | Contaminants from materials, equipment, or environment; media components | Allergen controls, aseptic procedures, cGMP, documentation, environmental monitoring, food safety assessment[4], material handling and positive release program, materials risk assessment, sterilization, supply-chain controls, testing program |
| Cell Fattening Phase | Contaminants from materials, equipment, or environment; media components | Allergen controls, aseptic procedures, cGMP, documentation, environmental monitoring, food safety assessment, foreign materials management program, sterilization, supply-chain controls, testing program |
| Harvest of Cell Material | Contaminants from materials, equipment, or environment; media components | Allergen controls, aseptic procedures, cGMP, compositional analysis, controlled temperature conditions, environmental monitoring, foreign materials management program, food safety assessment, specifications, sterilization, supply-chain controls, testing program, washing step |

[4] "Food safety assessment" indicates evaluation of the use of substances or materials based on commonly established paradigms for evaluating chemical, biochemical, and toxicological data in conjunction with estimates of exposure for their intended use to assess whether such use is consistent with applicable safety standards.

Cell Banking

Mission Barns provides information about the establishment of cell banks used in the subsequent production process. A cell bank as described in the firm's manufacturing process is a collection of cryopreserved cells derived from a single tissue source in a single animal. The steps involved include:

- Cell isolation
- Establishment of cell lines
- Manufacturing cell bank establishment

*Cell Isolation*

The cells used to establish the cell banks are isolated from subcutaneous belly fat biopsied from a domestic Yorkshire pig (*Sus scrofa domesticus*) in a veterinary operating chamber by trained veterinary doctors. The collected tissue is transported to a cell isolation lab in Mission Barns' facility, where it is processed under aseptic conditions to isolate and culture individual cells to be used during the downstream cell line establishment process. Reagents used at this stage may include materials of animal origin (e.g., serum), in addition to cell culture media, media components, and antibiotics and antifungals.

Potential hazards and quality issues identified by Mission Barns at this stage include:

- Source animal health prior to tissue procurement resulting in cells contaminated by adventitious agents such as bacteria or viruses;
- Introduction of adventitious agents from contaminated non-animal sourced reagents or the local environment; and
- Introduction of adventitious agents from animal-derived reagents (e.g., serum).

Mission Barns documents all processing steps from animal sourcing to cell isolation. Animal source documentation includes the results of a complete medical exam of the source animal, the source animal vaccination history, results of 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 records identifying the time and place of harvest.

Mission Barns states that internal SOPs are followed and describes controls in place to prevent environmental contamination during the cell isolation process, including the use of authorized and trained personnel, aseptic procedures, and the use of biosafety cabinets during cell handling, cell passaging, and change of culture medium. Antibiotics and antifungals are used to support establishment of sterile culture conditions for subsequent steps in the development of the cell bank. During the first month after cells are isolated from tissue, the firm visually inspects cultures for signs of bacterial or fungal contamination. At the end of this period, cultures are tested for viral and *Mycoplasma* spp. contamination. The firm also implements supply-chain preventive controls, filtration of growth media, sanitation controls (including

water quality monitoring, equipment cleaning, and facility sanitation), and an environmental monitoring program[5] to control for the introduction of adventitious agents.

The firm states that all processing reagents, including animal-derived substances, are food grade (when available), pharmaceutical grade, or the highest-quality material that is commercially available. All animal derived raw materials are either sterile as received or filtration-sterilized prior to their use in cell culture. Food safety and quality management systems are in place to account for the potential risks associated with the use of animal-derived substances, including a Materials Risk Assessment, Supplier Approval Program, and a Material Handling and Positive Release Program. All bovine-derived substances are verified to be sourced from bovine spongiform encephalopathy (BSE)-free/risk-negligible herds and compliant with 21 CFR 189.5, prohibited cattle materials. The firm notes that controls in place are adequate to manage contamination risk from sera[6] and any other animal-derived substances that could be used in production.

Records for each cell line include animal source documentation, methods used for originating tissue isolation, subculturing history, and substances used, including the cell culture medium and food contact surfaces such as that used for adherent culture.

*Establishment of Cell Lines*

Mission Barns passages the isolated cells to select for, or induce, individual cells that have desired characteristics, including the ability to grow in a serum-free medium, the ability to exhibit a stable phenotype with repeated, linear growth (cell immortalization), the ability to acquire characteristics of fat cells (adipocytes), and the ability to grow on solid substrates (adherent culture). The firm divides the cell line establishment process into two phases: preliminary cell bank establishment in a serum-supplemented medium and transition (adaptation) to a serum-free medium. During the preliminary cell line establishment phase, cell lines are expanded in a serum-supplemented medium. Preliminary cell lines are documented, expanded, and cryopreserved before the transition to the serum-free medium. During the transition to the serum-free medium, frozen cells are thawed and then transitioned to grow in serum-free medium. Population doubling time is used to monitor the proliferation rate and health of the cell line during the adaptation to the serum-free medium.

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[5] Mission Barns initially identified *Listeria monocytogenes* and *Staphylococcus* spp. as potential biological hazards from the environment or human sources. The firm revised its risk assessment after conducting a year of regular environmental monitoring program testing at its GMP-compliant manufacturing facility and identifying zero occurrences of *L. monocytogenes* contamination. Based on this data, the firm concluded that *L. monocytogenes* is not a meaningful food safety risk in the harvested cell material due to the firm's sourcing and production process. However, the firm detected *Bacillus cereus* and *Ralstonia insidiosa* in the processing environment. As the environmental sources of *Listeria* spp. overlap with environmental sources of *B. cereus*, and as *R. insidiosa* is a waterborne bacterium that is capable of survival in wet environments, Mission Barns agreed to test for *Listeria* spp. in its environmental monitoring program.

[6] Mission Barns provides a detailed description of the process used to verify that animal-derived sera are free of adventitious agents identified by Mission Barns as hazards during this stage of the production process to FDA as supporting, corroborative information in the supplemental, confidential material.

The cell lines described in CCC 000008 exhibit cell immortalization due to spontaneous immortalization through selection in culture. Mission Barns notes that genetic engineering is not employed at any point during the production process. Reagents used at this stage may include materials of animal origin (e.g., serum), in addition to cell culture medium, culture vessel coating reagents, medium components, and antibiotics and antifungals.

Potential hazards and quality issues identified by Mission Barns at this stage include:

- Introduction of adventitious agents from contaminated non-animal sourced reagents or the local environment;
- Introduction of adventitious agents from animal-derived reagents (e.g., serum); and
- Unintended effects of adaptation to culture (e.g., genetic instability).

Mission Barns describes controls to prevent environmental contamination during serum-free medium adaptation, including the use of trained personnel, antimicrobials, filter-sterilized media, aseptic procedures, and biosafety cabinets. Additionally, as noted above, Mission Barns implements supply-chain controls, sanitation controls, and environmental monitoring.

