Mission Barns Cultivated Animal Fat Cells (Pork)
Complete Consultation Data Package
Public Version

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Submitted to:

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Part 1. Executive Summary

1.1 Introduction

Established in Berkeley, California in 2018, Mission Barns focuses on cultivating animal fat cells. Its novel technology platform enables the rapid production of high-quality animal fat cells. Starting with a selection of non-genetically modified cells, these cells are fed a proprietary media inside a proprietary cultivator under controlled conditions. Over the course of days, this process creates a final product that can deliver the rich flavor of animal fats, without the need to raise and slaughter livestock. Upon harvesting the fattened cells, they can be added to plant-derived ingredients to make a variety of delicious and sustainable food products such as alternatives to bacon, sausages, and meatballs. The production process is visually summarized in **Figure 1**, below.

Mission Barns' cultivation process is expected to result in significantly reduced carbon emissions and requires only a fraction of the water and land that conventional animal agriculture methods require. Additionally, the process reduces public health risks relating to the consumption of animal products contaminated with foodborne pathogens from sources including contaminated manure, irrigation water, soil, and other environmental factors commonly associated with raising farm animals.

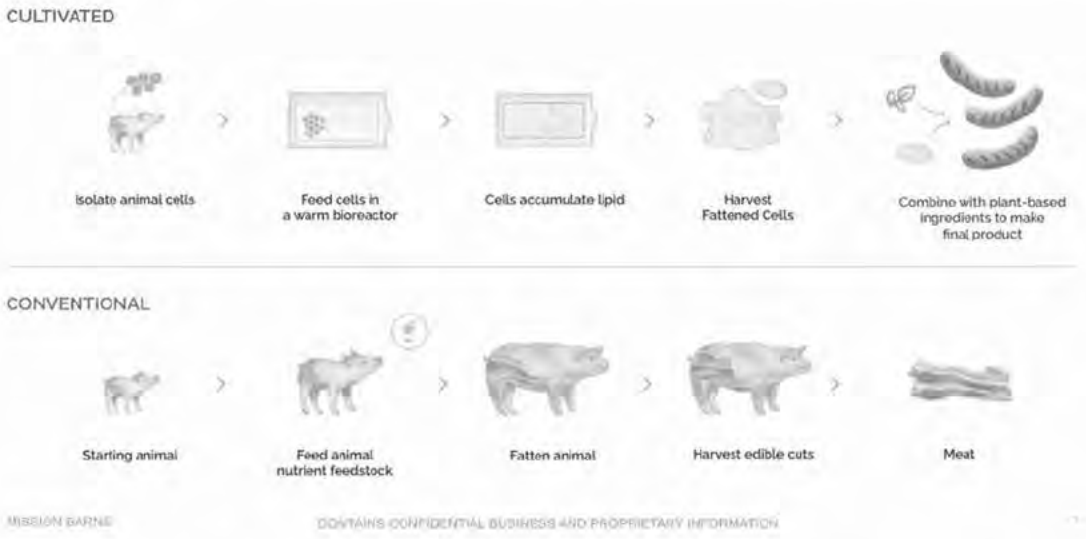

Figure 1. Summary of Cultivated Pork Fat Cell Production Process

This document describes Mission Barns' basis for determining that the cultivated animal fat cells and the category of finished food products that will be produced using the cultivated animal fat cells are safe for human consumption. In this premarket submission, we present the manufacturing process and biological, chemical, and physical safety assessment for cultivated pork fat cells, intended for commercial use in the US.

1.2 Production Process Overview

Mission Barns' production process for cultivated fat cells comprises two main phases: (I) cell banking, during which frozen proliferative cell culture stocks are established; (II) cultivated fat cell manufacturing, during which cells are thawed from the stocks established in Phase I, proliferated, fattened, and harvested for food applications.

I. Cell Banking Phase: 1/

- Tissue sourcing
- Cell isolation
- Preliminary cell bank establishment
- Proprietary media transition
- Manufacturing cell bank establishment

II. Manufacturing Phase:

- Cell culture expansion
- Cell fattening
- Cell harvest
- Harvested cell storage and release

All manufacturing activities described above will take place in current good manufacturing practice (cGMP)-compliant food processing facilities, in compliance with applicable FDA food regulations including 21 CFR Part 117 ("CURRENT GOOD MANUFACTURING PRACTICE, HAZARD ANALYSIS, AND RISK-BASED PREVENTIVE CONTROLS FOR HUMAN FOOD"). All the processing reagents used are food grade (when available), high-quality chemical or pharmaceutical grades, or the highest-quality material that is commercially available.

1/ The cell banking phase only occurs once for each starting cell population.

1.3 Safety Summary

To control for potential hazards in the cultivated pork fat cells, each step of the production process has been assessed for biological (microbiological), chemical, and physical risks. **Figure 2** shows the cultivated pork fat cell production process flow.

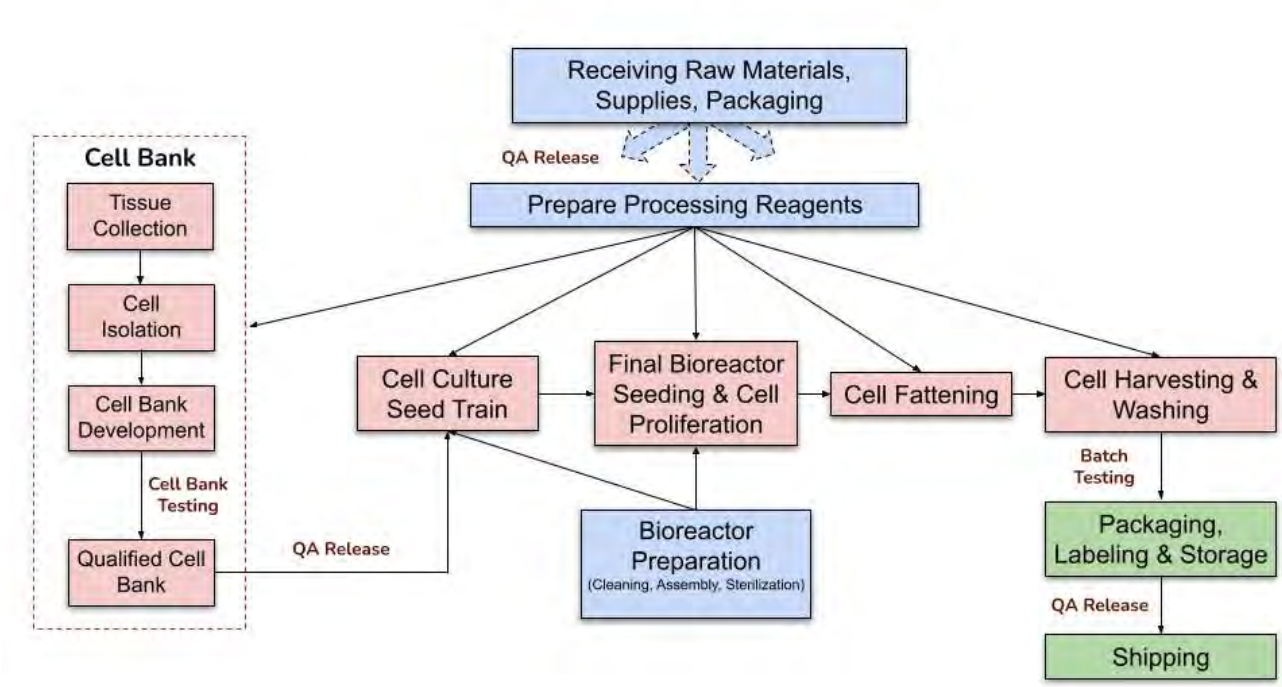

Figure 2. Cultivated Pork Fat Cell Production Process Flow Overview

1.3.1 Biological (Microbiological) Safety

In comparison to traditional meat processing, Mission Barns' process is inherently safer from a microbiological perspective, because products are cultivated and harvested under aseptic conditions. This eliminates the possibility of exposure to pathogens that are present in an animal's digestive tract and fecal matter which sometimes lead to contamination of meat products during animal slaughter. For sourcing some of Mission Barns' cell cultures, tissue is biopsied from a domestic pig in a veterinary operating chamber by trained veterinary doctors. The tissue collected is then transported to a cell isolation lab in Mission Barns' facilities following

procedures to minimize any potential microbial contamination. All the water used during the process is purified through distillation, reverse osmosis, or some other means. All processing reagents used are either sterile when provided by suppliers or sterilized by Mission Barns on-site by 0.2 micron membrane sterilization, autoclaving, or other appropriate means depending on material stability. Cell cultures are closely observed and monitored through microscopes for any signs of potential microbial contamination. At the end of the cell banking process, sterility is further verified by testing cell cultures for the absence of bacterial, fungal, viral and mycoplasma contamination.

During cultivated pork fat cell manufacturing, Mission Barns will also implement a Food Safety Plan in compliance with Food Safety Modernization Act (FSMA) regulations for Hazard Analysis Risk Based Prevent Controls (HARPC). The hazard analysis has evaluated biological, chemical, and physical hazards associated with each manufacturing step from receipt of raw materials to cell harvest and storage. The finished cultivated pork fat cells will also be tested for microbiological contamination.

1.3.2 Chemical Safety

The raw materials used in the cell banking and the manufacturing of cultivated pork fat cells have been evaluated and are safe and suitable for use in the manufacture of a food ingredient. Most of the raw materials are common nutrients such as amino acids, vitamins and their derivatives, and minerals that are needed by the cells to proliferate. All raw materials are considered safe and suitable for this use. Most of the raw materials are commonly used food ingredients that are considered generally recognized as safe (GRAS), approved food additives, or have been the subject of a safety assessment confirming the raw material is safe for this use. For those substances warranting a more detailed safety evaluation, Mission Barns established “worst-case” estimated daily intakes (EDIs) based on the highest concentrations of these components in the final cell culture media or harvest solution and it is further assumed conservatively that 100% of the conventional pork fat in the US will be replaced by the cultivated pork fat cells manufactured by Mission Barns. These theoretical EDIs are then compared to appropriate safety threshold levels identified through public literature to demonstrate that the very low theoretical residual levels of these substances in the cultivated pork fat cells do not pose any human safety concerns.

A search of relevant safety literature was conducted to identify the appropriate safety threshold levels using databases including PubMed, Google, and Google Scholar, and we also referenced applicable safety reviews conducted by FDA, the US Environmental Protection

Agency (EPA), the Agency for Toxic Substances and Disease Registry (ATSDR), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the National Academy of Science (NAS), and the European Food Safety Authority (EFSA).

1.3.3 Physical Safety

While the cultivators and other parts of the manufacturing equipment are made from metal, Mission Barns has evaluated its manufacturing process and the proprietary cultivator system, and determined there are no physical hazards such as the potential for “hard/sharp” physical hazards or “choking” hazards.

1.3.4 Genetic Stability

Mission Barns does not use any type of genetic engineering at any point in its production process. Mission Barns has focused on potential features of the process which would be most likely to introduce risk of causing genetic differences. Karyotyping has been conducted on cell banks and cultures to confirm that chromosomal abnormalities are absent in the cell cultures. Furthermore, Mission Barns has designed an analytical regime focusing on any unintended presence of residual processing aids or other naturally-occurring components in the cells to ensure that their presence (if any) does not pose any human safety concern.

1.3.5 Allergenicity Safety

Mission Barns’ cultivated pork fat cells will present the same allergenicity concern to consumers who may be allergic to conventional pork fat. This concern will be addressed through product labeling. Cell culture basal media and supplements that are added to the cells consist of nutritional components including amino acids, sugars, vitamins and their derivatives, and minerals. Mission Barns also uses other proprietary processing aids. None of these are or contain major food allergens as identified under the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). We also discuss why any residual levels of the cell culture components or other processing aids would not elicit an allergic reaction.

Part 2. Production Process Description

2.1 Cell Banking Process

2.1.1 Tissue Sourcing

Mission Barns has developed one particular cell population ("Manufacturing Cell Bank") for cultivated pork fat cell. The cell population was isolated from a domestic Yorkshire pig (*Sus scrofa domesticus*) in a veterinary operating chamber by trained veterinary doctors. Yorkshire, also called Large White or Large White Yorkshire, is a breed of swine produced in the 18th century by crossing the large indigenous white pig of Northern England with the smaller, fatter, white Chinese pig. 2/ It is the most common pig breed consumed by people around the world today. 3/ Tissue was removed from the animal, placed in a sterile, ice-chilled solution, and then transported to a cell isolation lab in Mission Barns' facilities.

2.1.2 Cell Isolation

Once the tissue arrived at the facility it was washed several times in a saline solution with a proprietary antibiotic blend, and then processed to isolate and culture cells from it. The entire process was performed in a controlled environment under lab procedures that minimize the chance of microbial contamination. Additionally, all processing reagents or equipment that came into primary or secondary contact with tissues/cells were sterile.

2.1.3 Preliminary Cell Bank Establishment

Isolated cells were passaged from a single culture vessel into multiple/larger vessels to expand the total number of cells in culture, via standard cell culture passaging protocols using saline solution, harvest reagents, and other proprietary processing reagents. The passaging process was repeated until enough cells were grown. The cells were then frozen and stored for further processing.

2/ Shringi, Nikhil, et al. "Morphometry of Spleen in White Yorkshire Pig (Sus scrofa)." *Int. J. Pure App. Biosci* 5.4 (2017): 755-757.
3/ Zinovieva, N. A., et al. "Evaluation of the contribution of different pig populations to the genetic diversity of the large white breed." *Сельскохозяйственная биология* 6 (eng) (2012).