As stated previously, animal-derived substances are either sterile as received or filtration-sterilized prior to their use in cell culture and the firm implements process controls to manage contamination risk from serum and any other animal-derived substances that could be used in production.

Mission Barns evaluates the genetic stability of early and late passages of cell lines under development by karyotyping, a procedure used to identify chromosomal abnormalities. Karyotyped cells of early and late passages demonstrate normal female porcine chromosome numbers and staining patterns for each chromosome indicating that genetic stability of cell lines is maintained over the duration of the cell line establishment process. Mission Barns also measures parameters related to cell proliferation and viability to confirm stability of the cells during the transition to serum-free medium.

*Manufacturing Cell Bank Establishment*

Mission Barns states that individual cell lines displaying the desired properties described in the previous section are expanded and then prepared for storage in a manufacturing cell bank.

Potential hazards and quality issues identified by Mission Barns at this stage include:

- Use of an unintended cell line due to documentation or handling errors;
- Use of cell lines that that do not exhibit desired growth characteristics;
- Contamination with microorganisms, zoonotic viruses, or other adventitious agents from the original animal source of cells;
- Introduction of adventitious agents from contaminated non-animal sourced reagents or the local environment; and
- Introduction of adventitious agents from animal-derived reagents (e.g., serum) used in cell line establishment.

Mission Barns describes quality and safety testing for each manufacturing cell bank. These include tests for species verification, genetic stability, and for sterility and the absence of adventitious agent contamination, which, the firm states, are validated for their intended purpose. The firm confirms the species identity of the cell lines in the manufacturing cell bank as *Sus scrofa domesticus* using a PCR method, the GeneScan DNAnimal Ident Pork IPC kit, which is designed to detect the presence of porcine genomic sequences in DNA extracted from food and feed matrices.[7] The firm also notes that research cell lines derived from other species (i.e., chicken, duck, cow) are physically separated from the manufacturing cell bank vials (i.e., research cell lines are stored in a separate on-site cryogenic freezer) and confirms that only cell lines derived from *Sus scrofa domesticus* are cultured in the manufacturing facility. Further, the firm has implemented cell bank inventory controls, including vial labeling and material and lot coding, to ensure only *Sus scrofa domesticus* derived manufacturing cell bank vials are used for production. The firm also karyotypes cells to confirm the genetic stability (i.e., normal female porcine chromosome numbers and staining patterns) of cells used in the cell banks.

Mission Barns' adventitious agent testing is intended to address common public health hazards that have the potential to propagate in cell culture in cultured animal cells. At the end of the cell banking process, the firm screens spent media for the presence of aerobic bacteria (i.e., aerobic plate count), *Enterobacteriaceae*[8], *Mycoplasma* spp., coliforms, and yeast and mold. Mission Barns discusses the details of these adventitious agent tests, which are conducted in-house using validated methods, and describes the methods as either observation of potential microbial growth under permissive conditions (aerobic plate count, *Enterobacteriaceae*, coliforms, or yeast and mold), or real-time PCR analysis (*Mycoplasma* spp.). To account for the use of animal-derived sera, cells of the manufacturing cell bank are tested for the presence of animal adventitious agent viruses identified by the firm as potential hazards.[9]

Mission Barns describes controls to prevent environmental contamination during cell banking, including, aseptic procedures, trained personnel, and the use of biosafety cabinets. Additionally, as noted above, Mission Barns implements other programs and controls such as, environmental monitoring, filtration of growth media, sanitation controls and supply chain controls to mitigate the risks. The firm ensures that each manufacturing cell bank meets identity, purity, safety, and stability standards before being released for use in the production process.

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[7] Mission Barns confirmed that DNA from other animal species, including those found in Mission Barns' research cell banks (i.e., chicken, duck, cow), is absent from the manufacturing cell bank using separate multi-species PCR analysis assays testing for cow, pig, horse, sheep, goat, chicken, turkey, and duck DNA sequences.

[8] A large family of Gram-negative bacteria that includes pathogens such as *Salmonella* serovars, *Escherichia coli*, *Klebsiella* spp., and *Shigella* spp.

[9] Animal viruses are tested using the fluorescent antibody testing method found in 9 CFR §113.47: bovine viral diarrhea virus, porcine parvovirus, porcine adenovirus, porcine hemagglutinating encephalitis virus, transmissible gastroenteritis virus, reovirus, and rabies virus.

Production Process

Mission Barns provides information about its production process, including:

- The proliferation phase using adherent culture;
- The cell fattening phase using adherent culture; and
- Harvest of cell material.

Mission Barns states that the firm's food safety and quality systems are based on the requirements of 21 CFR part 117 ("Current Good Manufacturing, Hazard Analysis, and Risk-based Preventative Controls for Human Food"), including the establishment of a facility food safety plan in compliance with the regulations.

Batch records will be maintained to provide traceability of all raw materials used, operations, and testing during the production process. Mission Barns also states that all incoming dry powdered culture media as well as raw materials used for culture media are placed on hold until they are approved for release by a Preventive Controls Qualified Individual following review of supplier certificates of analysis/certificates of conformance, testing results, and/or production records. Liquid media is sterilized with an appropriate filter (0.2 micron) and stored at 2-8 °C.[10] Mission Barns states that the firm uses appropriate and authorized food contact materials throughout the production process.[11] The firm further states that single use disposable sterile components are used for the seed train expansion and cell growth processes. The process also uses proprietary cell culture bioreactors[12] that are cleaned and sterilized using high temperature steam (>121°C).

Mission Barns states that the production process is a highly controlled aseptic process. Cell handling, cell passaging, and change of culture media are described as being performed under Class II biosafety cabinets or in a filtered air positive pressure environment. Mission Barns states that single use disposable sterile components or cleaned/sterilized stainless steel are used for all bioreactor surfaces that come in direct contact with the harvested cell material. Cultures are described as being sampled under sterile conditions at both pre- and post-harvest and tested for adventitious agent contamination. The firm also states that sanitation controls and an environmental monitoring program are in place to assess the effectiveness of overall hygienic practices in the manufacturing facility.

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[10] Mission Barns provides a detailed description of the filter-sterilized liquid media storage conditions (i.e., temperature) to FDA as supporting, corroborative information in the supplemental, confidential material.

[11] The production conditions described by the firm would be consistent with food type 1 (nonacid, aqueous products; may contain salt or sugar or both (pH above 5.0)) and conditions of use type D (hot filled or pasteurized below 66 °C). The various food types and conditions of use are described in Appendix V of FDA's "Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances (Chemistry Recommendations)."

[12] Mission Barns provides a detailed description of the proprietary cell culture bioreactors (which the firm refers to as "cultivators" and "bioreactors") to FDA as supporting, corroborative information in the supplemental, confidential material. This information includes a description of the bioreactor design, materials, and sanitation process controls.

*Proliferation Phase Using Adherent Culture*

Cells from a qualified cell bank are thawed and placed in sterile culture medium using aseptic technique. The culture is transferred to subsequently larger vessels to accumulate the desired quantity of cells. Mission Barns states that vessels used for cell expansion and growth are single-use, disposable sterile systems and that bioreactor components which come into direct contact with cultured cells are either single-use, disposable, and sterile or cleaned/sterilized stainless steel. The firm states that bioreactors are assembled, cleaned, and sterilized before each production run.

The potential hazards identified by the firm associated with this production stage include:

- Introduction of adventitious agents present in the local environment of the production facility during passaging from one vessel to another;
- Introduction of adventitious agents via contaminated culture medium components or inadequate sterilization of bioreactors; and
- Introduction of adventitious agents via personnel or the environment.

Mission Barns manages risk associated with these hazards through sterile procedures and monitoring programs discussed at the beginning of the "Production Process" section. Multiple parameters monitored during culture that reflect performance of the culture and serve as indirect indicators of absence of adventitious agent contamination are also described by the firm.

*Cell Fattening Using Adherent Culture*

Once enough cells are obtained after the proliferation phase of cell culture, Mission Barns introduces additional medium components, including a concentrated, defined mixture of lipids, to induce the cells to form and accumulate intracellular lipid droplets. The firm describes this process as "cell fattening" and evaluated the phenotype of its cultured pork cells at the end of the cell fattening stage by observing lipid droplet formation using fluorescent microscopy and by measuring the amount of accumulated lipids using an assay designed to quantify the levels of intracellular lipids.[13]

The potential hazards identified by the firm associated with this production stage include:

- Introduction of adventitious agents via contaminated culture media components; and
- Introduction of adventitious agents via personnel or the environment.

Mission Barns manages risks associated with the introduction of adventitious agents from personnel or the environment through aseptic procedures and monitoring programs discussed at the beginning of the "Production Process" section, and through the tests and specifications discussed in the "Characterization of Harvested Cell Material" section. Safety considerations

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[13] Mission Barns provides a detailed assessment of the cellular characteristics its cultured pork cells at the end of the cell fattening stage to FDA as supporting, corroborative information in the supplemental, confidential material.

associated with the use of media components that could be present as residues after washing are discussed in the subsequent section, "Substances Used in the Production Process."

*Harvest of Cell Material*

Mission Barns states that at the end of the cell fattening phase, spent media is drained and an aqueous solution containing harvest reagents is added to collect cells from the bioreactor. The cells and harvest solution are removed from the bioreactor and centrifuged to pellet the cells. The cell pellet is washed with saline to remove media components.

The potential hazards identified by the firm associated with this production stage include:

- Introduction of adventitious agents from personnel or the environment; and
- Media components that could be present as residues after washing.

Mission Barns manages risks associated with the introduction of adventitious agents from personnel or the environment through aseptic procedures and monitoring programs discussed at the beginning of the "Production Process" section, and through the product release program tests and specifications discussed in the "Characterization of Harvested Cell Material" section. Additionally, the firm states that washing is performed in sterile, single-use consumables. Safety considerations associated with the use of media components that could be present as residues after washing are discussed in the subsequent section, "Substances Used in the Production Process."

The firm states that samples of the washed cell pellet are aseptically transferred to sterile containers and submitted for product release testing. The remaining harvested biomass is transferred to food-grade, sterile containers, stored at 2-8°C, and quarantined until release is approved by Quality Assurance personnel, following receipt and review of final harvested cell material specification testing results, for further processing into finished food products.

**Substances Used in the Production Process**

Mission Barns provides information about the substances used during its production process in the form of cell culture media and other components, including:

- nutrients used to support primary cell metabolism;
- substances to manage properties of the culture medium;
- substances intended to support cell proliferation in culture; and
- substances used to harvest the cell material.

For each substance, information about the identity, the basis for its safety conclusion, and information about estimated consumer exposure was provided.[14]

[14] A complete list of substances was provided by the firm to FDA as supporting, corroborative information in the supplemental, confidential material.

The firm's cell culture medium is described as consisting of a basal medium, which includes amino acids, vitamins, antioxidants, inorganic salts[15], nucleic acids, fatty acids, and energy substrates (e.g., sugars). The firm states that these substances are used to meet the fundamental nutritional requirements of the cells. Additional substances used by the firm during the production phase (i.e., cell proliferation, cell fattening, and harvest stages) of cell culture include media management factors (e.g., buffers, food safe surfactants/emulsifiers), media supplements (e.g., recombinant porcine and bovine growth factors and steroid hormones) that support cell growth and proliferation, cell fattening reagents (a nutrient source of triglyceride components), culture vessel coating reagents, harvest reagents, and a saline wash solution. Mission Barns explains that most of these substances are already widely consumed in the U.S. food supply and notes that many are present in commonly consumed, commercially available pork products or animal milk. The firm states that the non-nutrient substances listed above are largely removed from the harvested cell material by washing prior to conventional food processing techniques[16], that residual levels in the product do not present concerns given the available toxicological information and existing use or presence in the food supply, and that the substances have no technical or functional effect in the finished food. No antibiotic agents were identified by the firm as being used during the cell culture process.

In the amendment dated June 3, 2024, Mission Barns notified FDA of updates to its production process, including the removal of specific substances (i.e., 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), Pluronic-F68, and tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl)) from the basal cell culture medium and the replacement of recombinant growth factors derived from the human genome (rHPs) with recombinant growth factors derived from porcine or bovine genomes. FDA's evaluation of the Mission Barns' safety conclusions regarding substances used during the production process considered analytical data (e.g., residual levels of certain substances used in cell culture) for the harvested cell material produced using the firm's current production process (i.e., harvested cell material manufactured without HEPES, Pluronic-F68, or Tris-HCl and with recombinant porcine and bovine growth factors). Mission Barns states that rHPs are not used during the production process.

Mission Barns describes its general framework for evaluating substances intended for use during the proliferation, cell fattening, and harvest stages of production, including whether substances used during proliferation, cell fattening, and harvest are currently authorized by FDA for use in human food as a result of a food additive regulation or effective food contact notification, or FDA evaluation of a generally recognized as safe (GRAS) notice. The firm also considered prior use in or natural presence in conventional food, and anticipated dietary exposure. In particular, Mission Barns discusses the firm's intended use of serum-free media

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[15] Sodium selenite is used as a nutrient to support primary cell metabolism. FDA notes in the amendment dated June 3, 2024, that sodium selenite is a substance "… for which no authorization for use in conventional food exists." We wish to clarify that sodium selenite is present in the U.S. food supply as a source of selenium used in infant formula.