2.1.4 Proprietary Media Transition

Frozen cells were thawed and suspended in the proprietary media they were originally cultured in and displaced into a new culture vessel. After the cells began to proliferate in the new vessel, a new proprietary media was used to culture the cells from that point on. No anomalous apoptotic or cell selection events took place during this transition.

2.1.5 Manufacturing Cell Bank Establishment

The cell culture was then expanded over the course of a few passages and once enough culture was grown, it was all again frozen and stored. The cell stocks from this bank are used to initiate manufacturing runs. A set of tests were done on the Manufacturing Cell Bank to confirm that it is acceptable:

Species verification testing was conducted to verify that the established bank is indeed of the desired species, in this case – *Sus scrofa domesticus*. Cell stability testing (karyotyping) was conducted to verify that the cell culture environment that the cells were exposed to did not compromise the genetic stability of the cells stored in the bank. Further, no bacteria or fungi were found in the Manufacturing Cell Bank cultures when conducting USP <71> sterility tests, demonstrating that our processing environment was sufficiently aseptic. Likewise, no mycoplasma was detected in the cell culture through PCR testing.

Released products should not be contaminated with animal adventitious viruses. Given the use of certain proprietary processing aids, and the source of porcine cells, testing of porcine-derived and other viruses is an important component of providing this assurance. We tested cell bank following the procedure in 9 CFR §113.47 (“Detection of extraneous viruses by the fluorescent antibody technique.”) Testing confirmed that cultures contain no bovine viral diarrhea virus, reovirus, rabies virus, porcine adenovirus, porcine parvovirus, transmissible gastroenteritis virus, and porcine hemagglutinating encephalitis virus, the viruses that would most likely be contaminants.

The tests and cell bank results described above are summarized in **Table 1** below.

Table 1. Analytical Testing Results with Manufacturing Cell Bank			
Test Item	**Test/Assay**	**Acceptable Criteria**	**Result**
Species Verification	PCR (Porcine DNA)	*Sus scrofa domesticus* (pig) DNA confirmed	Conforms
Cell Stability	Karyotyping	Normal chromosome spreads	Conforms
Microbiological contamination	Viral panel via cytopathology, hemadsorption, and fluorescent antibody including: • Bovine viral diarrhea virus • Porcine parvovirus • Adenovirus • Hemagglutinating encephalitis virus • Transmissible gastroenteritis virus • Reovirus • Rabies virus	Not detected	Pass
	PCR (Mycoplasma via genus)	Negative	Negative
Sterility test	USP <71> the sterility tests include evaluation of bacteria and fungi	Negative	Negative

2.2 Manufacturing Process

2.2.1 Cell Culture Expansion

To commence each manufacturing batch, a cell population is thawed from a Manufacturing Cell Bank stock into a cell culture vessel containing a proprietary media. Cells are proliferated in the vessel until meeting the vessel's capacity to support growth and then passaged to other vessels. This process is repeated until enough cells are grown to meet production needs.

2.2.2 Cell Fattening

Once enough proliferative cells are obtained, they are induced to form lipid droplets. Proprietary food-grade reagents are added to the cell culture media to conduct this process.

2.2.3 Cell Harvest

After cells are fattened, they are then displaced from the cultivator, washed multiple times to substantially reduce any residual levels of processing reagents, and harvested with saline solution to reduce the concentration of all processing reagent residuals.

2.2.4 Harvested Cell Storage and Release

Harvested and washed cell pellets are collected into sterile containers labeled with lot identifiers and marked with quarantine indicators. Samples of cell pellet are aseptically aliquoted into suitably sized sample containers and submitted for release testing. Sterile containers of the remaining quarantined product are transferred to an access-controlled space designated for storage of quarantined product within a temperature-monitored cold room or refrigerator at 2-8 ˚C, where they remain until released in accordance with the specifications summarized below in **Table 2**.

In addition, to further support the safety of the harvested cells, similarly labeled sterile containers of (a) final spent media and (b) final wash solution are aseptically aliquoted into suitably sized sterile sampling containers and submitted for further testing. Additional analytical tests including antibiotics residuals and hormones were conducted. Upon receipt and review of release specification testing results in **Table 2**, sterile containers of harvested cell pellet will be released by the Quality department, labeled as released, and transferred into an access-controlled, temperature-monitored refrigerator at 2-8 ˚C that has been designated for released product.

Below we discuss the analytical testing rationale in greater detail:

- Mycoplasma and Sterility testing is conducted because there is a theoretical potential for contamination by common adventitious reagents including bacteria, fungi, and mycoplasma from the manufacturing environment or processing reagents. Mycoplasma testing via PCR and USP <71> sterility testing are conducted on final spent media, which is the last cell culture media to be in contact with harvested cells, to ensure we have maintained a sterile environment throughout the process.

- Heavy metals (i.e., cadmium, lead, arsenic, and mercury) testing is performed on the harvested and washed cell pellet, in accordance with AOAC 2013.06. Of note, heavy metals testing is performed out of an abundance of caution as they are not expected to be present in the cultured cells.

- Cell culture media and harvest reagent residuals were tested with an ELISA assay that specifically measures a surrogate protein molecule. The residual protein levels obtained through this assay can be used as a surrogate for any residual processing reagents in the cultivated pork fat cells.

- Hormones analytical testing was performed on the harvested and washed cell pellet by ELISA assays for specific hormones to confirm that the concentrations of such components present in the cultivated fat cells are within safe limits.

- Antibiotic residuals were tested for, even though antibiotics are only used in the cell banking process, and not manufacturing process. Testing was performed on the final wash solution, in accordance with USP <81>, to confirm that antibiotic residuals are undetectable.

Test results from three non-consecutive batches of cultivated pork fat cell production are summarized in **Table 2** below. Mission Barns intends to use the following specifications in **Table 2** before releasing each commercial batch in the future.

Table 2. Specifications and Batch Analysis of Cultivated Pork Fat Cell Production					
Item		**Specifications**	**Batch #1**	**Batch #2**	**Batch #3**
Mycoplasma		Negative	Negative	Negative	Negative
Heavy metals	Cadmium	< 100 ppb	< 10 ppb	< 10 ppb	< 10 ppb
	Lead	< 100 ppb	< 10 ppb	< 10 ppb	< 10 ppb
	Arsenic	< 100 ppb	< 10 ppb	< 10 ppb	< 10 ppb
	Mercury	< 50 ppb	< 10 ppb	< 10 ppb	< 10 ppb
Sterility testing [4]/		Negative	Negative	Negative	Negative

In addition, to further support the safety of the cultivated fat cells, Mission Barns conducted additional analytical testing of antibiotics and hormones as part of the safety assessment to support its safety conclusions. The levels of antibiotics from three non-consecutive batches are non-detectable. The levels of hormones from three non-consecutive batches of harvested cells are either non-detectable or well below their respective safety limits.

2.3 Food Applications

The harvested cells can then be mixed with plant-based ingredients to formulate final products such as sausage and bacon alternatives, which are expected to be cooked for at least 4 minutes at a minimum internal temperature of 165 °F. The cooked products are then frozen and stored according to safe food handling procedures.

Mission Barns has analyzed the fatty acid profiles of the cultivated pork fat cells from three non-consecutive batches, and find them to be consistent with those reported in conventional

4/ The sterility tests include evaluation of bacteria and fungi.

pork fat. The typical intended use levels of cultivated pork fat cells are summarized in **Table 3** below:

Table 3. Food Applications with Cultivated Pork Fat Cells	
Food Applications	**Typical Use Levels**
Ground meat	Up to 20%
Formed products (e.g., burgers, meatballs)	Up to 30%
Encased products (e.g., hot dog, sausage)	Up to 40%

Part 3. Safety Discussion

3.1 Dietary Exposure Assessment

For the purposes of this dietary exposure assessment, it is conservatively assumed that cultivated pork fat cell consumption will be equivalent to 100% of the existing conventional pork fat consumption in the US. Most of the US population's fat intake is derived from unprocessed foods, including meat, poultry, fish, and plant-based sources. Cultivated fat cells are intended to be used in alternatives for such products, and will not increase overall consumer exposure to animal fat.

Pork fat consumption estimates for the US population and various subpopulation are based on food consumption data from the National Health and Nutrition Examination Survey (NHANES). NHANES is conducted by the Department of Health and Human Services (DHHS) to assess the health and nutritional status of a nationally representative sample of children and adults in the US. The data are representative of the civilian, non-institutionalized U.S. population. What We Eat in America (WWEIA), and its predecessor, the Continuing Survey of Food Intake by Individuals (CSFII), are the dietary intake interview component of the NHANES. It is conducted as a partnership between USDA and DHHS. WWEIA consists of two non-consecutive days of 24-hour dietary recall data.

WWEIA-Food Commodity Intake Database (FCID) 2005-10 was developed by EPA's Office of Pesticide Programs (OPP) to improve the utility of the WWEIA food consumption survey for pesticide dietary exposure assessment. These data are also used by EPA's Office of Research and Development to update food consumption rates presented in EPA's Exposure Factors Handbook. WWEIA-FCID 2005-10 translates food consumption as reported eaten in WWEIA (2005-2010 survey cycles) into consumption of EPA-defined food commodities. Such food commodity intakes are expressed as grams of food commodity consumed per day or per kg bodyweight per day for over 500 commodities derived from more than 7,000 different foods and beverages reported in the two surveys. WWEIA-FCID 2005-10 is intended to complement the CSFII and NHANES/WWEIA databases in that it provides estimates of food consumption expressed as food commodities as opposed to foods per se (i.e., "as eaten") which can in some exposure and other situations be of more utility.

Specifically, below the FCID 2005-10 was used to estimate pork fat consumption among the US population, which are summarized below in **Table 4**:

Table 4. Dietary Exposure Assessment for Pork Fat from FCID 2005-10				
Population	**Average intake**	**90th percentile Intake**	**Average intake (per bw)**	**90th percentile Intake (per bw)**
All ages	6.93 g/day	15.7 g/day	0.11 g/kg bw/day	0.2 g/kg bw/day
2 years and older	6.99 g/day	15.8 g/day	0.11 g/kg bw/day	0.2 g/kg bw/day
2 years to 12 years	4.70 g/day	10.5 g/day	0.19 g/kg bw/day	0.4 g/kg bw/day
13 years to 18 years	6.71 g/day	14.5 g/day	0.11 g/kg bw/day	0.2 g/kg bw/day
19 years and above	7.46 g/day	16.7 g/day	0.09 g/kg bw/day	0.2 g/kg bw/day

As indicated by the table above, the average and 90th percentile intake of pork fat among US consumers are 6.93 g/day and 15.7 g/day, respectively. For the purpose of this safety discussion, the highest 90th percentile intake of any of the subpopulations is **16.7 g/day** for 19 years and above, and on a per body weight basis **0.4 g/kg bw/day** for 2 years – 12 years. These values will be used as the most conservative intake for the purpose of calculating the EDI. This is based on the assumption that cultivated pork fat cells manufactured by Mission Barns will replace 100% of conventional pork fat in the marketplace today.

3.2 Safety Assessment

3.2.1 Biological (Microbiological) Risk Assessment

Our process is inherently safer from a microbiological perspective than traditional meat processing, because our products are not expected to be in contact with common foodborne pathogens. Further, during the entire process discussed below, all operations are conducted in cGMP-compliant facilities. Stringent controls are required to successfully maintain an aseptic environment for cell culture for the entirety of the production process, from culture establishment through harvesting and storage. In particular, all cell culture operations which involve primary or secondary exposure to air are conducted in aseptic air environments, such as those provided by a Class II biosafety cabinet. Any non-sterile materials introduced into this environment are first sanitized with 70% isopropyl alcohol or ethanol solutions. All cell work is performed with trained operators in lab coats and nitrile gloves as personal protective equipment (PPE).

In addition, all cell culture vessels are controlled for sterility. Before their introduction to the process, the vessels are either sterilized on-site via pressurized steam or purchased pre-sterilized via gamma-irradiation or pressurized steam from a third party with accompanying certificates of analysis. Additionally, the interior of all containers that are used to transfer cells from one culture vessel to another are controlled to meet the same sterility standards using the same sterilization methods. All processing reagents used are either sterile when provided by the suppliers or sterilized by Mission Barns on-site by heat or other appropriate means depending on material stability.

Below, potential points of compromise of cell culture environment aseptic conditions that may pose microbiological risks are discussed, together with preventive measures to mitigate them.

3.2.1.1 Cell Banking Process

During tissue collection, if the animal from which the tissue is removed has a microbial infection, the microbes that are present in the biopsied tissue could in theory contaminate the cell culture isolated from it. To mitigate this risk, only healthy animals without any signs of microbial infection are selected for the tissue biopsy. Animal tissues and cells are also handled under hygienic conditions to prevent contamination.

Further, to prevent microbial contamination introduced during operation, a combination of antimicrobial reagents including antibiotics and antifungals are used.

Out of an abundance of caution, to take potential sterile barrier failures into account during the manufacturing process, and to account for any unusually slow-growing contaminants, sterility testing is also conducted on the cell culture at the end of this process to assess if any fungal or bacterial contaminants are present. Biochemical assays are used to detect microbial contaminants that could be introduced during the culture establishment procedures. A list of such tests can be found in **Table 1** above.

3.2.1.2 Manufacturing Process

During the cultivated pork fat cell manufacturing process, Mission Barns also implements a Food Safety Plan in compliance with Food Safety Modernization Act (FSMA) for Hazard Analysis Risk Based Prevent Controls (HARPC). The analysis identified biological and chemical hazards associated with each manufacturing step from receipt of raw materials to cell harvest and storage. All operations at this stage are conducted in a cGMP compliant facility.