[16] Mission Barns provides exposure estimates (i.e., estimated daily intakes (EDIs)) for substances used in the production process as supporting, corroborative information in the supplemental, confidential material. Mission Barns' theoretical EDIs are based on the conservative assumption that the level of a substance in the harvested cell material is the same as the use level of the substance in the cell culture medium. Analytical EDIs are based on the residual levels of substances in three, non-consecutive batches of harvested cell material.

supplements to support proliferation of cells in culture, including recombinant porcine and bovine growth factors and steroid hormones. The firm also publicly discloses data and information regarding substances for which the intended uses are not addressed by an existing, authorizing regulation, effective food contact notification, FDA evaluation of an applicable GRAS notice, or another authorization, including the identity, toxicological studies or other relevant safety data, and estimates of consumer exposure informed by batch analysis of its harvested cell material. In addition to its discussion on the relevant safety-related information of these components of the cell culture medium, the firm also considered the estimated intake level derived from its analytical data from the harvested cell material for each component with reference to levels present in one or more currently consumed comparator foods. This information provided by Mission Barns is described in more detail below.

Recombinant bovine/porcine growth factors

Recombinant growth factors are used in cell culture to replace native, naturally occurring growth factors that are normally available to animal cells *in vivo*. Mission Barns states that the firm uses recombinant growth factors in production to maintain long-term cell proliferation and viability. The firm identifies the species origin of the gene sequence of each recombinant protein (i.e., bovine and/or porcine)[17] and states that these sequences produce amino acid sequences (e.g., growth factor functional domain) similar to the native bovine or porcine growth factor, and, therefore, these proteins are similar to the native proteins present in conventional beef or pork products that are commonly consumed by humans.[18] Mission Barns' assessment of the firm's intended uses of recombinant growth factors considered several factors including analytical data on the presence of two, representative growth factors and a surrogate protein molecule in the harvested cell material[19] as well as published data on the levels of the growth factors in commonly consumed agricultural species. Mission Barns states

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[17] Mission Barns provides a complete list of recombinant bovine and porcine proteins to FDA as supporting, corroborative information in the supplemental, confidential material.

[18] For each recombinant bovine or porcine growth factor used in the cell culture process, Mission Barns provides published data on the naturally occurring levels of the non-recombinant growth factor in commonly consumed food (e.g., milk) from common agricultural species as supporting, corroborative information in the supplemental, confidential material. Further, the firm provided certificates of analysis (CoAs) for each recombinant protein used during the production process, including information about a functional modification to the amino acid sequence of a single growth factor, to FDA as supporting, corroborative information in the supplemental, confidential material.

[19] Mission Barns conducted testing to support its conclusions regarding anticipated residual levels of the recombinant bovine or porcine growth factors used in the firm's cell culture process. The firm measured the levels of a particular surrogate protein molecule in spent media and the final wash solution from three, non-consecutive batches of harvested cell material. The identity of the surrogate protein molecule was provided to FDA as supporting, corroborative information in the supplemental, confidential material. The firm also found undetectable levels of two recombinant cell culture medium growth factors (the growth factor with highest use level and the most thermostable growth factor in use) in three, non-consecutive batches of harvested cellular material. The firm measured the levels of all cell culture medium growth factors in the final wash solution from three, non-consecutive batches of harvested cell material. The test results demonstrated low or undetectable levels of these proteins in the spent media and final wash solution. Mission Barns states that these results are consistent with the conclusion that these proteins would be present at very low or undetectable levels in the harvested cell material. FDA notes that analytical testing using spent media or wash solution has limitations as a proxy for residual presence of protein-based ingredients in the harvested cell material.

that, based on analytical data from the harvested cell material, spent media, and final wash solution, the growth factors used in the production process are present at very low or undetectable levels in the harvested cell material and that these levels are much lower than the native growth factors' naturally occurring levels in conventional animal milk. The firm also states that, given the firm's production process, these growth factors would likely be broken down by heat (e.g., cooking steps) prior to consumption. Mission Barns utilized *in silico* bioinformatics tools and conducted a literature search to evaluate the allergenicity potential of a genetically engineered recombinant porcine growth factor used during the production process. The firm states that the modification, which increases the recombinant growth factor's affinity for its cognate cell surface receptor, is not expected to alter the allergenic potential of the modified protein compared to the growth factor that naturally occurs in pork.

Hormones

Hormones are used in serum-free media formulations to replace naturally occurring hormones that are normally available to animal cells *in vivo*. Mission Barns states that the firm uses steroid hormones[20], which are chemical signaling molecules produced in the bodies of all animals, and in cell culture they support cell growth and differentiation. The firm reports that the hormones used during the cell culture process are normally present in foods, including conventional pork, that are commonly consumed by humans. Mission Barns' assessment of the firm's intended uses of hormones considered several factors including analytical data on the presence of the hormones in the harvested cell material[21] and published data on the levels of the hormones in conventional pork or in other commonly consumed foods (e.g., cow milk, fruit). Mission Barns states that, based on analytical data from the harvested cell material, each of the hormones used in production are present at very low levels in the harvested cell material and that these levels are lower than the naturally occurring levels of hormones in commonly consumed foods. To further support its safety conclusions for the use of hormone A, Mission Barns also considered a safety limit (i.e., acceptable daily intake (ADI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)). The firm notes that the estimated daily intake (EDI) of hormone A from the harvested cell material is orders of magnitude lower than the ADI established by JECFA. Moreover, with respect to all hormones used in the production process, the firm explains that additional processing/cooking may further reduce the activity of residual hormones in the harvested cell material, and states that certain cooking methods can reduce the levels of certain hormones in meat.

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[20] Mission Barns provides the identity of the three hormones used in the firm's culture process as supporting, corroborative information in the supplemental, confidential material shared with FDA. The hormones are identified as "hormone A," "hormone B," and "hormone C" in the disclosable safety narrative. The firm notes that these steroid hormones are non-protein chemical molecules that have conserved structures across animal species.

[21] Mission Barns conducted testing to support its conclusions regarding anticipated residual levels of the hormones used in the firm's culture process. The firm measured the levels of the hormones in harvested, washed cell material from three, non-consecutive batches. While the test results demonstrated that these hormones are detectable at very low levels (just above LOD) in the harvested cell material, the levels reported in the dataset for Mission Barns' cultured pork fat cells was lower than ranges of all three hormones reported in conventional, commonly consumed foods.

Nutrients used to support primary cell metabolism

As discussed below, Mission Barns considered relevant data and information on substances used to support primary cell metabolism, including available toxicological data, presence in conventional food, and presence in the firm's harvested cell material. The firm reports that these substances are present in the harvested cell material at levels comparable to those found in conventional pork fat, or at levels found in other commonly consumed foods while also being well below safe reference exposure values identified by various regulatory bodies that assess the safety of food, or both.