The cell bank used for the manufacturing process has already been tested to confirm the absence of any viral and mycoplasma contamination as summarized in **Table 1**. While all equipment and reagents used during this process are sterile, and the operations are conducted in an aseptic environment, the possibility of potential microbial contamination including fungal and bacterial contamination introduced through adventitious agents or the environment still exists. In the event that cell culture system sterility is compromised during the production process, the introduction of fungal or bacterial contamination would be detected via microscopic visual inspection of cultures. The cultivated pork fat cells will also be subject to sterility testing and mycoplasma testing as described in **Table 2**.

3.2.2 Chemical Risk Assessment

3.2.2.1 Substance Classification and Safety Rationale

All raw materials used in both the cell banking and manufacturing processes are considered safe and suitable for use. The raw materials are either approved food additives, considered GRAS when added directly to foods as an ingredient, or have been evaluated to confirm they are safe and suitable for this use.

For the purpose of the chemical risk assessment, for the substances that are mostly common nutrients such as amino acids, vitamins and their derivatives, and minerals that are needed by the cells to proliferate, when applicable, the relevant references to FDA food additives regulations for the raw materials used in the cell banking and the cultivated pork fat cell manufacturing process are provided to the FDA. Those that are believed to be GRAS for their intended use, either based on FDA's GRAS regulations, GRAS notice programs, or GRAS self-determination (self-GRAS), are identified. For the small number of chemical substances that are not nutrients (e.g., hormones and growth factors), Mission Barns conducted a safety assessment to support these raw materials are suitable for use in the manufacturing process. A more detailed toxicity assessment is provided based on conservative "worst-case" dietary intake scenarios. In particular, it is assumed, conservatively, that 100% of the conventional pork fat in the US will be replaced by the cultivated pork fat cells manufactured by Mission Barns. These EDIs are compared to appropriate safety threshold levels identified in publicly available literature to demonstrate that the low residual levels in the finished cultivated pork fat cells do not pose any human safety concern. [5]/

3.2.2.2 Dietary Intake Calculation

Multiple washing steps upon harvest substantially reduce the concentration of all residuals in the cultivated animal fat cells.

Based on an evaluation of all the components that may contact cultured cells in the manufacturing process, we chose one particular protein molecule to serve as a useful surrogate to analyze the potential presence of residual processing reagents in the cell culture product after washing. This is because, first, this particular surrogate protein molecule is present in the cell

5/ Databases including PubMed, Google, and Google Scholar, and applicable safety reviews conducted by FDA, the US Environmental Protection Agency (EPA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA) are reviewed to identify applicable safety threshold levels.

culture media at relatively high concentrations; and second, an established Enzyme-Linked Immunosorbent Assay (ELISA) that is very sensitive and specific for this molecule enables us to detect even very low levels after multiple washing steps. The surrogate protein molecule levels from three non-conservative batches are reported in **Table 5** below:

Table 5.Results of Surrogate Protein Molecule for Three Non-Consecutive Runs

Test Item	Batch #1	Batch #2	Batch #3
Spent Media (at the end of the culturing process)	3.5 ± 0.2 mg/mL	3.4 ± 0.1 mg/mL	3.0 ± 0.1 mg/mL
Final wash solution	0.000027 ± 0.000001 mg/mL	0.000036 ± 0.000002 mg/mL	0.000006 ± 0.000001 mg/mL

As the above table indicates, the surrogate protein molecule levels measured in spent media from the three non-consecutive runs 1-3 were: 3.5 ± 0.2 mg/mL, 3.4 ± 0.1 mg/mL, and 3.0 ± 0.1 mg/mL, respectively. The average residual protein concentrations measured in the final wash solution from the non-consecutive batches 1, 2, and 3 are 0.000027 mg/mL, 0.000036 mg/mL, and 0.000006 mg/mL, respectively. As such, the actual dilution factors for the protein molecule can be calculated as below:

- Batch #1: $0.000027 \div 3.5 = 7.71 \times 10^{-6}$
- Batch #2: $0.000036 \div 3.4 = 1.06 \times 10^{-5}$
- Batch #3: $0.000006 \div 3.0 = 2 \times 10^{-6}$

As the above calculations show, the actual dilution factors we have calculated for this surrogate protein molecule range from 2×10^{-6} to 1.06×10^{-5}. We believe this analytical data supports the use of a conservative dilution factor in our exposure calculations of approximately 10^{-5}, meaning that as compared with reagent quantities remaining in final spent media after the cell culture process is complete, residual levels of components present after the washing steps are expected to be lower by approximately at least 10^{-5}.

Other than the surrogate protein molecule, a few other cell culture media reagents may be present at levels above 0.1 g/L in the cell culture media. Most of these components are common nutrients such as amino acids and salts that have maximum use levels of no more than 10 g/L in the cell culture media, and are either approved by FDA for various food applications or well-characterized for their safety when used in foods.

All other cell culture media reagents are present at concentrations below 0.1 g/L. Therefore, the "worst-case" residual level of any other component from cell culture media can be calculated as:

$(0.1\ g/L) * 10^{-5} * 1\ L/kg = 10^{-6}\ g/kg$

Given the pork fat 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:

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

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

Based on the above worst-case estimation, the EDIs for all these components are well within appropriate margins of safety, as further discussed below. In the interest of providing context, we compare the exposure levels to reference values that have been established by FDA and other regulatory bodies. FDA has established a threshold of regulation for substances used in food-contact articles of 1.5 µg/day, provided the substance has not been shown to be carcinogenic, does not have any structural alerts, and otherwise complies with the regulations. EFSA and WHO have established a threshold of toxicological concern (TTC) of 0.15 µg/day or 0.0025 µg/kg bw/day based on a 60-kg body weight for compounds with a structural alert for genotoxicity. 6/ EFSA and WHO adopted the TTC as a science-based screening tool useful for assessing low dose chemical exposures, and to distinguish those for which further data are required to assess the human health risk from those with no appreciable risk. The TTC value of 0.15 µg/person/day for potential genotoxic carcinogens based on structural alerts for genotoxicity (excluding aflatoxin-like, nitrosamine and azoxy-compounds) was considered conservative because it was derived by linear extrapolation from the TD50 values combined with the analysis of the proportions of chemicals with each structural alert that had an upper-bound estimated lifetime cancer risk of greater than one in a million.

6/ European Food Safety Authority and World Health Organization. "Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree." *EFSA Supporting Publications* 13.3 (2016): 1006E.

We note the TTC approach is mainly used as a screening tool. The TTC value of 0.0025 µg/kg bw is aimed at potential genotoxic chemicals that are DNA-reactive mutagens, and we recognize this does not cover all mechanisms of genotoxicity such as clastogenicity (structural chromosomal aberrations) or aneugenicity (numerical chromosomal aberrations). [7]/ Another limitation for the TTC approach is that it uses toxicological data from oral doses administered to experimental animals to estimate an equivalent human external exposure. [8]/ Finally, when the TTC approach is applied, one of the biggest practical challenges remains the accurate calculation of the relevant exposure. [9]/ As such, the TTC lowest threshold level of 0.15 µg/day or 0.0025 µg/kg bw/day was not referenced as the safety threshold for any residual impurities. Rather, by comparing the estimated daily intake of any residual impurities to the TTC lowest threshold level, we aim to demonstrate that at these vanishingly low levels, the components would not be expected to pose any human safety concern.

3.2.2.3 Cell culture media

Proprietary cell culture media is made by combining food or pharma-grade components, the majority of which can already be found in some form in existing food applications. All cell culture media raw materials used in both the cell banking and manufacturing processes are considered safe and suitable for use. [10]/ All cell culture media components, including mostly common nutrients such as amino acids, nucleic acids, vitamins and their derivatives, and minerals, are either approved food additives, considered GRAS when added directly to foods as an ingredient, or have been evaluated to confirm they are safe and suitable for this use. For example, 21 CFR 172.320 establishes that the direct addition of many amino acids to food are safe. Further, in many cases, the calculated worst-case EDI of 0.0004 µg/kg bw/day in Section **3.2.2.2** is several orders of magnitude lower than the respective safety threshold levels of these components. When appropriate, Mission Barns also analyzed the particular residual levels of cell culture media components in the cultivated cells as part of the risk assessment. Using a similar approach, Mission Barns has demonstrated the residual levels of these components in the cultivated fat cells do not pose any human safety concern. We also note that none of the components from the basal cell culture media is a color additive.

7/ Serafimova, R., T. Coja, and G. E. N. Kass. "Application of the Threshold of Toxicological Concern (TTC) in Food Safety: Challenges and Opportunities." *Front. Toxicol. 3: 655951. doi: 10.3389/ftox* (2021).

8/ *See id.*

9/ *See id.*

10/ The chemical identity, regulatory basis, and when appropriate more detailed toxicology assessment for each component in the proprietary cell culture media is provided in a separate confidential submission on file with FDA.

3.2.2.4 Media supplements

Mission Barns' proprietary media formulation contains hormones and growth factors that are commonly used in the biotech industry and known to be essential for cellular proliferation and growth. Many of these compounds are ubiquitous in nature. The composition of the media supplement has been optimized to provide the appropriate ratios of growth factors to cells; therefore, the levels present in the cell culture media are well below those that would induce apoptosis or toxic effects. Some have a nominal concentration even in the media that is low enough to be comparable to their nominal concentration in commonly consumed animal products like milk. Through cell cultivation, washing, and downstream processing steps, the concentration of these components is further diminished. And as discussed above, the theoretical EDI levels calculated (i.e., 0.0167 µg/day and 0.0004 µg/kg bw/day) are well below the TTC of 0.15 µg/day for compounds with structural alert for genotoxicity. 11/ Additionally, the bioactivity of components would be expected to be completely lost from the cooking steps (i.e., at least 4 minutes at a minimum internal temperature of 165 °F).

More detailed toxicity assessment for these components is provided below.

a) Hormones

Hormones are chemicals that are produced naturally in the bodies of all animals, including humans. They are chemical message molecules released into the blood by hormone-producing organs, and travel to and affect different parts of the body. 12/ Harvested cells are mixed with plant-based ingredients to formulate final products, which are then cooked for at least 4 minutes at a minimum internal temperature of 165 °F. Any residual hormones will lose their biological activity after the cooking.

For the 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. The calculated EDIs (i.e., all well below 0.1 µg/day) for the residual hormones are several orders of magnitude lower than the respective safety threshold levels or naturally-occurring levels commonly found in foods

11/ The chemical identity, regulatory basis, and when appropriate more detailed toxicology assessment for each component in the proprietary media supplements is provided in a separate confidential submission on file with FDA.

12/ Gandhi, Renu, and Suzanne M. Snedeker. "Consumer Concerns About Hormones in Food, BCERF Fact Sheet No. 37." (2000).

such as animal milk. As such, any residual levels of hormones in the cultivated fat cells do not pose any human safety concern.

b) Growth Factors

Growth factors, which are generally considered a subset of cytokines, refer to the diffusible signaling proteins that stimulate cell growth, differentiation, survival, inflammation, and tissue repair. Normal cells require several growth factors to maintain proliferation and viability. Mission Barns' media supplements include several growth factors that are commonly used in the biotech industry. Mission Barns has conducted analytical testing of these growth factors in final wash solutions, and none of the growth factors were detectable in the final wash solution, indicating that the harvested cultivated pork fat cells do not contain any residual growth factors. To confirm the testing results reported above from the final wash solution are also representative for cell pellets, the cell pellets were also directly analyzed themselves for two representative growth factors, which were also not detected in the cell pellets. As such, for the purpose of our safety assessment, we will use the theoretical EDI level calculated from their potential presence in cultivated pork fat cells of 0.0167 µg/day.

Because the growth factors are non-detectable after cell harvest, and they are naturally-occurring in human plasma and breast milk, we do not view their use during the manufacturing process as posing any human safety concern. Further, because the harvested cultivated pork fat cells are mixed with plant-based ingredients to formulate final products such as sausage and bacon alternatives, which are then cooked for at least 4 minutes at a minimum internal temperature of 165 °F, the molecules would be completely denatured and no biological activity would be expected for residual growth factors. In addition, for most of these growth factors, the theoretical EDI level calculated from each growth factor's potential presence in cultivated pork fat cells of 0.0167 µg/day is much lower than the native growth factors' naturally-occurring levels found in one serving of 240 mL animal milk. This comparison further demonstrates any residual growth factors in cultivated pork fat cells do not pose any human safety concern.

c) Antimicrobials

As discussed above, Mission Barns conducted additional analytical testing of antibiotics in cultivated cells as part of the safety assessment to support its safety conclusions. The levels of antibiotics from three non-consecutive batches are non-detectable. Further, the calculated worst-case EDI of 0.0167 µg/day is several orders of magnitude lower than the respective safety threshold levels of the antibiotics. As such, any residual antibiotics in the cultivated pork fat cells do not pose any human safety concern.

d) Others

Other than hormones, growth factors, and antimicrobials, a few other components (such as vitamin derivatives) are also used in the supplements. These are all common cell culture supplements widely used in the biotech industry. Mission Barns has evaluated and concluded any residual levels of these components used in the cultivated pork fat cell production processes do not pose any human safety concern.

3.2.2.5 Food contact substances

During the manufacturing process, cells are grown in cultivators constructed of materials commonly used in the food industry and compliant with applicable FDA food additive regulations. They are either in compliance with applicable food additive regulations or considered GRAS.

3.2.2.6 Cell Fattening Reagents

Proprietary fattening reagents are used during the cell fattening process that signal the cells to grow and provide a nutrient source of triglyceride components. The ingredients are food-grade, and Mission Barns concluded their intended use does not pose any human safety concern.

3.2.2.7 Coating reagents

Food-grade ingredients are used to coat cell culture vessel surfaces, and Mission Barns concluded their intended use does not pose any human safety concern.