Folic acid is used as a nutrient to support primary cell metabolism in culture. Folic acid is a synthetic form of folate, a water-soluble B vitamin that is an essential nutrient with crucial roles in nucleic acid (DNA and RNA) biosynthesis and amino acid metabolism. Folate is present in all cells and in many foods, including legumes and vegetables. Folic acid is a regulated food additive for use in fortification of specified foods at limited use levels. Mission Barns analyzed the folic acid levels in three, non-consecutive batches of its harvested cell material using an enzyme-linked immunosorbent assay (ELISA), and reported an average folic acid level of 13.3 ng/g in harvested cell material, which is orders of magnitude lower than the levels specified in 21 CRF 172.345. The firm notes that the harvested cell material is intended to be a replacement for conventional pork fat in the market and states that folic acid from the harvested cell material would not be considered an additional source of folic acid in the diet.

Ferric nitrate, which is one of two iron-containing substances used in Mission Barns' cell culture process, is used as a nutrient to support primary cell metabolism. Ferric nitrate is not the subject of an authorizing U.S. food additive regulation or a GRAS notice evaluated by FDA. Mission Barns notes that the substance dissociates into ferric ($Fe^{3+}$ (iron)) and nitrate ($NO3^-$) ions in the aqueous cell culture medium. Iron and nitrate ions are components of other substances permitted for use in human food in the U.S. and are naturally present in many foods. Mission Barns' analytical data indicates that the average level of iron per 100 grams of its harvested cell material is 0.49 mg. The firm states that this value is comparable to the range of iron reported in conventional pork fat (0.09 – 0.47 mg/100g). The firm also states that the theoretical EDI for nitrate from the harvested cell material is $1.82 \times 10^{-4}$ mg/kg body weight (bw)/d, and that JECFA has established an ADI for nitrates of 3.7 mg/kg bw/d. The firm notes that the theoretical EDI is several orders of magnitude lower than the JECFA ADI, and, as such, exposure to nitrate from the harvested cell material does not pose a safety concern.

Nickel chloride is an inorganic salt that is used as a nutrient to support primary cell metabolism. Nickel chloride dissociates into nickel ($Ni^{2+}$) and chloride ($Cl^-$) ions in aqueous solution, and nickel is a trace element that plays a role in various biological processes in animals, including protein synthesis. Nickel chloride is not the subject of an authorizing U.S. food additive regulation or a GRAS notice evaluated by FDA. While Mission Barns cites existing direct and indirect food additive, as well as GRAS affirmation regulations for elemental nickel, FDA notes that these regulations do not apply to the use of nickel chloride as nickel is present in the ionic form in nickel chloride, and the nickel ion has higher bioavailability and toxic potential compared to elemental nickel. The firm analyzed the level of nickel in the harvested cell material and reported a value of 0.06 ppm. Based on this analytical data and the

serving size of 16.7 g, FDA calculated an EDI[22] of 1.7 x $10^{-5}$ mg/kg bw/d, which is orders of magnitude lower than the EPA established reference dose (RfD) of 0.02 mg nickel/kg bw/d. The firm cites an inductively coupled plasma-mass spectrometry (ICP-MS) study of the elemental composition of pork belly fat in the U.S. that reports an average of 0.201 ppm for nickel. Mission Barns set a specification of <0.200 ppm of nickel to ensure that the nickel in the harvested cell material is comparable to the levels found in conventional pork belly fat from the cited study.

Ammonium metavanadate is an inorganic salt that is used as a nutrient to support primary cell metabolism. Ammonium metavanadate is not the subject of an authorizing U.S. food additive regulation or a GRAS notice evaluated by FDA. In aqueous solutions, ammonium metavanadate dissociates into ammonium ions ($NH_4^+$) and metavanadate ions ($VO_3^-$), which is a source of vanadium in Mission Barns' cell culture process. FDA notes that naturally occurring levels of ammonium in conventional food are low and pose no health risk. Ammonium salts, such as ammonium bicarbonate (21 CFR §184.1135) and ammonium chloride (21 CFR §184.1138), are affirmed as GRAS for their intended use. The firm analyzed the level of vanadium in the harvested cell material and reported a value of 0.02 ppm. The firm cites an ICP-MS study of the elemental composition of pork belly fat in the U.S. that reports an average of 0.034 ppm for vanadium. Mission Barns set a specification of <0.03 ppm of vanadium to ensure that the vanadium in the harvested cell material is comparable to the levels found in conventional pork belly fat from the cited study. To further support the safety of vanadium, the firm cites a toxicological profile for vanadium from the Agency for Toxic Substances and Disease Registry. According to this evaluation, the intermediate-duration minimum risk level was determined to be 0.01 mg vanadium/kg bw/d. The EDI for vanadium in Mission Barns' harvested cell material is 5.6 x $10^{-6}$ mg vanadium/kg bw/d, which is approximately three orders of magnitude lower than the intermediate-duration minimum risk level.

Ammonium molybdate tetrahydrate is an inorganic salt that is used as a nutrient to support primary cell metabolism. Ammonium molybdate tetrahydrate is not the subject of an authorizing U.S. food additive regulation or a GRAS notice evaluated by FDA. Ammonium molybdate tetrahydrate serves as a source of molybdenum, which is an essential trace element for microorganisms, plants, and animals. When ammonium molybdate tetrahydrate dissolves in water, it dissociates into ammonium ions and molybdate ions. However, the specific molybdate species present in solution depends on the pH. As noted above, naturally occurring levels of ammonium in conventional food are low and pose no health risk and certain ammonium salts, as discussed above, are affirmed as GRAS for their intended use. Mission Barns analyzed the level of molybdenum in the harvested cell material and reported a value of 0.01 ppm and sets a specification of <0.1 ppm for molybdenum in the harvested cell material. The firm cites 21 CFR §101.9(c), which establishes a daily value (DV) of 45 µg molybdenum for

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[22] Mission Barns provided analytical data for the levels of nickel in three non-consecutive batches of harvested cell material, a safety narrative for the use of nickel chloride during the production process, and a specification for nickel in the harvested cell material. Mission Barns did not calculate an EDI for nickel based on the analytical data it provided for nickel. As such, FDA calculated the EDI for nickel (based on the analytical data provided by Mission Barns) and compared this EDI to the safe reference level provided by the firm (i.e., EPA established reference dose (RfD)).

adults and children ≥ 4 years old. Mission Barns notes that in the most conservative scenario conducted by the firm, where it is assumed that molybdenum is present in the harvested cell material at the level set by the specification (<0.1 ppm), exposure to molybdenum would be 1.6 µg/person (p)/d, which is <5% of the DV at 45 µg specified under 21 CFR §101.9(c). To further support the safety of molybdenum, the firm cites a study of the levels of various metals in conventional food in the U.S. that reports ranges of 104 µg, 9 µg, and 8 µg of molybdenum for 3 ounces servings of beef liver, chicken meat, and ground beef, respectively. The EDI for molybdenum in Mission Barns' harvested cell material is 1.6 µg molybdenum/p/d, which is 5-56 times lower than the values reported in the study.

**Characterization of Harvested Cell Material**

Identity

As described above, Mission Barns uses a PCR assay to verify the species identity of the manufacturing cell bank as the domestic Yorkshire pig (*Sus scrofa domesticus*). The firm also carries out karyotyping of early and late-stage cell lines during the cell banking process to verify the genetic stability of the cell lines used in the production process. At the end of the cell fattening stage, Mission Barns assessed the accumulation of lipid droplets within the harvested cell material using techniques designed to observe and quantify lipids in cultured cells. The firm reported that fatty acid compositional analyses from a single batch of harvested cell material produced using its current production process using the HEPES-free medium is 5.58%. This value is consistent with the of range of total fat reported for three independent batches of harvested cell material manufactured using the HEPES-containing medium, 4.99-6.78%. The firm concluded that the collected data confirms the species identity, expected phenotype, and stability of its cell line throughout cell culture adaptation and manufacturing of the harvested cell material, and that there was no information that would raise questions about the safety of the harvested cell material.

Adventitious Agents and Contaminants

Mission Barns discusses adventitious agent testing for the harvested cell material, including specified bacteria[23] and fungi. The firm conducts batch release microbial testing on spent media collected immediately prior to harvest, noting that potential microbial contamination of the cell material during the cell culture process would likewise be present in the surrounding media. The firm provided specifications intended for use in routine microbiological testing of each production batch and results from three independent batches demonstrating conformance with the stated specifications. Microbial specifications include:

- Aerobic plate count (<10 colony-forming units (CFU)/mL)

[23] Mission Barns states that *S. aureus* is the only species of *Staphylococcus* identified as a potential hazard from the environment or human sources, and therefore, the firm maintained a specification for *S. aureus*. The firm does not identify the production of staphylococcal enterotoxins as a safety concern in its production process.

- *Enterobacteriaceae* (<10 CFU/mL)[24]
- *Staphylococcus aureus* (<10 CFU/g)
- Coliforms (<10 CFU/mL)
- *Mycoplasma* spp. (not detected in 0.2 mL)
- Yeast (<10 CFU/mL)
- Mold (<10 CFU/mL)

Microbial testing was performed using methods validated for their intended purposes.

Mission Barns provides specifications for toxic heavy metals that are commonly considered in conventional food manufacturing and could potentially be present as contaminants in the harvested cell material. Using analytical methods validated for their intended purposes, the firm analyzed three independent production batches for these toxic heavy metals, demonstrating conformance with the stated specifications. Heavy metal specifications include:

- Arsenic (<0.05 ppm)
- Lead (<0.05 ppm)
- Mercury (<0.025 ppm)
- Cadmium (<0.05 ppm)

As discussed in the previous section, "Substances Used in the Production Process," Mission Barns provides batch release testing specifications for certain trace metal salts used during production. Testing for trace metals was performed on three non-consecutive batches of the harvested cell material using methods validated for their intended purposes, demonstrating conformance with the stated specifications. The firm states that the specifications for these trace metal salts are either at or below the levels reported to be present in conventional U.S. pork belly fat (nickel and vanadium) or in meat from commonly consumed agricultural species (molybdenum). The firm also states that the consumption of the harvested cell material is not expected to lead to a significant increase in consumers' cumulative exposures of these trace metals. Trace metal specifications include:

- Nickel (<0.2 ppm)
- Vanadium (<0.03 ppm)
- Molybdenum (<0.1 ppm)

[24] Mission Barns states that for any non-conforming batches that fail to pass the microbial testing plan acceptance criteria, it 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 to determine the source of the contamination, and corrective and preventative actions, as needed.

Composition

Mission Barns conducted a compositional analysis of three independent production batches of harvested cell material, including proximates, amino acids, vitamins, and minerals.[25] Proximates include moisture, protein, fat, ash, and carbohydrate content. The cell material is washed with a saline solution during harvest, resulting in the introduction of additional sodium and moisture content. As a point of reference, Mission Barns also presents nutrition data from a U.S. Department of Agriculture (USDA) database on conventional pork products, including cooked and raw pork fat.[26] Protein and amino acid percentages were similar between the harvested cell material and conventional pork fat. The total fat content was lower in the harvested material (4.99-6.78%) versus the conventional comparators[27] (53% to 65.7% total lipid (fat)). The relative levels of minerals were similar between the harvested material and conventional pork fat. The relative levels of vitamins were similar between the harvested material and conventional pork fat, with modest increases in the levels of riboflavin (vitamin B2), pyridoxine (vitamin B6), and alpha-tocopherol (vitamin E) in the harvested cell material.

Mission Barns measured the levels of fatty acids in three, non-consecutive batches of the harvested cell material produced using a defined in-house lipid mixture and a HEPES-containing basal cell culture medium without Pluornic-F68 and Tris-HCl and reported the results in the amendment dated November 13, 2024. Relative proportions of saturated, monounsaturated, and polyunsaturated fats were similar in the harvested material relative to reference data for conventional comparators from the USDA FoodData Central database, "pork, fresh, separable fat, raw" (USDA FoodData Central, NDB Number:10006) and "pork, fresh, composite of separable fat, with added solution raw" (USDA FoodData Central, NDB Number:10942). The firm notes that no *trans* fats were detected in the harvested cell

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[25] As noted in the previous section, "Substances Used in the Production Process," Mission Barns notified FDA of updates to its production process in the amendment dated June 3, 2024. In the amendment dated November 13, 2024, the firm provides analytical data for proximates (moisture, total fat, protein, ash, carbohydrates), vitamins, minerals, toxic heavy metals, trace metals, and fatty acids for three non-consecutive batches of harvested cell material produced using a basal cell culture medium containing HEPES (due to supply chain limitations), but without Pluronic-F68 or Tris-HCl (HEPES-containing medium). The firm also provides limited analytical data for proximates, toxic heavy metals, and trace metals from a single batch of harvested cell material produced without HEPES, Pluronic F-68, or Tris-HCl (HEPES-free medium). Mission Barns concludes that batches of the harvested cell material produced with media containing HEPES is sufficiently representative of its current production process (i.e., HEPES-free medium) to assess safety, based on the consistency of the analytical data between both methods of production.