3.2.2.8 Harvest reagents

During the final step of the production process, an aqueous solution containing harvest reagents is added to collect fattened cells from the cultivator. Multiple washing steps upon harvest significantly reduce concentration of all residuals. Mission Barns concluded their intended use does not pose any human safety concern.

3.2.2.9 Heavy metals

While unlikely, out of an abundance of caution, we evaluate the potential for heavy metals to be present in the cells from the cultivators, which are made from metal, and from any plant-based processing reagents used. As such, close monitoring of heavy metals including lead, cadmium, arsenic, and mercury in the finished cultivated fat cell products will be performed. We have established a specification requiring non-detectable levels of lead, arsenic, cadmium, and mercury. As demonstrated in **Table 2**, heavy metal levels in three non-consecutive batches of cultivated pork fat cell production are reported as "non-detected." We, nonetheless, provide an assessment of heavy metals.

Below, more detailed toxicity assessments for these heavy metals are provided, and safety threshold levels Mission Barns plans to rely on for risk assessment are identified. At the outset, it is noted that inorganic metals, including lead, arsenic, cadmium, and mercury are ubiquitous in the environment due to natural occurrence and in some circumstances as the result of environmental pollution. Metals are found in air, water, and soil where they can be taken up by plants and incorporated into the foods we consume. Regulatory agencies including FDA have long acknowledged this dietary route of exposure and have set exposure limits or safety benchmarks.

Lead

Lead is widely present in the environment due to its natural occurrence and human activities such as the use of leaded gasoline. Because lead may be present in environments where food crops are grown and animals used for food are raised, FDA states that various foods may contain unavoidable but small amounts of lead that do not pose a significant risk to human health. [13]/ The Centers for Disease Control and Prevention (CDC) has identified a blood reference level of 5 micrograms of lead per deciliter of whole blood (ug/dL) as the level at which they recommend clinical monitoring of lead exposure in children. Using the CDC's level as a biomarker, in 2018 the FDA calculated a maximum daily intake for lead from food, termed the interim reference level (IRL). [14]/ The IRL is the calculated amount of dietary lead intake that would be required to reach the CDC's blood reference level, including a 10x safety factor. The calculated IRLs are 3 µg per day for children and 12.5 µg per day for adults.

13/ FDA, *Supporting Document for Recommended Maximum Level for Lead in Candy Likely To Be Consumed Frequently by Small Children* (November 2006), *available at*: https://www.fda.gov/Food/FoodbornelllnessContaminants/Metals/ucm172050.htm#over (accessed on September 17, 2021).

14/ FDA, *Lead in Food, Foodwares, and Dietary Supplements, available at*: Lead in Food, Foodwares, and Dietary Supplements | FDA (accessed on September 17, 2021).

Cadmium

Cadmium is a naturally-occurring environmental toxicant. Food is the primary source of cadmium exposure among the general population as a consequence of the bio-concentration of the heavy metal from the soil. [15]/ JECFA set a tolerable dietary intake level for cadmium and a threshold to safeguard population health. [16]/ In particular, JECFA estimated that a lower bound of the 5th population percentile dietary cadmium exposure of 0.8 µg/kg bw/d or 25 µg/kg bw/ month would result in a urinary cadmium concentration of 5.24 µg cadmium/gram creatinine. The PTMI established was therefore 25 µg/kg bw. Satarug et al. (2017) noted that the FAO/WHO tolerable intake was established at 25 µg per kg body weight per month or 58 µg/day for a 70-kg adult. [17]/

Arsenic

Arsenic is a naturally occurring element in the environment that can enter the food supply through soil, water or air. Organic arsenic compounds contain arsenic with carbon, and are not related to organic farming practices. Inorganic arsenic compounds contain arsenic with a non-carbon element such as oxygen. FDA notes that this distinction is important because current research indicates that the level of toxicity and the associated health effects are more severe from exposure to inorganic arsenic as compared with organic arsenic. [18]/ FDA also assessed potential cancer risks of inorganic arsenic exposure over a lifetime from low dose exposures by extrapolating risks of lung and bladder cancer from populations that were historically exposed to high naturally-occurring levels of arsenic in drinking water ranging as high as 3,000 ppb in Taiwan. [19]/ In 2009, the EFSA Panel concluded that an overall range of the lower benchmark dose (at 1% chance of an increase in effect; $BMDL_{01}$) values of 0.3 and 8 µg/kg bw/day should be used instead of a single reference point in the risk characterization for inorganic arsenic. [20]/

Mercury

15/ Chunhabundit, Rodjana. "Cadmium exposure and potential health risk from foods in contaminated area, Thailand." *Toxicological research* 32.1 (2016): 65.

16/ TRS 983 JECFA 77, *available at*: https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1376 (accessed on September 17, 2021).

17/ Satarug, Soisungwan, David A. Vesey, and Glenda C. Gobe. "Current health risk assessment practice for dietary cadmium: Data from different countries." *Food and Chemical Toxicology*106 (2017): 430-445.

18/ FDA, *Arsenic in Food and Dietary Supplements*, *available at*: Arsenic in Food and Dietary Supplements | FDA (accessed on September 17, 2021).

19/ FDA, *Arsenic in Rice and Rice Products Risk Assessment Report (March 2016)*, *available at*: Arsenic-in-Rice-and-Rice-Products-Risk-Assessment-Report-PDF.pdf (fda.gov) (accessed on September 17, 2021).

20/ EFSA Journal 2009; 7(10):1351.

Mercury occurs naturally in the Earth's crust and is released into the environment through natural and anthropogenic processes. The most common form of mercury in the environment is methylmercury. Methylmercury is the most toxic form of mercury in food and almost all dietary exposure to methylmercury is from seafood. [21]/ JECFA considered kidney weight changes, which occurred at doses similar to or lower than other renal effects, the critical endpoint and 6-month exposure to be sufficient to establish a health-based guidance value, given that steady-state renal mercury concentrations were reached by 4–6 months in rats dosed via their drinking water and exposures in the same dose range for longer durations produced early mortality. [22]/ The $BMDL_{10}$ for relative kidney weight increase in male rats was 0.11 mg mercuric chloride/kg bw/d, corresponding to 0.06 mg/kg bw/d as mercury after adjustment for the 5 days/week dosing schedule used in the study & for the molar percent of mercury in mercury(II) chloride. After application of a 100-fold uncertainty factor, JECFA established a PTWI for inorganic mercury of 4 µg/kg bw.

Therefore, we believe that non-detectable levels (i.e., < 10 ppb) of lead, arsenic, cadmium, and mercury in the cultivated pork fat cells would not pose any human safety concern.

3.2.2.10 Additional cell banking processing reagents

Additional processing reagents used during cell banking include other components commonly used in the biotech industry in the cell banking process.

Because these reagents are used exclusively during the cell banking process, and given the remoteness of these reagents from the final harvested cells, their potential residual levels in the cultivated pork fat cells, if any, would be vanishingly small. And as discussed above, the theoretical EDI levels calculated (i.e., 0.0167 µg/day and 0.0004 µg/kg bw/day) are well below the TTC of 0.15 µg/day for compounds with structural alert for genotoxicity. As such, any residual levels of these processing reagents used during cell banking, if any, will not pose any human safety concern.

3.2.3 Physical Risk Assessment

Mission Barns has evaluated its manufacturing process and determined there are no physical hazards. As there are no manufacturing operations such as cutting or blending, the limited

21/ Díez, Sergi. "Human health effects of methylmercury exposure." *Reviews of environmental contamination and toxicology* (2008): 111-132.
22/ TRS 959-JECFA 72, *available at*: EVALUATION OF CERTAIN CONTAMINANTS IN FOOD (who.int) (accessed on September 17, 2021).

metal-to-metal contact during processing will not introduce metal fragments into product. There are also no glass or hard plastics fragments that can be introduced during the manufacturing process. Therefore, there are no "hard/sharp" physical hazards or "choking" hazards.

In particular, Mission Barns' proprietary cultivator system is designed explicitly for its intended purpose in cultivated fat cell production. The food-contact parts of the cultivators are made from materials permitted for such uses under FDA's food additive regulations, and they are designed to have robust resistance to process reagents and conditions used. Mission Barns' cultivator is intended to be largely reusable and has been designed to comply with food industry standards.

3.2.4 Genetic Stability

At no point in the production process, including before cell banking, is genetic engineering or modification employed, or other process steps introduced to alter the cells' genome.

To ensure the cells cultured to produce cultivated pork fat maintain a stable genomic structure, karyotyping is applied to compare the genomes of early passaged cells to late passaged cells. A typical karyotyping procedure involves staining condensed chromosomes from cells and pairing and comparing the staining patterns on each chromosome. Karyotyping analyzes the number and staining patterns of all the chromosomes of a cell and identifies genomic structure changes including aneuploidy, deletions, duplications and rearrangements at a resolution of 5Mb of DNA. Karyotyping has been used in research and clinical settings to identify chromosomal abnormalities. [23]/ Karyotyping has confirmed that the cultured porcine cells are indistinguishable from cells freshly established from animal tissues, indicating genetic stability over the duration of culturing.

23/ *See* "Karyotyping." n.d. Accessed September 9, 2021. https://www.nature.com/scitable/topicpage/karyotyping-for-chromosomal-abnormalities-298/. Stultz, Brian G., Kathleen McGinnis, Elaine E. Thompson, Jessica L. Lo Surdo, Steven R. Bauer, and Deborah A. Hursh. 2016. "Chromosomal Stability of Mesenchymal Stromal Cells during in Vitro Culture." *Cytotherapy* 18 (3): 336–43. Halevy, Tomer, Shira Akov, Martina Bohndorf, Barbara Mlody, James Adjaye, Nissim Benvenisty, and Michal Goldberg. 2016. "Chromosomal Instability and Molecular Defects in Induced Pluripotent Stem Cells from Nijmegen Breakage Syndrome Patients." *Cell Reports* 16 (9): 2499–2511.

Part 4. Allergenicity Safety

4.1 Intrinsic to Pork

The cultivated pork fat cells will present the same allergenicity concern to consumers who may be allergic to conventional pork. This concern will be addressed through product labeling.

4.2 Introduced during Manufacturing

For background, Congress passed the Food Allergen Labeling and Consumer Protection Act (FALCPA) in 2004 to require that the label of a food that contains an ingredient that is or contains protein from a "major food allergen" declare the presence of the allergen in the manner described by the law. FALCPA identifies eight foods or food groups as the major food allergens. They are milk, eggs, fish (e.g., bass, flounder, cod), crustacean shellfish (e.g., crab, lobster, shrimp), tree nuts (e.g., almonds, walnuts, pecans), peanuts, wheat, and soybeans. FDA also issued a draft guidance in 2020 encouraging food manufacturers to voluntarily declare sesame in the ingredient list on food labels and Congress has since passed the FASTER Act that defines sesame as a major food allergen. [24]/ Culture media and supplements that are added to the cells consist of nutritional components including amino acids, sugars, vitamins and their derivatives, and minerals. None of these are or contain major food allergens as identified under FALCPA.

We also discuss why any residual levels of certain proprietary protein components, given their source, would not elicit an allergic reaction. For the purpose of this assessment, we continue to use the conservative EDI of 0.0167 µg/day for the components of cell culture media with a use level in culture media lower than 0.1 g/L, which greatly overestimates the actual residual levels of these protein components.

Threshold levels for allergenic proteins can be defined as the maximum amount of an allergenic protein that can be tolerated without producing any adverse reaction. An individual threshold is the maximum amount of an allergenic food that can be tolerated by a specific food-allergic individual. A population threshold is the maximum amount of an allergenic food that can be tolerated by the entire population (or a representative sub-population) of individuals with a specific type of food allergy. The most sensitive individuals dictate the low end of the range for the population threshold. The population threshold is often described in terms of percentages

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[24]/ FDA, "*Voluntary Disclosure of Sesame as an Allergen: Guidance for Industry*," (November 2020). Food Allergy Safety, Treatment, Education, and Research (FASTER) Act of 2021 (S. 578).

e.g. ED_{10} (the eliciting dose predicted to provoke a reaction in 10% of individuals with a specific food allergy). The calculated EDI is several orders of magnitude lower than the allergenic threshold levels for these proprietary protein components. As such, any residual level of these proprietary protein components in cultivated pork fat cells will not elicit any allergic reaction in allergic individuals.

Requests for Information to be Added to the Disclosable Safety Narrative

Cell Procurement

1. Information Requested

The disclosable safety narrative states that tissue is biopsied from a domestic pig in a veterinary operating chamber by trained veterinary doctors. For addition to the disclosable safety narrative, please provide additional information about potential microbiological hazards (e.g., salmonellae, *Campylobacter coli. Yersinia enterocolitica*, parasites) that you have identified for your selected cell sourcing method and how the controls used during cell procurement, cell line establishment and cell bank establishment are sufficient to address any risks arising from these hazards.

Significance

Discussion of the relationship between specific microbiological hazards and the controls used to address any risks arising from these hazards provides additional support for the overall public safety conclusion made by the firm.

Mission Barns' Response:

Mission Barns has thoroughly assessed the microbiological hazards of concern during cell sourcing, as well as implemented appropriate controls to address any potential risk from such hazards. As discussed in detail in our response to information request #19 below, our process is different from a microbiological perspective than traditional meat processing because our process does not involve animal slaughtering, during which meat products may be exposed to common pathogens present in an animal's digestive tract and fecal matter. Instead, the source tissue is generally isolated from a healthy pig by a trained veterinarian under hygienic conditions. Biopsied tissue is subsequently washed with an antibiotic-containing saline solution to mitigate potential microbial contamination prior to establishing a cell bank. Mission Barns' cell culture is conducted under aseptic conditions by trained personnel with appropriate personal protective equipment and all operations are performed under a Class II biosafety cabinet or equivalent environment. All processing agents and equipment with primary or secondary contact to the tissues/cells are sterile. Mission Barns uses microscopy to monitor the cell cultures to identify instances of potential microbial contamination and verifies the absence of microbial contamination in cultivated pork fat cells through manufacturing cell bank analytical testing. Below, the relationship between specific microbiological hazards and the controls used to address any risks arising from these hazards is discussed in more detail.

During tissue sourcing, if the animal from which the tissue is removed has a microbial infection, the microbes that are present in the biopsied tissue could, in theory, contaminate the cell culture isolated from it. To mitigate this risk, as discussed in more detail in our response to information request #2 below, only healthy animals without any signs of microbial infection are selected for the tissue biopsy. Animal tissues and cells are also handled under hygienic conditions to prevent contamination. Experienced veterinary doctors remove hair from the animal's skin first, apply disinfecting reagents to the region of the animal's skin where an incision is made for the subcutaneous adipose tissue biopsy, and use sterilized instruments to conduct the biopsy.

Further, to prevent microbial contamination introduced during operation, a combination of antimicrobial reagents are used and is included in both the saline that is used to extensively wash the biopsied tissue and the cell culture media that is used to establish the culture of cells extracted from the tissue.

The biopsied tissue is quickly displaced, while minimizing air contact duration, into a sterilized container and fully submerged in sterile antimicrobial-containing cell culture media. During approximately the first month after cells are isolated from tissue, cultures are regularly visually examined for any bacterial or fungal

contamination. Additionally, the cultures are tested for viral and mycoplasma contamination at the end of this duration.

Any microbial contamination, if present, is expected to overtake animal cell cultures. Out of an abundance of caution, to take potential sterile barrier failures into account during the cell procurement, cell line establishment, and cell bank establishment process, and to account for any unusually slow-growing contaminants, testing is performed at the end of the cell banking process for animal-specific and environmental microbiological organisms of concern. Specifically, Mission Barns tests for Aerobic Plate Counts, coliforms, enterobacteria, mycoplasma and yeast/mold to ensure that no adventitious agents of concern are introduced into the manufacturing stream. These tests were selected by Mission Barns as they are common methods used in the food industry for microbial hazards screening. To account for the use of animal sera, cell banks are also all tested and verified to be free of common animal-derived viruses.

2. Information Requested

Page 18 of the disclosable safety narrative describes the cell banking process, "During tissue collection, if the animal from which the tissue is removed has a microbial infection, the microbes that are present in the biopsied tissue could in theory contaminate the cell culture isolated from it. To mitigate this risk, only healthy animals without any signs of microbial infection are selected for the tissue biopsy"; however, verification, certification, and documentation of this is not provided. For addition to the disclosable safety narrative, please describe how this is verified or certified, and provide relevant examples of documentation, as appropriate.

Significance

Discussion of procedures that control this hazard provide relevant information when evaluating the overall safety conclusion provided by Mission Barns.

Mission Barns' Response:

To verify the health of the animals that Mission Barns derives its cell cultures from, a set of tests and examinations are conducted on the animals. Viral and bacterial screening is conducted for Porcine Reproductive & Respiratory Syndrome Virus (PRRS), Transmissible Gastroenteritis Virus (TGV), Influenza A, *Brucella*, *Leptospira*, Pseudorabies Virus (PSR), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* (APP), and Porcine Epidemic Diarrhea Virus (PEDV) and the results from the screens are compiled in a test report. The results of a complete medical exam, including verifying that the source animal's vaccinations are current, are captured in a general workup report, vaccination history certificate, and in a Certificate of Veterinary Inspection from an applicable governmental agency (*e.g.*, the California Department of Food and Agriculture). Source animal health documents are stored as records as part of the company's safety and quality system.

3. Information Requested

For addition to the disclosable safety narrative, please incorporate the last two sentences of the first complete paragraph on page 25, Section 3.2.1.1, of the supplemental confidential material.

Significance

This information provides significant additional information with respect to a substantive control step, and thus support to the firm's overall safety conclusion.

Mission Barns' Response:

As requested, Mission Barns includes the following to the disclosable safety narrative:

During approximately the first month after cells are isolated from tissue, cultures are continuously visually examined for any bacterial or fungal contamination. Additionally, the cultures are tested for viral and mycoplasma contamination at the end of this duration.

Cell Bank Establishment

Identity

4. Information Requested

For addition to the disclosable safety narrative, provide a statement of tissue source and cell type used in the production process.

Significance

To clearly establish the identity of the process and product evaluated in CCC 000008, it is helpful to explicitly state certain attributes of the cell lines used for production that are relevant to the identity of the harvested cell material.

Mission Barns' Response:

The cell population that Mission Barns has developed for cultivated pork fat cell production was isolated from subcutaneous belly fat tissue biopsied from a domestic Yorkshire pig (*Sus scrofa domesticus*) in a veterinary operating chamber by trained veterinary doctors.

Cell Line Adaptation and Selection

5. Information Requested

For addition to the disclosable safety narrative, please provide as much as possible of the information presented in Sections 3.2.4.1 and 3.2.4.2 (page 63) of the supplementary confidential material.

Significance

A discussion of the specific considerations, metrics, and outcomes related to stability that you considered in developing the cell line for production provides additional support for the firm's overall safety conclusion.

Mission Barns' Response:

Genetic engineering, genetic modification, or other process steps introduced to alter a cell population's genome are not used at any point in Mission Barns' production process, including before cell banking. Normal cells have multiple mechanisms including cell cycle checkpoints to ensure genome integrity.[1] Similarly to what happens to cells in the body, cells cultured ex vivo can lose genetic stability when these safeguarding mechanisms fail. The paragraph below focuses on the feature of the process most likely to introduce the risk of causing genetic differences and how genetic changes in the process are measured.

When Mission Barns' cells are transitioned from serum-containing to serum-free media, the proliferation rate of cells transiently drops for some passages, but gradually increases back to a stable rate. No drops in cell viability and no mass death events are observed during this transition, which can occur during the serum-free

[1] Aguilera, A. and Gómez-González, B. (2008) "Genome Instability: A Mechanistic View of Its Causes and Consequences," *Nature Reviews Genetics* 9 (3):204–17.

weaning and adaptation processes with common substantiated biopharmaceutical cell lines like CHO. To test for significant genetic changes that could be introduced in this process and in the production process generally, karyotyping is conducted on preliminary cell bank stocks and on cells cultured from stocks that supply the manufacturing process. A typical karyotyping procedure involves staining condensed chromosomes from cells and pairing and comparing the staining patterns on each chromosome. Karyotyping has been used in research and clinical settings to identify chromosomal abnormalities.[2]

As demonstrated in Section 3.2.4.2 of the supplementary confidential material, cells of early and late passages show normal female porcine chromosome numbers and staining patterns for each of the chromosomes, as analyzed by trained cytogeneticists. Therefore, the cells do not show detectable genetic instability in vitro over the duration of culturing.

Testing Methods

6. Information Requested

Table 1 on page 11 of the disclosable safety narrative provides the analytical testing results of the manufacturing cell bank, however, the specific methods used are not provided for most analyses presented in the table (e.g., internally developed methods, AOAC, ISO). Please provide complete citations of all analytical methods used. Please also specify, for analyses with a "positive/negative" result, the sample size analyzed (e.g., negative in 25 g). Further, please provide a statement that all analytical methods, including internally developed methods, are validated for their intended purpose.

Significance

A clear understanding of the identity and validity of the methods used to generate data described in the disclosable safety narrative provides additional support for the overall safety conclusion.

Mission Barns' Response:

Per the FDA's request, Mission Barns has updated Table 1 of the disclosable safety narrative pertaining to the methods, sample sizes, and results of testing performed on manufacturing cell banks. Please note that sterility testing performed on cell banks is further discussed in our response to information request #8 below.

Further, Mission Barns confirms that all analytical methods, including internally developed methods, are validated for their intended purpose.

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[2] *See* "Karyotyping." n.d. Accessed September 9, 2021 https://www.nature.com/scitable/topicpage/karyotyping-for-chromosomal-abnormalities-298/. Stultz, Brian G., Kathleen McGinnis, Elaine E. Thompson, Jessica L. Lo Surdo, Steven R. Bauer, and Deborah A. Hursh. 2016. "Chromosomal Stability of Mesenchymal Stromal Cells during in Vitro Culture." *Cytotherapy* 18 (3): 336–43. Halevy, Tomer, Shira Akov, Martina Bohndorf, Barbara Mlody, James Adjaye, Nissim Benvenisty, and Michal Goldberg. 2016. "Chromosomal Instability and Molecular Defects in Induced Pluripotent Stem Cells from Nijmegen Breakage Syndrome Patients." *Cell Reports* 16 (9): 2499–2511.

Table 1. Manufacturing Cell Bank Analytical Testing Results			
Test	**Method**	**Sample Size**	**Specification**
Species Verification	Porcine (Pork) DNA by validated PCR method Method developed at 3rd party ISO-17025 certified lab	400,000 cells	*Sus scrofa domesticus* (pig) DNA confirmed
Cell Stability	Karyotyping (GTG banding)[3]	2x 10^6 cells	Normal chromosome spreads
Viral Panel: • Bovine viral diarrhea virus • Porcine parvovirus • Adenovirus • Hemagglutinating encephalitis virus • Transmissible gastroenteritis virus • Reovirus • Rabies virus	9 CFR Part 113: • 113.46 - Detection of cytopathogenic and/or hemadsorbing agents) • 113.47 - Detection of extraneous viruses by the fluorescent antibody technique	75 cm^2 of a cell culture monolayer	Not Detected
Mycoplasma	Mycoplasma Genus TaqMan® PCR, or equivalent[4]	200 µL of spent media	Negative

Adventitious Agent Hazard Assessment

7. Information Requested

For addition to the disclosable safety narrative, please state what adventitious agent hazards would be associated with use of any animal-derived substances other than the cells themselves in cell isolation, cell line establishment, or cell bank establishment and your basis for concluding that any potential risks would be adequately addressed by the controls implemented by the firm.

Significance

Animal-derived substances represent a distinct potential source of adventitious agent contamination. An

[3] *See* Howe, B., Umrigar, A. and Tsien, F. (2014) "Chromosome preparation from cultured cells," *Journal of Visualized Experiments* 83.

[4] *See* Holland, P.M. et al. (1991) "Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of Thermus aquaticus DNA polymerase," *Proc Natl Acad Sci USA*. 88(16).

explanation in the disclosable safety narrative regarding the adequacy of production controls implemented by Mission Barns to address any risk arising such hazards will provide support for the overall public safety conclusion made by the firm.

Mission Barns' Response:

The primary hazards identified with the use of animal-derived materials used in Mission Barns' cell isolation, cell line establishment, or cell bank establishment activities are the potential risks of propagation and/or transmission of microorganisms such as bacteria, viruses, or, in the case of bovine-derived components, prions, which are the cause of transmissible spongiform encephalopathies (TSE), such as bovine spongiform encephalopathy (BSE) in cows and Creutzfeldt-Jakob Disease (CJD) in humans.

As discussed in Mission Barns' response to information request #11, all processing reagents used, including animal derived substances, are food grade (when available), pharmaceutical grade, or high-quality chemical grade. All animal-derived substances are either sterile as received or filtration-sterilized by Mission Barns prior to their introduction into the manufacturing process. Mission Barns 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 such substances. These controls include the following:

Materials Risk Assessment

Product specifications are established for each material based on the risks identified and mitigating controls, such as special handling and batch testing, are implemented where appropriate and are required to be reported on a Certificate of Analysis (COA) or equivalent quality document received with each product shipment. In particular, any bovine-derived components used in Mission Barns' manufacturing process have associated certifications/statements that affirm they are sourced from geographies that are classified as negligible risk for BSE under OIE Resolution[5] and/or have certifications/statements that state they present negligible/minimal TSE risk. To the extent any bovine-derived components used are imported into the United States, they will be sourced only from countries that are classified as BSE negligible risk by the Animal and Plant Health Inspection Service (APHIS) in accordance with 9 CFR § 92.5.

Supplier Approval Program

As another level of control, Mission Barns has implemented a supplier approval program that utilizes a set of procedures and controls for ensuring the safety of all incoming materials, including any animal-derived substances used.

Material Handling and Positive Release Program

All incoming raw materials and finished products are placed on hold by Quality until they are approved for release following a review of certificates of analysis/certificates of conformance, testing results and/or production records by a Preventive Controls Qualified Individual (PCQI). All non-conforming materials or batches are quarantined and removed from production.

Finally, any microbial contamination, if present, is expected to overtake animal cell cultures. During approximately the first month after cells are isolated from tissue, cultures are continuously visually examined for any bacterial or fungal contamination. Out of an abundance of caution, testing is performed at the end of cell banking process for animal-specific and environmental microbiological organisms of concern, including Aerobic Plate Counts, coliforms, enterobacteria, mycoplasma and yeast and mold to ensure that no adventitious agents of concern are introduced into the manufacturing stream. To account for the use of animal sera, cell banks are also all tested and verified to be free of common animal-derived viruses as

[5] *Available at* /https://www.woah.org/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Resolutions/2019/A_R19_BSE_risk.pdf (accessed March 1, 2023)

discussed in our response to information request #6 above.

8. Information Requested

Page 10 of the disclosable safety narrative states that the USP 71 sterility test are used for aseptic process confirmation at the cell bank stage. Page 11 presents test criteria and results. Page 13 provides the rationale for the analyses. You include sterility testing, and reference USP 71 as the analytical method used. USP 71 states, "These Pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedures. *The test is applied to substances, preparations, or articles which, according to the Pharmacopeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined under the conditions of the test*." Further, you do not specify the media used for this analysis, nor the incubation parameters (i.e., time and temperature). Not all bacteria and fungi optimally utilize the same media, nor do they maintain the same growth parameters. Additionally, this is not a common method used for products destined for the human food supply. Common analyses, such as aerobic plate count, yeast and mold, coliforms, *Enterobacteriaceae*, or more specific analyses for foodborne pathogens, such as *Salmonella* serovars, are not provided. For addition to the disclosable safety narrative, please provide additional discussion regarding your **rationale for the suitability** and **adequacy of USP 71** (including the media used, and the growth parameters). Alternatively, we strongly recommend that you consider expanding your testing strategy to encompass validated microbial detection methods for use in human foods.

Significance

Given the novelty of this test for food safety applications, further consideration and discussion regarding the suitability and adequacy of the method will provide additional support for the overall public safety conclusion made by the firm.

Mission Barns' Response:

In accordance with FDA's recommendation as specified above, Mission Barns has amended its testing plan for cell banks to replace the USP 71 sterility test with microbial detection methods commonly used for human food products.

At the outset, we would like to note that, as discussed in detail in our response to information request #19 below, our process is different from a microbiological perspective than traditional meat processing because our process does not involve animal slaughtering, during which meat products may be exposed to common pathogens present in an animal's digestive tract and fecal matter. Further, as discussed in our response to information request # 1 above, Mission Barns' cell culture is conducted under aseptic conditions by trained personnel with appropriate personal protective equipment. Mission Barns uses microscopy to monitor the cell cultures to identify instances of potential microbial contamination. Any microbial contamination, if present, is expected to overtake animal cell cultures. Out of an abundance of caution, to take potential sterile barrier failures into account during the cell procurement, cell line establishment, and cell bank establishment process, and to account for any unusually slow-growing contaminants, testing is performed at the end of the cell banking process for animal-specific and environmental microbiological organisms of concern, including Aerobic Plate Counts, coliforms, enterobacteria, mycoplasma and yeast/mold to ensure that no adventitious agents of concern are introduced into the manufacturing stream. The tests for adventitious agents of concern which have been identified as appropriate are summarized in the table below. These tests were selected by Mission Barns as they are common methods used in the food industry for microbial hazards screening. Further, Mission Barns confirms that all analytical methods are validated for their intended purpose.

Mission Barns' updated cell bank microbial testing plan is included below for reference:

Cell Bank Microbial Testing Plan			
Test	**Method**	**Sample Size**	**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)

Substances Used During Cell Culture

Exposure Estimation

9. Information Requested

For addition to the disclosable safety narrative, provide the discussion of washing steps contained in the first paragraph of Section 3.2.2.2 (pages 26-27) of the supplementary confidential material.

Significance

This description provides important context for the overall exposure model.

Mission Barns' Response:

Multiple washing steps upon harvest substantially reduce the concentration of all residuals in the cultivated animal fat cells. In the beginning of the harvesting process of the final cultivator, all the media is drained, leaving an estimated 5% of the total volume of media within the cultivator. A harvest solution is then added, collected along with the dissociated cells, and centrifugated. Once the cells are pelleted, the supernatant is removed, leaving an estimated 1-2% of the total volume of the media in the centrifuging tube. Finally, at least two additional washing and centrifugation steps are performed to get the final cell culture product. Each of those washes leaves an estimated level of 1-2% of the total volume of solution in the tube.

Substance Identity and Criteria for Evaluation

10. Information Requested

Page 26 of the disclosable safety narrative states that several substances are used for cell fattening, to coat cell culture vessel surfaces, and at the harvest step. The narrative describes these substances as food-grade and asserts that their intended use does not pose any human safety concern. Please provide additional

information about the basis upon which you concluded that their use did not pose any food safety concern. This could include statements about prior exposure or presence in food as an ingredient or constituent, estimated exposure, prior authorization or evaluation, or other information that would provide insight into Mission Barns' assessment process and decision criteria.

Significance

The absence of any general information on identity, decision criteria, or grounds for safety conclusions in this section of the disclosable safety narrative does not provide a sufficient basis for FDA to discuss in documenting our evaluation.

Mission Barns' Response:

All reagents used in the fattening, coating, and harvest processes of Mission Barns' cultivated pork fat cell production process either have an existing regulation that affirms them as GRAS for various specified food uses, which Mission Barns references as an indicator of underlying publicly available data relevant to Mission Barns' intended use, or are common components found in food (e.g., proteins, polyphenols, and inorganic salts) that are considered by Mission Barns as appropriate for the intended uses. Further, as discussed before, multiple washing steps upon harvest substantially reduce the concentration of all residuals, including any residual cell fattening, coating, or harvest reagents, in the cultivated animal fat cells. Any residual reagents in the harvested cells will not be present at amounts that would pose any human safety concern.

In addition, the chemical identity, detailed regulatory basis of each proprietary component for the cell fattening, culture vessel coating, and harvest reagents is provided in the confidential submission on file with FDA.

Material Sourcing

11. Information Requested

The disclosable safety narrative states that "All the processing reagents used are food grade (when available), high-quality chemical or pharmaceutical grades, or the highest-quality material that is commercially available." For addition to the disclosable safety narrative, please describe the controls, processes, and systems used to address potential hazards associated with impurities in substances that are not food grade, high-quality chemical, or pharmaceutical grades.

Significance

The overall safety conclusion will receive additional support from a discussion of both procedural and specific information on the firm's management of potential hazards associated with substance quality and purity in circumstances where food-grade or other high-quality grade materials are not available.

Mission Barns' Response:

Mission Barns would like to clarify that all the processing reagents used are actually food grade (when available), pharmaceutical grade, or "high-quality chemical," which is equivalent to the term "highest-quality material that is commercially available." For those high-quality chemicals (or highest-quality material that is commercially available) used in Mission Barns' cell manufacturing process that cannot be procured as food grade or pharmaceutical grade, 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, such as impurities, associated with the use of those substances.

Materials Risk Assessment

As a first level of quality control, all raw materials used throughout Mission Barns' cell cultivation process, regardless of material grade, undergo a hazards risk assessment to identify potential biological, chemical and physical safety risks those materials may present. Product specifications are established for each material based on the risks identified and mitigating controls, such as special handling and batch testing, are implemented where appropriate and are required to be reported on a Certificate of Analysis (COA) or equivalent quality document received with each product shipment.

Supplier Approval Program

As another level of control, Mission Barns has implemented a supplier approval program that utilizes a set of procedures and controls for ensuring the safety of all incoming materials entering the manufacturing stream. Depending on the risk level, suppliers are qualified through supplier questionnaires which assess the suppliers' food safety and quality systems, testing plans, quality certifications and registrations; through supplier audits; and/or through testing and sampling plans performed by Mission Barns. Suppliers are monitored on an ongoing basis to ensure compliance through periodic verifications, sampling, testing and/or corrective actions (SCARs).

Material Handling and Positive Release Program

All incoming raw materials and finished products are placed on hold by Quality until they are approved for release following a review of certificates of analysis/certificates of conformance, testing results and/or production records by a Preventive Controls Qualified Individual (PCQI). All non-conforming materials or batches are quarantined and removed from production.

Hormones and Growth Factors

12. Information Requested

On pages 24-25 of the disclosable safety narrative, you state that "The calculated EDIs (i.e., all well below 0.1 µg/day) for the residual hormones are several orders of magnitude lower than the respective safety threshold levels…" For addition to the disclosable safety narrative, please provide some additional discussion of the kinds of studies or data relied on to support this statement about safety threshold levels. We note that relevant toxicological endpoints for hormones are not addressed by the genotoxicity threshold of toxicological concern.

Significance

Where possible, clarifying the nature of the information relied on to reach a safety conclusion provides additional support for the overall disclosable safety narrative.

Mission Barns' Response:

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, ADI) 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 1,000-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 FDA-established tolerance levels in other foods or published reports on the natural-occurrence and concentration of the specific hormones in commonly consumed foods such as cow's milk or fruit.

For the specific hormones that Mission Barns adds as part of the cell culture media, the company has analyzed levels of such hormones in cultivated pork fat cells for three non-consecutive batches and has shown the estimated daily intake (EDI) levels are all several orders of magnitude lower than the respective safe limits. As such, Mission Barns concludes that any residual levels of hormones in the cultivated fat cells do not pose any human safety concern.

The threshold of toxicological concern (TTC) was not applied as the "TTC approach should be used only in cases where the available chemical-specific data are inadequate for normal risk characterization".[6]

13. Information Requested

Page 24 of the disclosable safety narrative and page 44 of the supplementary confidential material state that "Harvested cells are mixed with plant-based ingredients to formulate final products, which are then cooked for at least 4 minutes at a minimum internal temperature of 165 F. Any residual hormones will lose their biological activity after the cooking." For addition to the disclosable safety narrative, please provide references or other data to support or clarify the statement that substances in these general chemical classes are deactivated or degraded by thermal treatment or otherwise clarify this statement.

Significance

The overall public safety conclusion associated with the use of these substances as part of Mission Barns' production process will receive additional support by providing a basis for this element of the weight of evidence.

Mission Barns' Response:

For clarification, while Mission Barns expects cooking/heating will further deactivate or degrade any hormones present in the cultivated pork fat cells, Mission Barns is not relying on the claim that cooking conditions alone may be sufficient to completely deactivate or degrade all hormones. However, it is reported that certain cooking methods can significantly reduce the levels of certain hormones in meat.[7]

As the agency indicates, our safety rationale of residual hormones (as well as other processing aids) is based on a weight-of-evidence approach considering (1) the levels of residual hormones as shown by test data from three non-consecutive, representative batches, (2) the naturally occurring levels of these hormones from common food intake, (3) any safety limits established by regulatory or scientific bodies such as JECFA or FDA, and (4) any additional processing/cooking that may further mitigate potential activity/chemical risk of the processing aids used during the manufacturing process.

14. Information Requested

On page 13 of the disclosable safety narrative, residue testing is described, including residues from cell culture media and the harvest reagent, hormones, and antibiotics; however, results from these analyses are not provided in Table 2 on page 14. For addition to the disclosable safety narrative, please address why these analyses are not included in some form in Table 2 and clarify at what stage in your production process these

[6] Kroes, R., Renwick, A., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., Van Schothorst, F. and Vos, J. (2004) "Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet," *Food and chemical toxicology* 42(1):65-83.

[7] *See e.g.*, Zeitoun, M.M. and Ahmed, S.M. (2011) "Effect of Cooking Method on the Residues of Natural Sex Steroid Hormones in Local and Imported Meats and Meat Products in Al-Qassim Region," *Journal of Agricultural and Veterinary Sciences* 4.2.

analyses are performed and how often they are performed.

Significance

It is helpful to have a clear discussion of the firm's approach to testing and setting specifications for various aspects of the production process, particularly those specific to this kind of food production.

Mission Barns' Response:

Mission Barns' Approach to Setting Specifications

Mission Barns has developed batch release specifications for cultivated pork fat cells after cell harvest to ensure that a safe and suitable product is released for further food processing. Table 2 on page 13 of the disclosable narrative specifies Mission Barns' batch release criteria, and therefore outlines the release testing that is performed on every batch of cultivated pork fat cells. These include heavy metals and common methods for detecting microbial contamination. Further, as discussed in our response to information request #23 below, Mission Barns will also include certain trace metal limits as part of the product specification. Our revised Mission Barns' cultivated pork fat cells batch release criteria are restated below.

Mission Barns' Cultivated Pork Fat Cells Batch Release Criteria			
Test		**Method**	**Specification**
Aerobic Plate Count		APHA CMMEF CHP 8 or equivalent	Negative (< 10 CFU/mL)
Enterobacteriaceae		APHA CMMEF CHP 9 or equivalent	Negative (< 10 CFU/mL)
Coliforms		FDA BAM ONLINE CHP 4 or equivalent	Negative (< 10 CFU/mL)
Mycoplasma		Mycoplasma Genus TaqMan® PCR, or equivalent	Negative
Yeast and Mold		FDA-BAM, 7th ed., AOAC Official Method 2014.05, or equivalent	Negative (< 10 CFU/mL)
Heavy Metals	**Cadmium**	FDA EAM 4.7 or equivalent ICP-MS method.	< 50 ppb
	Lead		< 50 ppb
	Arsenic		< 50 ppb
	Mercury		< 25 ppb
Trace metals (Confidential)		FDA EAM 4.7 or equivalent ICP-MS method	Confidential (all < 0.2 ppm)

Mission Barns' Approach to Residuals Testing

Unlike the batch release criteria discussed above, antibiotics, hormones, and other residuals are not tested for in each manufacturing batch, and therefore not included in Table 2. As part of its overall safety

assessment, Mission Barns has previously tested three non-consecutive batches for antibiotics, hormones, and other residuals after cell harvest to characterize the amount of process residuals that can be expected from manufacturing runs. Mission Barns expects these process residuals to be present (if at all) in the harvested cells at levels that do not pose any human safety concern. Test results have shown that the levels of residual antibiotics from three non-consecutive batches are not detected. The levels of residual hormones from three non-consecutive batches of harvested cells are either not detected or well below their respective safety limits, as discussed further in our response to information request #12.

Instead of testing every batch, Mission Barns will assess the risk of any major manufacturing process change that may result in materially increased levels of process residuals and retest for these residuals as appropriate after cell harvest.

Cell Culture Process

15. Information Requested

For additional to the disclosable safety narrative, please provide additional information about monitoring or testing that is conducted during the cell culture food production process (e.g., culture parameters, growth curves, sampling, visual or microscopic inspection) and the information it provides with respect to microbiological or other aspects of food safety.

Significance

Discussion of any monitoring or testing that is conducted during this key step and the significance of the information it conveys will provide additional support for the overall public safety conclusion.

Mission Barns' Response:

Prior to commencing the manufacturing process, cell banks have already been tested to confirm the absence of any adventitious agents of concern. While all equipment and reagents used during the manufacturing process are sterile and the operations are conducted using aseptic techniques, the possibility of potential microbial contamination including fungal and bacterial contamination from handling or the environment still exists. In the event that cell culture system sterility is compromised during the manufacturing process, Mission Barns expects contamination would be detected via visual inspection (including microscopic inspection) of cultures.

In addition to the information already provided in the disclosable safety narrative, Mission Barns particularly monitors for potential contamination during manufacturing by collecting various liquid samples (*e.g.*, media and wash solutions) in direct contact with the cells at multiple points. These include:

- At each passage during the cell culture seed train
- Daily during cell proliferation
- At the removal of final spent media
- Following introduction of the harvest solution
- After each of the wash steps

Further, as discussed in our response to information request #16 below, if contamination is detected through visual inspection, additional confirmatory microbial testing is performed. Every contamination event is managed and tracked per an incident management system that entails a root cause analysis and appropriate corrective and preventive actions, as required.

16. Information Requested

On page 19 of the disclosable safety narrative, you state “In the event that cell culture system sterility is

compromised during the production process, the introduction of fungal or bacterial contamination would be detected via microscopic visual inspection of cultures." Please describe, for addition to the disclosable safety narrative, the steps carried out if fungal or bacterial contamination is detected using microscopy.

Significance

Discussion of the specific measures that would be applied in the event of detection of a contamination event provides additional support for the firm's overall safety conclusion.

Mission Barns' Response:

If contamination is detected, additional confirmatory microbial testing is performed. Every contamination event is managed and tracked per an incident management system that entails a root cause analysis and appropriate corrective and preventive actions, as required. As an immediate corrective action, any product, equipment, and/or materials that are confirmed to be contaminated are immediately quarantined and removed from the production environment, with appropriate decontamination activities performed afterwards to mitigate the spread of contamination agents.

Product Characterization

Adventitious Agent Hazard Assessment

17. Information Requested

For addition to the disclosable safety narrative, please state what adventitious agent hazards could be associated with use of any animal-derived substances other than the cells themselves in cell culture or cell harvesting and your basis for concluding that any potential risks would be adequately addressed by the controls implemented by the firm.

Significance

Animal-derived substances represent a distinct potential source of adventitious agent contamination. An explanation in the disclosable safety narrative regarding the adequacy of production controls implemented by Mission Barns to address any risk arising such hazards will provide support for the overall public safety conclusion made by the firm.

Mission Barns' Response:

Mission Barns refers the FDA to its response to information request #7 above for the adventitious agent hazards associated with animal-derived substances. All controls mentioned in response #7 also apply to the manufacturing process.

18. Information Requested

On page 19 of the disclosable safety narrative, you state "Out of an abundance of caution, to take potential sterile barrier failures into account during the manufacturing process, and to account for any unusually slow-growing contaminants, sterility testing is also conducted on the cell culture at the end of this process to assess if any fungal or bacterial contaminants are present. Biochemical assays are used to detect microbial contaminants that could be introduced during the culture establishment procedures. A list of such tests can be found in Table 1 above." For addition to the disclosable safety narrative, please specify what specific biochemical assays are used to detect the microorganisms presented in Table 1.

Significance

A description of methods used to generate data that support a safety conclusion provide additional confidence in that conclusion.

Mission Barns' Response:

Mission Barns would like to clarify that the reference to "biochemical assays" as used on page 19 of the disclosable narrative refers to: (1) the PCR assay for detecting mycoplasma in cell banks and (2) the fluorescent antibody testing method (9 CFR 113) used to detect species-specific viruses in cell banks. Both assays are already included in Table 1.

19. Information Requested

On page 6 of the disclosable safety narrative, you state "In comparison to traditional meat processing, Mission Barns' process…eliminates the possibility of exposure to pathogens that are present in an animal's digestive tract and fecal matter which sometimes lead to contamination of meat products during animal slaughter"; however, you do not discuss the possibility of adventitious agents introduced during processing or other handling. Furthermore, you do not provide a robust discussion (with appropriate citations to the publicly available literature) discussing adventitious agents of concern in a conventional comparator, and why they may not be of concern in your production process. For addition to the disclosable safety narrative, please elaborate on your microbiological risk assessment on page 18 in more detail, with appropriate citations, regarding adventitious agents of concern in a conventional comparator, whether they are of concern in your production process, as well as a discussion of adventitious agents they may be introduced during each processing stage (including handling) and how the presence of these adventitious agents is mitigated during your production process.

Significance

An explanation in the disclosable safety narrative regarding the adventitious agents of concern during production, as well as the adequacy of production controls implemented by Mission Barns to address any risk arising such hazards will provide support for the overall public safety conclusion made by the firm.

Mission Barns' Response:

Often, contamination by foodborne pathogens and parasites in meat is attributed to inadequate sanitation or from slaughtering, meat cutting, and meat handling. As noted by Rubio et al.,[8] "[b]y removing animals from the manufacturing process, several externalities can be alleviated." These "externalities" include exposure to and contamination by adventitious agents, which often occurs during slaughter, where hide removal, evisceration, and cutting of the animal carcass presents an opportunity for contamination through exposure to contaminants in the digestive tract and fecal matter. As these carcass processing steps are not present in cultivated meat manufacturing, exposure to these adventitious agents from contaminants in the animals' digestive tract and fecal matter is completely eliminated.

To identify common pathogens of concern in Mission Barns' production process, the company refers to existing scientific references, including USDA's "Fresh Pork from Farm to Table"[9]. It is reported that conventional pork must be adequately cooked to eliminate disease-causing parasites and bacteria that may be present. For example, humans may contract trichinosis (caused by the parasite, *Trichinella spiralis*) by eating undercooked pork. Other foodborne microorganisms that can be found in conventional pork include *Escherichia coli, Salmonella, Staphylococcus aureus, Yersinia enterocolitica* and *Listeria monocytogenes*. These bacteria can infect individuals if they consume raw or undercooked pork, or if they come into contact with contaminated surfaces such as cutting boards, countertops, and utensils. Risks from these adventitious

[8] Rubio, N. R., Xiang, N. and Kaplan, D. L. (2020) "Plant-based and cell-based approaches to meat production," *Nature Communications*, 11(1), pp. 6276.

[9] *Available at* https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/meat/fresh-pork-farm-table

organisms are typically mitigated by proper handling and thorough cooking.

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. As discussed in Mission Barns' responses to information requests #1 and #2 above, during tissue sourcing, if the animal from which the tissue is removed has a microbial infection, the microbes that are present in the biopsied tissue could, in theory, contaminate the cell culture isolated from it. To mitigate this risk, as discussed in more detail in our response to information request #2 above, only healthy animals without any signs of microbial infection are selected for the tissue biopsy. Animal tissues and cells are also handled under hygienic conditions to prevent contamination. Experienced veterinary doctors remove hair from the animal's skin first, apply disinfecting reagents to the region of the animal's skin where an incision is made for the subcutaneous adipose tissue biopsy, and use sterilized instruments to conduct the biopsy.

Mission Barns' approach to adventitious agents during the cell banking stage is further discussed in detail in its response to information request #1.

With respect to the manufacturing process, although all cell banks are verified to be free of adventitious agents of concern, process equipment and reagents used are sterile and aseptic techniques are employed, personal protective equipment is worn, and manufacturing activities are carried out in a clean room environment, there is still a potential risk of contamination from environmental or human sources. As such, cell cultures are monitored on a regular basis for potential contamination. In the event that cell culture sterility is compromised during the production process, any fungal or bacterial contamination would be readily detected through microscopic visual examination as discussed in Mission Barns' response to information request #15 above. 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.

20. Information Requested

Table 2 on pages 19-20 of the disclosable safety narrative indicates that sterility testing is conducted at the harvest stage. Given your discussion of USP 71 elsewhere in the narrative, we again note that, "These Pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedures. *The test is applied to substances, preparations, or articles which, according to the Pharmacopeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined under the conditions of the test*." Further, you do not specify the media used for this analysis, nor the incubation parameters (i.e., time and temperature). Not all bacteria and fungi optimally utilize the same media, nor do they maintain the same growth parameters. Additionally, this is not a common method used for products destined for the human food supply. Common analyses, such as aerobic plate count, yeast and mold, coliforms, *Enterobacteriaceae*, or more specific analyses for foodborne pathogens, such as *Salmonella* serovars, are not provided. For addition to the disclosable safety narrative, please provide additional discussion regarding your rationale for the suitability and adequacy of USP 71 (including the media used, and the growth parameters). Alternatively, we strongly recommend that you consider expanding your testing strategy to encompass validated microbial detection methods for use in human food.

Significance

Given the novelty of this test for food safety applications, further consideration and discussion regarding the suitability and adequacy of the method will provide additional support for the overall public safety conclusion made by the firm.

Mission Barns' Response:

In accordance with FDA's recommendation as specified above, Mission Barns has amended its testing plan for cultivated pork fat cells batch release testing to replace the USP 71 sterility test with microbial detection methods commonly used for human food products. Mission Barns' updated cultivated pork fat cells batch release microbial testing plan has been included below for reference:

Cultivated Pork Fat Cells Bacterial and Fungal Testing Plan			
Test	**Method**	**Sample Size**	**Specification**
Aerobic Plate Count	APHA CMMEF CHP 8 or equivalent	25mL of spent media	Negative (< 10 CFU/mL)
Enterobacteriaceae	APHA CMMEF CHP 9 or equivalent	25mL of spent media	Negative (< 10 CFU/mL)
Coliforms	FDA BAM ONLINE CHP 4 or equivalent	25mL 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	50mL of spent media	Negative (< 10 CFU/mL)

Composition

21. Information Requested

Table 2 on page 14 of the disclosable safety narrative provides specification and the results of three batch analyses for the harvested cellular material. These analyses include *Mycoplasma* spp., toxic heavy metal analyses, and sterility testing, but do not include parameters regarding the identity of the harvested cellular material, such as fat content. On pages 14 and 15 of the disclosable safety narrative you state "Mission Barns has analyzed the fatty acid profiles of the cultivated pork fat cells from three non-consecutive batches, and find them to be consistent with those reported in conventional pork fat." For addition to the disclosable safety narrative, please provide product specifications and the results from three batches (preferably non-consecutive) characterizing the harvested cellular material.

Significance

To clearly establish the identity of the process and product evaluated in CCC 000008, it is helpful to provide a variety of parameters and attributes, including representative compositional data on the product at harvest.

Mission Barns' Response:

The fatty acid profiles of cultivated pork fat cells from three non-consecutive batches were determined via a gas chromatography method per AOCS CE 1F-96. The mass fractions of fatty acids commonly present in

conventional pork, including palmitic acid, stearic acid, oleic acid, and linoleic acid, were identified and quantified in all three batches as tabulated below together with reported comparator ranges for conventional pork (e.g., back fat, belly, etc.) in peer-reviewed public literature.

Mass fractions of common fatty acids from three representative batches (AOCS CE 1F-96)				
Batch #	**Palmitic acid** [16:0]	**Stearic acid** [18:0]	**Oleic acid** [18:1 cis]	**Linoleic acid** [18:2 cis]
1	14.7%	11.2%	48.1%	3.5%
2	13.8%	10.1%	46.8%	3.4%
3	11.7%	10.5%	48.5%	4.3%
Conventional pork (ranges)	4.79%[10] - 25.11%[11]	1.90%[12] - 13.42%[13]	6.22%[14] - 45.4%[15]	3.55%[16] - 30.73%[17]

As the table above indicates, Mission Barns finds the common fatty acid ratios to be consistent with those reported in conventional pork.

22. Information Requested

The disclosable safety narrative provides specifications for heavy metals in the harvested cell product. For cadmium, lead and arsenic, the specification provided was 10 times higher than the level reported in the batch analyses. For mercury, the specification was 5 times higher than the level reported in the batch analyses. To the extent feasible given your production process, consider providing revised specifications that more closely reflect your analytical results.

Significance

Use of the lowest reasonably achievable specifications for contaminants in foods supports FDA's overall public health mission.

Mission Barns' Response:

Mission Barns has considered the FDA's recommendation of lowering heavy metals specifications to more closely reflect the analytical results for our harvested cell product and agrees to lower our specifications for cadmium, lead, arsenic to be less than 50 ppb, and mercury to be less than 25 ppb.

[10] Jang, H.-L., Park, S.-Y., Lee, J.-H., Hwang, M.-J., Choi, Y., Kim, S.-N., Kim, J.-H., Hwang, J., Seo, D. and Nam, J.-S. (2017) "Comparison of fat content and fatty acid composition in different parts of Korean beef and pork," *Journal of the Korean Society of Food Science and Nutrition*, 46(6):703-712.

[11] Nevrkla, P., Kapelański, W., Václavková, E., Hadaš, Z., Cebulska, A. and Horký, P. (2017) "Meat quality and fatty acid profile of pork and backfat from an indigenous breed and a commercial hybrid of pigs," *Annals of Animal Science*, 17(4):1215.

[12] Jang et al. (2017).

[13] Grela, E., Kowalczuk-Vasilev, E. and Klebaniuk, R. (2013) "Performance, pork quality and fatty acid composition of entire males, surgically castrated or immunocastrated males, and female pigs reared under organic system," *Polish Journal of Veterinary Sciences*.

[14] Jang et al. (2017).

[15] Maw, S. J., Fowler, V. R., Hamilton, M. and Petchey, A. M. (2003) "Physical characteristics of pig fat and their relation to fatty acid composition," *Meat Science*, 63(2):185-190.

[16] Jang et al. (2017).

[17] Alencar, S. A. d. S., Kiefer, C., Nascimento, K. M. R. d. S., Viana, L. H., Corassa, A., Gomes, M. d. N. B., Marçal, D. A. and Farias, T. V. A. (2021) "Dietary soybean oil modulates fatty acid composition of pork," *Tropical Animal Health and Production*, 53(3):357.

23. Information Requested

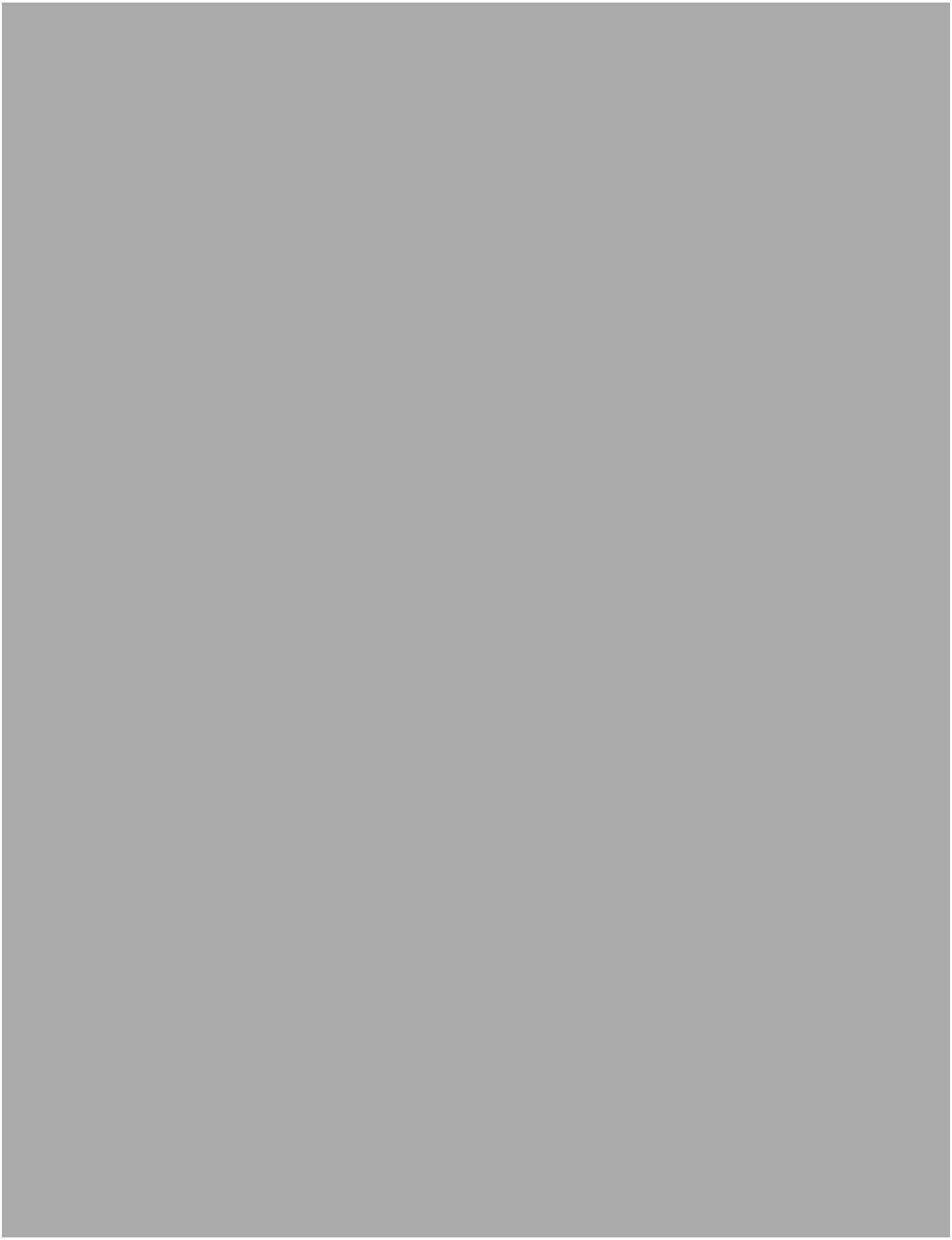
The supplementary confidential material discusses the use of certain metal salts during production. We request that you consider, for addition to the disclosable safety narrative, a specification for your harvested cell material that is based on or related to the firm's use of these substances during production.

Significance

In the context of safety assessments directly informed by cumulative exposure, specifications may provide useful additional support.

Mission Barns' Response:

A number of micronutrients, including salts of certain trace metals, are essential for cell growth and replication. In accordance with FDA's recommendation, Mission Barns has established additional specifications for certain trace metal salts used during production (*i.e.*, all limits < 0.2 ppm). Mission Barns notes all these 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.



24. Information Requested

Page 13 and page 21 of the disclosable safety narrative discuss quantification of a specific protein in a final wash solution as a proxy for residual presence of all protein-based ingredients in the harvested cell material. Please provide some additional discussion of any potential limitations of this approach and your basis for concluding that it is an appropriate proxy, which may include reference to the observations regarding direct testing discussed on page 25.

Significance

The final wash measurements are an important element of the overall exposure model, and discussion of your rationale for selecting this approach will provide additional support for the overall safety conclusion.

Mission Barns' Response:

Mission Barns notes the specific protein discussed on page 13 and page 21 of the disclosable safety narrative was selected because: (1) it was present in the cell culture media at relatively high concentrations; (2) an established Enzyme-Linked Immunosorbent Assay (ELISA) that is highly sensitive for the surrogate enables detection at very low levels of surrogate after multiple washing steps; and, (3) the specific protein is not naturally produced in cultivated pork fat cells. These three factors make the protein a suitable proxy for measuring a large range of dilution factors.

Mission Barns acknowledges that given the differences in protein concentrations and protein molecular structures, there are inherent limitations in using a representative protein at relatively high concentrations as a proxy for the residual presence of all protein-based ingredients. Mission Barns noted the adopted dilution factor of 10^{-5} is based on the actual dilution factors we have calculated for this surrogate protein molecule ranging from 2×10^{-6} to 1.06×10^{-5}. To account for the potential limitations, and to add another layer of conservativeness, Mission Barns intentionally chose the highest dilution factor, 10^{-5}, calculated from three test results. Indeed, in light of the extensive washing, Mission Barns does not expect any residual proteins, other than the specific surrogate protein, to be detected. As discussed on Page 25 of the disclosable safety narrative, when Mission Barns tested for the residual concentrations of growth factors (which are also protein-based ingredients in cell culture media) in final wash solutions of three non-consecutive batches, none were detected.

25. Information Requested

On page 13 of the disclosable safety narrative, you state “Hormone analytical testing was performed on the harvested and washed cell pellet by ELISA assays for specific hormones to confirm that the concentrations of such components present in the cultivated fat cells are within safe limits.” For addition to the disclosable safety narrative, please describe what is meant by “… within safe limits.”

Significance

Clarification of the underlying basis for a statement related to safety provides additional support for the firm's overall safety conclusion.

Mission Barns' Response:

Please see Mission Barns' response to information request #12 above.

Points of Clarification

26. Information Requested

Please provide a statement that there is an allergen control program in the facility to address potential cross-contamination issues and provide a brief description of the program.

Mission Barns' Response:

Mission Barns confirms there is an allergen control program in the facility to address potential cross-contamination issues.

Mission Barns' allergen control program includes measures to control allergens throughout the process stream – from incoming materials to final packaged products. Examples of these measures include:

- Identification and risk assessment of allergenic hazards for all incoming raw materials/ingredients.
- Controls over the storage, handling and processing of materials containing any allergens of concern.
- Cleaning of process equipment to prevent cross-contamination of products.
- Documentation and accurate labeling of finished goods.

27. Information Requested

The disclosable safety narrative states on page 18 that all processing reagents used are either sterile when provided by the suppliers or sterilized by Mission Barns on-site by heat or other appropriate means depending on material stability. Please provide additional information on what kind of heat process would be used.

Mission Barns' Response:

Currently, all processing reagents used in Mission Barns' production process are either sterile when provided by the suppliers or sterilized using membrane filtration by Mission Barns. Mission Barns would like to clarify it does not currently use heat for sterilization of processing reagents; it currently uses heat only for the sterilization of equipment.

28. Information Requested

On page 25 of the disclosable safety narrative, you state that "the calculated worst-case EDI of 0.0167 µg/day is several orders of magnitude lower than the respective safety threshold levels of the antibiotics." Please clarify what you mean by "respective safety threshold levels of the antibiotics" (i.e., whether these NOAEL values are from chronic studies, subchronic studies, TTC for genetic toxicity, or some other values).

Mission Barns' Response:

When establishing the appropriate safety threshold levels for antibiotics, Mission Barns takes into consideration established safe levels (e.g., acceptable daily intake, ADI) derived from a relevant authoritative body (e.g., U.S. FDA, Joint FAO/WHO Expert Committee on Food Additives, or EPA). If comparisons of anticipated dietary intakes relative to an authoritative reference intake value are not readily available, Mission Barns takes into consideration the published no-observed-adverse-effect levels (NOAELs) from animal

chronic and short-term toxicology studies to evaluate food safety risks. For a given antibiotic, a margin of exposure (MOE) of 100-fold or greater between the antibiotic's NOAEL and its estimated dietary intake is typically considered adequate for food safety. For all antibiotics with established NOAELs, the MOEs are well over 1,000-fold.

For the specific antibiotics Mission Barns uses as part of the cell banking process, the company has analyzed two representative antibiotics in three non-consecutive, representative batches and found them to be not detected. As such, Mission Barns has adopted a theoretical EDI of 0.0167 µg/day, which is several orders of magnitude lower than the respective safety threshold levels for these antibiotics. As discussed in more detail in the supplementary confidential material, the safety thresholds are established based on available data on allergic reactions, the NOAEL determined from a 2-year animal feeding study, or the NOAEL from a 28-day animal feeding study.

Based on this finding, Mission Barns concludes that any residual levels (if any) of antibiotics in its cultivated fat cells do not pose any human safety concern.

29. Information Requested

The disclosable narrative includes estimates of exposure. Please confirm for addition to the disclosable record that exposure analysis is based on an 'eaters only' estimate rather than a per capita estimate.

Mission Barns' Response:

Mission Barns confirms that our exposure analysis is based on an "eater only" estimate, rather than a per capita estimate.

30. Information Requested

The disclosable safety narrative states that there are no physical hazards such as the potential for "hard/sharp" physical hazards or "choking" hazards in the process. For addition to the disclosable narrative, please address why physical hazards such as foreign objects from employees or from environment will not be present in your process by adding brief discussion of the food safety management system to mitigate this risk in the facility.

Mission Barns' Response:

As part of the company's food safety and quality system, Mission Barns has implemented a foreign materials management program that is designed to prevent the introduction of physical hazards, such as glass and brittle plastic, into products. Mission Barns' team of Preventive Controls Qualified Individuals (PCQIs) has assessed the risk from a process and process equipment standpoint to be low. Other sources of physical hazards potentially introduced into product material by personnel or from the operating environment have also been considered.

As a control measure to prevent foreign material contamination from personnel, all persons entering the manufacturing environment are required to wear personal protective equipment (PPE) that includes sanitary clean room gowns, hairnets, gloves, protective eyewear, face/beard masks and shoe covers prior to entering the GMP manufacturing area. Personal items such as headphones/earbuds and jewelry such as earrings, finger rings, and necklaces are not permitted in and must be removed prior to entering the manufacturing area. Cell phones must be placed in and contained within a sanitary protective bag. Only personnel with proper PPE and aseptic technique training are permitted in the production environment.

Regular GMP audits of the manufacturing floor, environment and facility grounds are performed by PCQIs and include inspections of all equipment, components, materials, packaging, furniture, walls, floors, ceiling, light fixtures, piping and drains for damage or wear that could potentially lead to physical hazards being introduced into product materials. Any items of concern that are identified during an audit are documented and

addressed per the company's Corrective Action and Preventive Action process.

31. Information Requested

In the supplementary confidential material, there is some ambiguity about analytical method being used for detection of metals. Please clarify for addition to the disclosable safety narrative which method is used.

Mission Barns' Response:

For all metals testing Mission Barns uses either FDA EAM 4.7 method or equivalent ICP-MS method.

32. Information Requested

Figure 1 on page 4 of the disclosable safety narrative includes the following disclaimer, "Contains confidential business and proprietary information." For addition to the disclosable safety narrative, please provide a statement clarifying this discrepancy.

Mission Barns' Response:

The inclusion of the disclaimer, "Contains confidential business and proprietary information" on Figure 1 on page 4 of the disclosable safety narrative was included in error. Figure 1, as it stands on page 4, does not contain any confidential business or proprietary information.

33. Information Requested

For addition to the disclosable safety narrative, please provide an explicit statement that you will only use food contact materials which are authorized for their intended use.

Mission Barns' Response:

Mission Barns will only use food contact materials which are authorized for their intended use under the laws and regulations administered by FDA.

34. Information Requested

On page 6 of the disclosable safety narrative, you state "For sourcing some of Mission Barns' cell cultures, tissue is biopsied from a domestic pig in a veterinary operating chamber by trained veterinary doctors." For addition to the disclosable safety narrative, please clarify what is meant by "some" in this context.

Mission Barns' Response:

To clarify, all cell lines that are used for cultivated pork fat production are isolated under the conditions specified on page 6 of the disclosable safety narrative.