[26] Mission Barns provided reference ranges for amino acids, minerals, and vitamins for the highest and lowest values in the following USDA FoodData Central datasets: "Pork, fresh, backfat, raw" (NDB Number 10004), "pork, fresh, separable fat, raw" (NDB Number: 10006), "pork, fresh, composite of separable fat, with added solution raw" (NDB Number:10942), "Pork, fresh, variety meats and by-products, leaf fat, raw" (NDB Number:10109), and "Pork, fresh, separable fat, cooked" (NDB Number 10007). The term "conventional pork fat" includes data reported for samples including raw pork backfat, raw and cooked separable pork fat, raw separable pork fat with added solution, and other varieties of raw pork meat and by-products. Reference ranges for moisture, total fat, protein, ash, and carbohydrates were pulled from the five USDA Food Central datasets provided by Mission Barns.

[27] The range of total lipid (fat) in conventional comparators are the lowest and highest values from the USDA FoodData Central "pork, fresh, belly, raw" (NDB Number 10005), "pork, fresh, separable fat, raw" (NDB Number:10006), and "pork, fresh, composite of separable fat, with added solution raw" (NDB Number:10942).

material.[28] To further support its safety conclusions, Mission Barns set a specification of ≤1 g total *trans* fat/100 g fat to ensure that the total *trans* fat in the harvested cell material is comparable to the *trans* fat levels found in conventional raw pork fat (i.e., 0.9 g/100 g fat - 1.1 g/100 g fat)[29] as reported by the USDA FoodData Central data.

**FDA's Evaluation**

FDA evaluated the data and information provided by Mission Barns with respect to the established cell lines, cell banks, substances used in the production process, and properties of the harvested cell material that collectively are the subject of CCC 000008. The primary focus of FDA's evaluation is the information on which the firm relies to conclude that the harvested cell material is safe for use as food and does not contain substances or microorganisms that would adulterate the food.

Mission Barns provides information on the establishment of the cell lines used to produce the food that is the subject of CCC 000008. FDA considered the information on the source and lineages of the cell lines and the culture adaptation process. We also considered the information provided by Mission Barns with respect to the observed behavior of the cell lines in culture, as well as other information available to us with respect to the genetic capacity of animal cells to produce toxins or other potentially harmful substances, and the viability of cells following harvest.

The information reported was consistent with pork-derived cells that displayed enhanced replicative capacity under *in vitro* conditions. However, once removed from the protected and controlled environment of the bioreactor the cells die quickly, removing any replicative capacity. Subsequent food processing (such as cooking) would further break down cellular structures and contents. Digestion after consuming food made from the harvested cell material would also break down any residual cellular structure. No information presented by the firm or otherwise available to us indicated any mechanism by which the harvested cell material, once rendered non-living, heated, consumed, and digested, would retain any replicative capacity or the ability to induce replicative capacity in living cells exposed to this material.

Mission Barns notes the harvested cell material will present the same allergenicity concerns to consumers who may be allergic to conventional pork fat, given that each cell contains the complete domestic pig genome including genetic code for the relevant proteins. The firm states

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[28] Mission Barns initially detected elaidic acid (18:1 *trans*), a *trans* fatty acid, and nervonic acid (24:1), a monounsaturated fatty acid not typically found in pork, in the harvested cell material. The firm updated its manufacturing process by replacing the lipid source used during the cell fattening stage (i.e., replaced the third-party supplier chemically defined lipid mixture with an "in-house" version made by Mission Barns with the same ingredients with COAs that attest to high purity), removed several substances from its cell culture medium (i.e., HEPES, Pluronic-F68, and Tris-HCl), and replaced rHPs with recombinant bovine and porcine growth factors. After implementing these manufacturing changes, the firm no longer detected elaidic or nervonic acid in the harvested cell material.

[29] The range of total *trans* fat in conventional pork comparators is based on values reported for USDA FoodData Central "pork, fresh, separable fat, raw" (NDB Number:10006) and "pork, fresh, composite of separable fat, with added solution raw" (NDB Number:10942).

that it will address concerns regarding allergenicity of the harvested cell material through product labeling.

Mission Barns established a specification for *trans* fat which assures that exposure is equal to or lower than levels found in conventional pork. The FDA has taken major steps to reduce artificial *trans* fat in the food supply in the past[30]. Those actions have not included *trans* fat occurring naturally in conventional food products from ruminant animals (e.g., milk, butter, cheese, meat products). Human food made with cultured animal cells are expected to be consumed consistent with exposure to the conventional comparator. The specification established by Mission Barns ensures there will be no increase in exposure of *trans* fat in the diet. Foods containing the harvested cell material are also subject to the labeling requirements under either 21 CFR §101.9 (c)(2)(ii) or 9 CFR §381.462(c)(1)(i).

In summary, we did not identify any properties of the cells as described that would render them different from other animal cells with respect to safety for food use.

We did not identify any substance uses that would lead us to question Mission Barns' conclusion regarding the safety of its food given available information, existing uses or authorizations in food, and anticipated exposure. We noted moderate differences in the levels of several nutritional components relative to conventional pork products (discussed below); however, the information available to us from Mission Barns and from the available scientific literature indicates that these components are being used to support primary metabolism in cell culture rather than for inappropriate or indiscriminate food fortification. Regarding the use of any food contact materials throughout the production process, we note that the production conditions described by the firm during culture for food production and immediately subsequent to harvest are consistent with food type 1 (nonacid, aqueous products; may contain salt or sugar or both (pH above 5.0)) and conditions of use type D (hot filled or pasteurized below 66°C) save for post-harvest storage (conditions F or G for refrigeration or frozen storage, respectively). Thus, any food contact materials authorized for these conditions would be appropriate.[31]

FDA reviewed the data and information that was provided on the identity and composition of the harvested cell material, including genetic and cellular identity, batch test data for constituents and contaminants, and specifications. We considered the analytical data provided by Mission Barns on the composition of the harvested cell material from several production runs as one element in characterizing the identity of its product, as evidence of the firm's ability to conform to its stated specifications for food contaminants, and as relevant information in evaluating the relationship between the production process described in CCC 000008 and the properties of the harvested cell material produced through that process. We evaluated the firm's specifications for toxic heavy metals and trace metals to ensure they were as low as reasonably possible and were consistent with levels that are considered safe in food.

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[30] In 2015, FDA released its final determination that Partially Hydrogenated Oils (PHOs) are not GRAS (80 FR 34650).

[31] As noted earlier, the various food types and conditions of use are described in Appendix V of FDA's "Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances (Chemistry Recommendations)."

We also considered data and information relating to compositional analysis. In all cases, levels of analytes were within the range of those found in commonly consumed foods. We did not consider the establishment of exact equivalence of all nutrients and components relative to any particular conventional comparator as a necessary component of Mission Barns' safety conclusion, nor did we interpret the analytical data provided by the firm as definitive nutritional information regarding either harvested cell material produced through the process defined in CCC 000008 or food products that contain this material.

**Conclusions**

Based on our evaluation of the data and information that Mission Barns provides in CCC 000008, as well as other information available to FDA, we did not identify a basis for concluding that the production process as described would be expected to result in food that bears or contain any substance or microorganism that would adulterate the food. We have no questions at this time about Mission Barns' conclusion that foods comprised of, or containing, cultured pork fat cell material resulting from the production process defined in CCC 000008 are as safe as comparable foods produced by other methods.

ASHLEY E. NAZARIO-TOOLE -S

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Ashley Nazario Toole

**Appendix: Summary of potential identity, quality, and safety issues**

| Process Step | Potential Issues | Management Strategy |
|---|---|---|
| Tissue sourcing | Cell line identification, cells from different line or species inadvertently used | cGMP, cross-species testing, DNA testing, supplier documentation |
| Cell Isolation | Carryover of adventitious agents such as bacteria, fungi, viruses, parasites, and prions from source animal | Animal health documentation, antibiotics application, testing |
| Cell Isolation | Introduction of adventitious agents during isolation | Antibiotic solution, aseptic procedures, hygienic condition, testing (cell bank), visual observation |
| Cell Isolation | Introduction of contaminants from animal-derived reagents (e.g., serum, trypsin) | Material risk assessment including documentation (e.g., BSE-free certification), material handling and positive release program, supply-chain controls, adventitious agent testing (cell bank), visual observation |
| Cell Isolation | Introduction of contaminants in laboratory reagents | Sterilization, supply-chain controls, testing |
| Cell Isolation | Facility environment contamination | Aseptic procedures, hygienic condition |
| Establishment of Cell Lines | Cell line identification, cells from different line or species inadvertently used | PCR testing |
| Establishment of Cell Lines | Genetic instability | Cell stability testing (Karyotyping) |
| Establishment of Cell Lines | Cells do not display expected growth profile | Monitoring population doubling times |
| Establishment of Cell Lines | Contamination of adventitious agents | Aseptic procedures, hygienic condition, sterilization, adventitious agent testing, use of antimicrobial reagents |
| Establishment of Cell Lines | Contamination from animal-derived reagents (e.g., serum, trypsin) | Material risk assessment including documentation (e.g., BSE-free certification), material handling and positive release program, supply-chain controls, adventitious agent testing (cell bank), observation |
| Establishment of Cell Lines | Introduction of adventitious agents from media components | Aseptic procedures, environmental monitoring, sanitation controls, sterile filter, supply-chain controls, testing |

| | | |
|---|---|---|
| Establishment of Cell Lines | Introduction of chemical hazards | Allergen controls, cGMP, document control and training, sanitation controls, supply-chain controls |
| Establishment of Cell Lines | Introduction of physical hazards | cGMP, foreign materials management program, sanitation controls, sterile filter, supply-chain controls |
| Establishment of Cell Lines | Facility environment contamination | Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls |
| Manufacturing Cell Bank Establishment | Cells from different line or species inadvertently used | PCR testing |
| Manufacturing Cell Bank Establishment | Genetic instability | Cell stability testing (Karyotyping) |
| Manufacturing Cell Bank Establishment | Introduction of adventitious agents during manufacturing cell bank establishment process | Aseptic procedures, cGMP, environmental monitoring, sterilization, testing |
| Manufacturing Cell Bank Establishment | Contamination with adventitious agents from original animal source | Testing |
| Manufacturing Cell Bank Establishment | Contamination with adventitious agents from culture media components | Aseptic procedures, cGMP, environmental monitoring, sanitation controls, sterile filter, supply-chain controls, testing |
| Manufacturing Cell Bank Establishment | Introduction of chemical hazards | Allergen controls, cGMP, document control and training, sanitation controls, supply-chain controls |
| Manufacturing Cell Bank Establishment | Introduction of physical hazards | cGMP, foreign materials management program, sanitation controls, sterile filter, supplier control |
| Manufacturing Cell Bank Establishment | Facility environment contamination | Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls |
| Proliferation Phase | Introduction of adventitious agents during proliferation phase | Aseptic procedures, cGMP, environmental monitoring, sterilization, testing |
| Proliferation Phase | Contamination with adventitious agents from media components | Aseptic procedures, material handling and positive release program, materials risk assessment, supply-chain controls |

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|---|---|---|
| Proliferation Phase | Contamination with adventitious agents through inadequate sterilization of vessels and transferring between vessels | cGMP, validated sanitation processes and environmental monitoring |
| Proliferation Phase | Introduction of media components that could persist as residues in harvested cells | Food safety assessment |
| Proliferation Phase | Introduction of media components that could accumulate in the cells before harvest | Food safety assessment, compositional analysis at harvest |
| Proliferation Phase | Introduction of chemical hazards | Allergen controls, cGMP, document control and training, sanitation controls, supplier control |
| Proliferation Phase | Introduction of physical hazards | cGMP, foreign materials management program, sanitation controls, sterile filter, supply-chain controls |
| Proliferation Phase | Facility environment contamination | Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls |
| Cell Fattening Phase | Introduction of adventitious agents during fattening process | Aseptic procedures, cGMP, environmental monitoring, sterilization, testing |
| Cell Fattening Phase | Contamination with adventitious agents from culture media components | Batch records, cGMP, material handling and positive release program, materials risk assessment, sterilization, supply-chain controls |
| Cell Fattening Phase | Contamination with adventitious agents through inadequate sterilization of vessels/bioreactors and transferring between vessels | cGMP, validated sanitation processes, environmental monitoring |
| Cell Fattening Phase | Introduction of media components that could persist as residues in harvested cells | Food safety assessment |
| Cell Fattening Phase | Introduction of media components that could accumulate in the cells before harvest | Compositional analysis at harvest, food safety assessment |
| Cell Fattening Phase | Introduction of chemical hazards | Allergen controls, cGMP, document control and training, sanitation controls, supply- |

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| Cell Fattening Phase | Introduction of physical hazards | cGMP, foreign materials management program, sanitation controls, sterile filter, supply-chain controls |
| Cell Fattening Phase | Facility environment contamination | Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls |
| Harvest of Cell Material | Presence of adventitious agents from culture process | Culture monitoring, specifications, testing |
| Harvest of Cell Material | Migration of contaminants from food contact materials | Use of authorized food contact materials |
| Harvest of Cell Material | Presence of residual media components after harvest | Analytical testing, food safety assessment, wash steps |
| Harvest of Cell Material | Presence of elemental contaminants (metals) after harvest | Specifications, testing |
| Harvest of Cell Material | Introduction of chemical hazards | Allergen controls, cGMP, document control and training, sanitation controls, supply-chain controls |
| Harvest of Cell Material | Introduction of physical hazards | cGMP, foreign materials management program, sanitation controls, sterile filter, supply-chain controls |
| Harvest of Cell Material | Facility environment contamination | Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls |