FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

Memorandum

Date November 14, 2022

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Through

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Subject Cell Culture Consultation (CCC) 000002, Cultured *Gallus gallus* cell material

To Administrative File, CCC 000002

Submission Received Date October 1, 2021

Amendments Received Date: March 30, 2022; September 16, 2022; September 30, 2022; November 9, 2022

Sponsor: UPSIDE Foods, Inc. (UPSIDE, the firm)

Summary

- The Food and Drug Administration (FDA, we) evaluated the food that is the subject of CCC 000002 submitted by UPSIDE.
- This food is defined as the cell material at harvest, comprised of cultured *Gallus gallus* cells, with characteristics of myocytes and fibroblasts, in the form of sheets of cells, as produced by the method of manufacture described in CCC 000002.
- The cell lines are originally isolated from either adult chickens or mid-stage fertilized chicken eggs. The isolated cell lines are phenotypically characterized using standard methods validated for their intended purpose, including microscopy and immunostaining.
- The cell lines are established by selection of myoblast and fibroblast cells with demonstrated differentiation capacity that are then adapted to suspension culture. Immortalization is achieved either through selection in culture or through introduction of a cisgene expressing chicken telomerase reverse transcriptase (TERT).

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- The cells are cultured by first increasing total cell numbers in a suspension culture proliferation phase, followed by a subsequent adherent differentiation phase in which the cells are induced to assume characteristics of muscle cells supported by both specific medium factors as well as surface contact.
- The cells are harvested in the form of sheets of cells washed from the surface of the culture vessel into a collection basin, followed by subsequent washing and moisture adjustment in a temperature-controlled environment.
- The harvested material, following washing, is described as a coherent tissue of chicken (*Gallus gallus*) cells, similar in composition and nutritional characteristics to conventional poultry products.[1] Microbial and toxic heavy metal specifications are provided. Species identity and cell identity were verified in the final product using an enzyme-linked immunosorbent assay (ELISA).
- We evaluated information about the cell lines, the production process (including cell bank establishment), substances used in the production process, and properties of the harvested cell material, including information available in both the safety assessment as well as supporting, corroborative information in a confidential supplementary appendix.
- Based on the data and information presented in CCC 000002, we have no questions at this time about UPSIDE's conclusion that foods comprised of or containing cultured chicken cell material resulting from the production process defined in CCC 000002 are as safe as comparable foods[2] produced by other methods. Furthermore, at this time we have not identified any information indicating that the production process as described in CCC 000002 would be expected to result in food that bears or contains any substance or microorganism that would adulterate the food.[3]

Production Method

UPSIDE describes an overall production process involving the establishment of a cell bank that provides a standardized source of cells for food production, and a cell culture food production process including proliferation or multiplication of the cells, differentiation of the cells to

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[1] UPSIDE provided some analytical data on the composition of the harvested cell material from several production runs as one element in characterizing the identity of their product, in order to demonstrate the capability to meet or exceed specifications for food contaminants, and to demonstrate the consistency of their production process. Some variations in mineral, metal content, and somewhat higher levels of folate and cholesterol were observed in the data from these batch analyses of the harvested cell material, relative to a conventional poultry product. In all cases levels were within the range of those found in commonly consumed foods. UPSIDE's conclusions regarding the safety of their harvested cell material are not based on the establishment of exact equivalence of all nutrients and components relative to a particular conventional comparator.

[2] UPSIDE identifies "conventional poultry meat from a chicken carcass" as a comparator. In the firm's submission, skinless chicken breast is the most common comparator. Some analyses also include other chicken components.

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

acquire characteristics of muscle, and harvest or collection of the cell material for subsequent conventional food processing.

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

- A current good manufacturing practice (cGMP) program that includes all the items enumerated in 21 CFR 117 subpart B;
- Validated sanitation processes and an environmental monitoring program;
- A supplier approval program;
- Document and records control including material and product specifications;
- Controls for prevention of biological, chemical, and physical hazards;
- A product release system involving quality assurance review for incoming raw materials, intermediate products, and finished products;
- Batch record review; and
- Traceability of raw materials and finished products.

UPSIDE also notes the importance of use of supporting programs such as internal auditing, risk-based sampling of raw materials and products, and sanitary design of equipment and tools to ensure accessibility for clearing and minimization of harborage areas. Crisis Management with Product Recall, Food Defense & Food Fraud programs, and Employee Training programs are also included.

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

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

Process Step	Potential Issues	Management Strategies
Cell Isolation	Cell identity; contaminants from source, reagents, or environment	Antibiotics, aseptic procedures, documentation, sterilization, supplier management, testing program
Establishment of Cell Lines	Cell identity; contaminants from materials or environment; appropriate adaptation to culture; introduced genetic material and expression product	Aseptic procedures, documentation, genetic sequencing, food safety assessment[4], process and environmental monitoring, sterilization, supplier management, testing program
Establishment of Master Cell Banks (MCB)	Cell identity; contaminants from materials or environment; appropriate adaptation to culture	Aseptic procedures, documentation process and environmental monitoring, sterilization, supplier management, testing program
Proliferation Phase	Contaminants from materials, equipment, or environment; media components	Aseptic procedures, documentation, food safety assessment, process and environmental monitoring, sterilization, supplier management, testing program
Differentiation Phase	Contaminants from materials, equipment, or environment; media components	Aseptic procedures, documentation, food safety assessment, process and environmental monitoring, sterilization, supplier management testing program
Harvest of Cell Material	Contaminants from materials, equipment, or environment; media components	Compositional analysis, controlled temperature conditions, food safety assessment, specifications, sterilization, supplier management, testing program, washing

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

Cell Banking

UPSIDE provides information about the establishment of cell banks used in the subsequent cell culture food production process. UPSIDE defines a cell bank in the firm's manufacturing process as a collection of cryopreserved cells derived from a single tissue source in a single animal. UPSIDE uses a common system in which there is both a primary cell bank (the master cell bank; MCB) and secondary cell banks (the manufacturing working cell bank; MWCB) each derived from a subset of cells stored in the MCB. The steps involved include:

- Cell isolation
- Establishment of cell lines
- Establishment of MCBs
- Establishment of one or more MWCBs

Cell Isolation

The cells used to establish the cell banks are isolated either from adult chickens destined for human consumption (a myoblast cell line derived from muscle tissue) or from mid-stage fertilized chicken eggs (a fibroblast-like cell line derived from skin tissue). Hereafter, the two sources are referred to collectively as the "source animal" or "animal source." Reagents used at this stage may include materials of bovine or porcine origin (serum and trypsin, respectively), in addition to cell culture media, media components, and antibiotics and antimycotics.

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

- Incorrect source animal identity resulting in isolated cells of incorrect origin;
- Source animal health prior to tissue procurement resulting in cells contaminated by adventitious agents such as bacteria or viruses;
- Introduction of adventitious agents from contaminated non-animal sourced reagents or the local environment, including *Mycoplasma* spp., *Listeria monocytogenes*, *Salmonella* serovars, pathogenic *Escherichia coli*, *Enterobacteriaceae*, and *Campylobacter* spp.; and
- Introduction of adventitious agents from animal-derived reagents (e.g., bovine serum).

UPSIDE documents all processing steps from animal sourcing to cell isolation. Animal source documentation includes records identifying time and place of harvest, and any relevant inspection of the animal ante- or post-mortem. Records for each cell line include animal source documentation, methods used for originating tissue isolation, subculturing history, and substances used, including cell culture media or culture substrate. Bovine sera are verified to be sourced from bovine spongiform encephalopathy (BSE)-free/risk-negligible herds. Animal-derived raw materials are tested for species-specific adventitious agents. If trypsin is sourced from porcine origin, UPSIDE tests for porcine viruses that could survive in human cells. Antibiotics and antimycotics are used to support establishment of sterile culture conditions for subsequent steps in development of the cell bank.

Establishment of Cell Lines

UPSIDE screens the isolated cells to select for, or induce, individual cells that have desired characteristics, including the ability to exhibit a stable phenotype with repeated, linear growth (cell immortalization), the ability to acquire characteristics of muscle cells (myoblasts) or connective tissue cells (fibroblasts) through differentiation, the ability to grow in suspension within the cell culture media without the need for attachment to surfaces ("suspension adaptation"), and following suspension adaptation, verification that the cells can grow on solid substrates and produce an intact/integral cell culture product.

The cell lines described in CCC 000002 exhibit cell immortalization due either to spontaneous immortalization through selection in culture or due to induced immortalization through the introduction of a constitutively expressed TERT protein. Suspension adaptation is achieved using standard methods of media support and selection. Reagents used at this stage may include materials of bovine or porcine origin (bovine serum and trypsin, respectively), in addition to cell culture media, media components, and antibiotics and antimycotics.

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

- Introduction of adventitious agents from contaminated non-animal sourced reagents or the local environment, including *Mycoplasma* spp., *L. monocytogenes*, *Salmonella* serovars, pathogenic *E. coli*, *Enterobacteriaceae*, and *Campylobacter* spp.;
- Introduction of adventitious agents from animal-derived reagents (e.g., bovine serum);
- Phenotypic changes or loss of stability in cell lines;
- Residual bacterial selection genes not removed at completion of genetic engineering process; and
- Unintended effects of genetic engineering and/or adaptation to culture (pleiotropy and off-target effects).

Animal-derived raw materials are tested for species-specific adventitious agents as well as environmental adventitious agents that may have been introduced during cell culture. Bovine sera are verified to be sourced from BSE-free/risk-negligible herds. If trypsin is sourced from porcine origin, UPSIDE tests for porcine viruses that could survive in human cells. UPSIDE monitors viable cell density and cell viability (percent viability) as the cells adapt to suspension cell culture. Adapted cell lines are documented, expanded, and cryopreserved before progressing to the next stage in the manufacturing process.

UPSIDE states that cell lines under development are tested for adventitious agents including *Mycoplasma* spp., *L. monocytogenes*, *Salmonella* serovars, *E. coli*, *Enterobacteriaceae*, and *Campylobacter* spp. UPSIDE also states that cell lines are tested for sterility, including aerobic plate count, as well as yeast and mold.

To verify successful genetic integration and stability of the inserted TERT cisgene, UPSIDE determines the insertion location in the genome, the number of copies present, and the absence of extraneous DNA (including antibiotic resistance genes and other vector backbone

components) through polymerase chain reaction (PCR)-based methods. UPSIDE also confirms the desired phenotype resulting from the expression product of the inserted gene and its stability by monitoring growth and viability of immortalized cell lines.

UPSIDE discusses the TERT cisgene expressed in one of the cell lines that is the subject of CCC 000002. The protein expressed by this gene is a component of the telomere enzymatic complex,[5] which is responsible for the maintenance and lengthening of telomeres. The gene is already present in the chicken genome and is expressed in some chicken cells, including muscle cells. [6] UPSIDE uses constitutive expression of this protein to enable immortalization of the firm's cell lines in culture. UPSIDE states that TERT protein activity is subject to the constraints of normal cellular control mechanisms, and that cells expressing the introduced TERT protein exhibit similar growth requirements, cell-cycle checkpoints, and karyotypic stability to comparator cells.

UPSIDE discusses the features of immortalization generally and as they relate to the cell lines described in CCC 000002. UPSIDE notes that there are multiple ways to induce high levels of cell replication, including the methods described above used by the firm as well as a method historically used in research to study unregulated cell proliferation, known as transformation. UPSIDE describes cell transformation as an aberrant cellular state in which cells gain an unrestricted growth ability and resistant to normal cellular process to regulate cell replication in a local physiological context. UPSIDE contrasts cell transformation with the firm's immortalization strategies which they state do not result in features of transformation such as unregulated growth. UPSIDE notes that even transformed cells would not be expected to pose a food safety hazard[7]; however, the firm, as a quality control measure, nevertheless monitors

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[5] The telomere enzymatic complex is present in most eukaryotes including common agricultural species such as chicken.

[6] The TERT gene is present in most eukaryotes. Highest expression and activity are typically observed in replicating cells such as germ cells and embryonic cells. Expression in differentiated cells and tissues is rarer and less consistent, but does occur, as in the muscle cell example cited by UPSIDE.

[7] A hypothetical hazard could be associated in some way with the consumption of cells with enhanced proliferative capacity. However, once removed from the protected and controlled environment of the bioreactor, the cells would be expected to lose their proliferative capacity. Subsequent food processing (such as cooking) would further break down cellular structures and contents. Digestion after consuming food made from this cell material would also break down any residual cellular structure. FDA notes that dysregulation of telomerase activity in human cells has been observed as an aspect of the process of tumorigenesis. However, any residual TERT protein present in food would be rendered nonfunctional by heat processing (cooking) and subsequent gastrointestinal digestion by the consumer, and thus would be incapable of exhibiting its expected physiological function in a telomere enzymatic complex. Further, the food will be in contact with the gut lumen and any residual TERT protein present in the food matrix would not be in the correct biological context (embedded in a complex in the nuclear compartment) to influence proliferative activity even in a hypothetical scenario where it was not rendered non-functional by heat and digestion.

its immortalized cultures for two parameters (response to cues for differentiation as well as expected rates of growth) to detect any spontaneous transformation events.

UPSIDE discusses the potential for genetic engineering to result in unintended effects on the phenotype of the cell, and notes that naturally occurring transgenic events (e.g., via endogenous retroviral fragments) take place routinely in poultry without noticeable adverse effects[8]. UPSIDE also notes that while plants and microorganisms rely on toxin production for defense, this is not a common strategy for animals. Thus, animals traditionally consumed as food do not have a latent genetic capacity to produce endogenous toxins.

Establishment of MCB

UPSIDE states that individual cell lines displaying the desired properties described in the previous section are prepared for storage in an MCB, which is defined by UPSIDE as a collection of cryopreserved cells derived from a single tissue source from a single animal.

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

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

UPSIDE confirms the species identity of the cell lines in the MCB by PCR analysis of mitochondrial DNA for cytochrome c oxidase. UPSIDE also measures parameters related to cell growth to confirm stability of the immortalized phenotype. Finally, using a direct aliquot of the primary cell bank, UPSIDE tests for the presence of aerobic bacteria (i.e., aerobic plate count), yeast and mold, *Mycoplasma* spp., *Enterobacteriaceae*[9], *E. coli*, *Campylobacter* spp., *Salmonella* serovars, and *L. monocytogenes*, as well as bovine, porcine, and avian viruses identified by the firm as of potential concern (discussed further below). UPSIDE notes that human pathogens of clinical importance associated with conventional chicken include *E. coli*, *Campylobacter* spp., *Salmonella* serovars, and *L. monocytogenes*. Since specifications for these major pathogens are limited to "non-detect" results, UPSIDE does not further evaluate

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[8] UPSIDE states, for example, that integrated exogenous DNA in the form of retroviral fragments in the chicken genome are routinely passed vertically from one generation to another and that they are endemic in poultry consumed as food, without known pleiotropic or other deleterious effects that would indicate a food safety concern.

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

for specific pathogen serovars (e.g., *E. coli* O157:H7). UPSIDE also screens for additional adventitious agents and for sterility for purposes of quality and safety. UPSIDE discusses details of testing, which is conducted using validated methods by a third-party laboratory using either observation of potential microbial growth under permissive conditions (for aerobic plate count, yeast, and mold) or real-time PCR analysis (for *Mycoplasma* spp., *Enterobacteriaceae*, *E. coli*, *Campylobacter* spp., *Salmonella* serovars, *L. monocytogenes*, and certain viruses).

UPSIDE describes an adventitious agent testing plan that includes a component specifically addressing potential viral contamination. The firm notes that current food safety practices for the production and harvest of conventional meat products have proven sufficient to mitigate risks associated with transmission of zoonotic diseases from animal tissues to consumers, and viruses that may be endemic within animal populations consumed as food are typically innocuous to humans. UPSIDE also notes that lytic or latent viral infections in cultured cells would negatively affect the production capacity of the cell culture system, trigger quality control checks, and are thus inherently self-limiting. Notwithstanding these considerations, UPSIDE seeks to manage any potential viral risk both through selecting low-risk substances for use in the production process and through testing during production (including cell line development, cell bank establishment, and final harvest).

UPSIDE describes the design of a viral testing panel based on both the species which the virus can infect as well as the potential for exposure to viral agents. Here, UPSIDE focuses on testing for avian viruses that can cross the avian-human species barrier as well as certain mammalian viruses that are capable of infecting avian cells. Viruses in the second category are identified based on the animal-derived substances from other mammalian species that are used at any stage of production (e.g., porcine trypsin). UPSIDE states that this testing occurs both prior to cell bank establishment and during production.

Establishment of the MWCB

UPSIDE states that each MWCB is established directly from one or more aliquots of the MCB which are expanded by serial subculture. UPSIDE does not reevaluate the MWCB for adventitious agents prior to qualification given that it is derived from the MCB and that the production process is a highly controlled, aseptic process. However, for quality purposes, the MWCB is tested for sterility and *Mycoplasma* spp. prior to banking since a production run will fail if the starting seed cells are not sterile. UPSIDE describes the records that will document the creation of each MWCB, including information about the MCB aliquots used and testing results.

Cell Culture Food Production Process

UPSIDE provides information about its cell culture food production process, including:

- The proliferation phase using suspension culture;
- The differentiation and maturation phase using adherent culture; and
- Cell harvest.

UPSIDE states that the firm's food safety and quality systems are based on the requirements of 21 CFR part 117 and Global Food Safety Initiative-benchmarked standards, including the establishment of a facility food safety plan. Batch records will be maintained to provide traceability of all raw materials used, operations, and testing during the production process. UPSIDE also states that all incoming dry powdered culture media as well as raw materials used for culture media undergo testing and verification appropriate to the specific media component and are stored under appropriate conditions. Liquid media is sterilized with an appropriate filter and stored at 2-8 °C. UPSIDE states that the firm uses appropriate and authorized food contact materials throughout the production process.[10]

UPSIDE states that the production process is a highly controlled, aseptic process. The production process is monitored to support cell growth via a control system that supplies media that (1) delivers nutrients, (2) removes cell waste, and (3) facilitates gas exchange. UPSIDE explains that the controlled system and environment are similar to industrial food culture fermentation systems. Sterile procedures are used at all stages of cell culture. UPSIDE states that, for smaller-scale culture vessels, any cell passages or sampling are conducted in high-efficiency particulate air-filtered biological safety cabinets by operators trained on appropriate aseptic practices. Cultures in larger suspension bioreactors use aseptic procedures to reduce the risk of introducing contaminants, and stainless-steel culture vessels and transfer lines are sterilized using high temperature steam. UPSIDE states that cultures are periodically sampled and tested using a qualified assay to detect any contamination by adventitious agents. UPSIDE also states that an environmental monitoring program is in place to assess the effectiveness of overall hygienic practices in the manufacturing facility.

Proliferation Phase Using Suspension Culture

One or more vials of frozen cells from the MWCB are thawed and placed in sterile culture medium in a small vessel under sterile conditions. Following multiple rounds of cell division and growth (proliferation), the cells are transferred to a larger container (passaging). This process, under continued sterile conditions, is repeated with increasingly larger vessels to accumulate the desired quantity of cells (i.e., a seed train). The seed train cultures are agitated so that the cells are suspended in a homogeneous mixture within the liquid culture medium inside the vessel. UPSIDE states that all vessels are either pre-sterilized single-use, food grade plastic, or reusable vessels that can be cleaned and sterilized between uses.

UPSIDE identifies potential hazards associated with this production stage including:

- Introduction of adventitious agents via contaminated culture media components or inadequate sterilization of bioreactors;

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

- Introduction of adventitious agents present in the local environment of the production facility during passaging from one vessel to another;
- Introduction of media components that could persist in the cell material through proliferation, differentiation, and washing at harvest, thus being present as residues in the harvested cell material; and
- Direct bioaccumulation of media components in the cell material at later stages of proliferation.

UPSIDE manages risk associated with the first two hazards through the sterile procedures and monitoring programs discussed at the beginning of the section "Cell Culture Food Production Process." Safety considerations associated with the last two hazards are discussed in the subsequent section, "Substances Used in Cell Culture Food Production Process."

Differentiation and Maturation Using Adherent Culture

Undifferentiated cells from the seed train are passaged to a new culture vessel where they are encouraged to differentiate and assume characteristics of muscle cells (myocytes) and connective cells (fibrocytes) through adherence to the surface of the culture vessel and through introduction of additional medium components. UPSIDE notes that some continued cell division as well as increased individual cell mass (hypertrophy) and cell differentiation are expected during this phase. UPSIDE also states that during this phase the surface-adherent cells also adhere to each other to form a multicellular tissue.

UPSIDE identifies potential hazards associated with this production stage including:

- Introduction of adventitious agents via contaminated culture medium components or inadequate sterilization of bioreactors;
- Introduction of adventitious agents present in the local environment of the production facility during passaging from one vessel to another; and
- Introduction of media components that could be present as residues after washing of cell material in subsequent harvest step; and
- Direct bioaccumulation of persistent media components in the cell material.

UPSIDE manages risk associated with the first two hazards through the sterile procedures and monitoring programs discussed at the beginning of the section "Cell Culture Food Production Process." Safety considerations associated with the last two hazards are discussed in the subsequent section, "Substances Used in Cell Culture Food Production Process."

Harvest of Cell Material

UPSIDE states that at the end of the maturation phase, the adherent cell material is removed from the culture vessel, collected in a wash basin, and washed to remove remaining culture media from the extracellular matrix. UPSIDE describes the wash solution as a sterile dilute phosphate-buffered saline (PBS) solution, commonly used in a wide variety of research,

clinical, and food applications.[11] After washing, the PBS solution is drained. UPSIDE states that analytical testing of the washed product indicates that the wash step does not introduce any additional phosphorus, potassium, or sodium. The moisture level is then reduced to render the material suitable for consumer product formulation. After harvest, the cells are no longer held in a warmed, aerated, and nutrient-rich environment. Cell viability is no longer maintained, and the cells are converted to non-living material prior to conventional food processing.

UPSIDE identifies potential hazards associated with this production stage including:

- Introduction of adventitious agents present in the local environment of the production facility during removal, washing, and transfer to receptacles for further conventional food processing;
- Migration of contaminants from food contact materials in the growth vessel; and
- Persistence of residual media components after washing of the harvested material.

Safety considerations associated with this last hazard are discussed in the subsequent section, "Substances Used in Cell Culture Food Production Process."

UPSIDE states that the harvested product is maintained in clean equipment under cold processing conditions for moisture adjustment and storage. UPSIDE notes that an environmental monitoring program assesses the effectiveness of the overall hygienic practices in the manufacturing facility and provides necessary information to prevent possible microbial contamination of food products. Samples of the product are periodically taken and tested using a qualified assay to inspect for contamination. UPSIDE also states that the firm uses appropriate and authorized food contact materials throughout the production process.[12]

Substances Used in Cell Culture Food Production Process

UPSIDE provides information about the substances used during its cell culture food production process in the form of cell culture media and components; including:

- nutrients used to support cell primary metabolism,
- substances to manage properties of the culture media,
- substances intended to support cell proliferation and differentiation during culture, as well as

[11] PBS is comprised of potassium chloride, potassium phosphate, sodium phosphate, and sodium chloride, with sodium chloride representing the major component.

[12] The production conditions described by the firm would be consistent with food type 1 (nonacid, aqueous products); may contain salt or sugar or both (pH above 5.0) and conditions of use type F or G (refrigerated or frozen storage). Any food contact substances authorized for use with food type 1 and conditions of use type F or G may be used.

- a cisgenic protein expressed in the cells and intended to support proliferation during culture.

For each substance, UPSIDE provides information about the identity and the basis for its safety conclusion, and in certain cases information about estimated consumer exposure. [13]

UPSIDE describes the firm's proprietary cell culture medium as containing substances commonly found in food including amino acids, fatty acids, sugars, nucleotides, trace elements, and vitamins, and states that these substances are metabolized and used for the fundamental nutritional requirements of the cells. Additional substances identified by UPSIDE during the production phase of cell culture include emulsifiers and surfactants, antioxidants, bovine serum albumin, growth factors,[14] and wash buffers. UPSIDE notes that most of these substances are widely used in traditional food production technologies such as microbial fermentation and algal cell culture. UPSIDE states that the components mentioned above are largely removed from the cell material by washing prior to conventional food processing, that residual levels in the product do not present concerns given available toxicological information and existing use in the food supply, and that they have no technical or functional effect in the finished food. UPSIDE also states that no antibiotics or antifungal agents are used during proliferation or differentiation phases of cell culture.

UPSIDE describes the firm's general framework for evaluating its intended use of a culture medium constituents, including consideration of existing authorizations, prior use in or presence in food, and anticipated dietary exposure. In particular, UPSIDE discusses the firm's intended use of recombinant proteins used to support proliferation or differentiation in culture.[15] UPSIDE states that the firm uses such proteins in production to replace biological sources that would ordinarily be available to animal cells *in vivo*, that the gene sequences are derived from agricultural species, and that these proteins are among those commonly present in animals that are consumed by humans. UPSIDE's assessment of the firm's intended uses considered several factors including:

- the residual presence of these proteins in foods from commonly consumed agricultural species;

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[13] A complete list of these substances was provided to FDA as supporting, corroborative information in a confidential, supplementary appendix.

[14] UPSIDE describes a version of the production process that also uses bovine serum as a source of growth factors, a common practice in research settings. UPSIDE notes that the components of bovine serum, a derivative of bovine blood, are present as a residue in conventional meat products due to residual blood in the carcass. As noted elsewhere, UPSIDE uses bovine serum that is filter-sterilized and obtained from animals originating from BSE-free or risk-negligible countries.

[15] UPSIDE provides a complete list of these recombinant proteins as supporting, corroborative information in a confidential, supplementary appendix shared with FDA.

- published information on the stability of growth factors; [16]
- published data indicating absence of bioactivity through the oral route;
- analytical data on the presence of these growth factors both in UPSIDE's cultured cell material after harvest and in a conventional comparator (ground chicken meat, whole muscle meat from a chicken leg, and skin from a chicken leg); and
- analytical data on stability of these growth factors subjected to cooking temperatures.

UPSIDE states that all growth factors used in the process described in CCC 000002 are present[17] in commercially available chicken products.[18] None of the proteins are derived from a major food allergen. UPSIDE notes that their assessment of the available scientific literature indicates that growth factors are generally recognized as having low stability, and do not exhibit bioactivity via the oral route. UPSIDE further states that, given the firm's production process, these proteins:

- are present in the harvested cell material at levels similar to those found in foods derived from animal muscle tissue, such as chicken meat;
- would be present at very low or undetectable levels in finished foods after conventional food processing; and
- would likewise be broken down by both heat (e.g., cooking) and gastric fluids in the gastrointestinal tract of the consumer.

As previously noted, with respect to the specific harvested cell material described in CCC 000002, UPSIDE states that, based on the firm's testing using appropriate assays in raw and cooked samples of both store-bought chicken and UPSIDE's harvested material, these factors are thermolabile and are typically not detected at any level following cooking. Proprietary data provided by UPSIDE corroborates these statements. [19]

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[16] UPSIDE notes that *in vitro* digestibility studies would typically be appropriate for further characterization of proteins that differ slightly from their endogenous counterparts (e.g., slight amino acid differences or differences in post-translational modification) or have been directly modified for stability or activity; however, none of the proteins used by UPSIDE meet these criteria. UPSIDE therefore concludes that *in vitro* digestibility studies are not needed. Further, in light of the poor stability of the proteins when subjected to conventional food processing (e.g., cooking) and gastric fluids in the gastrointestinal tract of the consumer, UPSIDE states that any *in vitro* digestibility studies would be corroborative.

[17] UPSIDE defines the identity of these proteins in this regard with respect to protein function or homology, rather than species origin (e.g., bovine or avian). UPSIDE notes that these proteins are highly conserved among vertebrates, and in some cases identical in amino acid sequence. The firm thus considers the proteins used in their process to be equivalent in a practical sense to those present in conventional chicken products from a food safety perspective.

[18] The exception is the previously noted alternative production process that uses bovine serum.

[19] UPSIDE conducted some analytical tests to provide additional support for their conclusions regarding anticipated residual levels of the recombinant proteins used in the firm's culture process in both raw and cooked

UPSIDE discusses the TERT cisgene expressed in one of the cell lines that is the subject of CCC 000002. As discussed in the previous section, "Establishment of Cell Lines," the protein expressed by this gene is a component of the telomere enzymatic complex, which is responsible for the maintenance and lengthening of telomeres. UPSIDE states that the protein is non-toxigenic, is not an allergen, is not heat-stable or resistant to digestion, and would be digested and metabolized like any other food protein upon consumption.

As discussed in the previous section, "Substances Used in Cell Culture Food Production Process," UPSIDE states that the firm uses appropriate and authorized food contact materials throughout the production process.[20]

Characterization of Harvested Cell Material

UPSIDE describes characterization of the harvested cell material through compositional analysis, verification of species identity, and markers for muscle cell differentiation. Species verification relies on a commercially available validated ELISA test for chicken proteins in cooked meat.[21] Presence of muscle cell characteristics in the food are assessed by a positive test for the expression of the tropomyosin protein, which is a major characteristic structural protein of muscle cells. The compositional analyses[22] of the harvested cell material includes major nutrients (protein and amino acids, fats, carbohydrates, minerals, and vitamins), and some residues of media components. UPSIDE provides information on potential contaminants including: aerobic plate count, mold, yeast, coliforms, *E. coli*, *Enterobacteriaceae*, *Salmonella* serovars, *Enterobacter cloacae* complex, and certain viruses (influenza type A and B); as well as toxic heavy metals (lead, arsenic, cadmium, and mercury), based on the analysis of three

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samples. All results were consistent with the conclusion that these proteins would be detectable at very low levels under certain conditions in both conventional poultry products and in the firm's product, but would be rendered undetectable by post-slaughter or post-harvest aging of the material as well as by cooking. The range of levels reported in the dataset overlapped for UPSIDE's product and the conventional comparator, but with some limited indication (given the sample size) that levels in UPSIDE's freshly harvested cell material may trend higher than in the conventional comparator. UPSIDE stated that given the low level of stability exhibited by these proteins, ongoing breakdown would be expected in any particular sample depending on duration after harvest or slaughter (e.g., samples of freshly harvested cell material or slaughtered chicken tissue relative to a commercial sample purchased at retail), consistent with their observations.

[20] As previously noted, the production conditions described by the firm would be consistent with food type 1 (nonacid, aqueous products); may contain salt or sugar or both (pH above 5.0) and conditions of use type D (hot filled or pasteurized below 66 °C) save for post-harvest storage (conditions F or G for refrigeration or frozen storage, respectively).

[21] As discussed in the previous section, "Establishment of MCB," species identity is also established at the cell bank stage through mitochondrial DNA sequencing of the cytochrome c oxidase gene, used as a common reference gene for this purpose.

[22] The production process defined in CCC 000002 may use or omit bovine serum as a source of growth factors in the cell culture food production phase. In the firm's submission, UPSIDE provides compositional data on material produced with and without serum. The firm notes that results from analysis of serum-based production are similar to the serum-free product.

nonconsecutive production runs. UPSIDE states that all microbial and viral tests were negative at the limit of detection, and that toxic heavy metals are below levels that would result in safety concerns. Data was concurrently generated using conventional ground chicken. UPSIDE states that all analytical methods are validated for their intended purpose. UPSIDE provides specifications for the harvested cell material, including specifications for microbial detection: total plate count (<100 colony forming units (CFU)/g), *Enterobacteriaceae* (<10 CFU/g), and *Salmonella* serovars (not detected in 25 g)[23]; as well as toxic heavy metals: lead (≤0.1 ppm), cadmium (<2 ppm), mercury (<2 ppm), and arsenic (<1 ppm).

As a point of reference, UPSIDE also presented nutrition data from a U.S. Department of Agriculture (USDA) database on skinless light meat chicken and on an aggregate of data on light and dark muscle products from chicken, including those with and without skin.[24] Protein and amino acid results were found to be similar; UPSIDE attributes this to the expression of genetically defined muscle proteins as a dominant feature of the cell material in each case. UPSIDE reports higher levels[25] of some components (including iron, potassium, phosphorus, folate, and cholesterol), relative to values reported in USDA data.[26] UPSIDE notes that some form of folate is an essential nutrient for cells in culture to support key aspects of primary metabolism including nucleotide synthesis, amino acid synthesis, and methylation. UPSIDE states that nutrient composition for the food is within expected and safe ranges.

FDA's Evaluation

FDA evaluated the information provided by UPSIDE with respect to the established cell lines, cell banks, substances used in the production process, and properties of the harvested cell material that collectively are the subject of CCC 000002. The primary focus of FDA's evaluation is the information on which the firm relies to conclude that the harvested cell material is safe for use as food and does not contain substances or microorganisms that would adulterate the food.

UPSIDE provides information on the establishment of the cell lines used to produce the food that is the subject of CCC 000002. FDA considered the information on the source and lineages of the cell lines, the culture adaptation process, the processes used to immortalize the cell lines (selection and genetic engineering), and the final differentiation of the cells. We also considered the information provided by UPSIDE with respect to the observed behavior of the

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[23] UPSIDE states that the analyses for *Enterobacteriaceae* and *Salmonella* serovars are only performed on production runs of the finished product that exceed the specification for aerobic plate count.

[24] This aggregate measure of "All USDA chicken (no organs)" includes data from 27 different published samples including light meat with and without skin, dark meat with and without skin, ground raw, and other chicken samples for roasting or stewing, but did not include data for skin only or chicken organs.

[25] Observed only in serum-free harvested cell material with respect to iron and potassium.

[26] These values are within ranges observed in other commonly consumed foods.

cell lines in culture, the genetic capacity of animal cells to produce toxins or other potentially harmful substances, and the viability of the cells following harvest.

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

Finally, while ectopic expression of egg protein allergens was a theoretical possibility given that each cell contained the complete chicken genome including genetic code for the relevant egg proteins, there was neither evidence of such expression nor a basis for anticipating it based on the selective pressures and engineered protein used.

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

Regarding the production process, FDA considered the information on hazard analysis for each production step and rationales for risk-based preventive controls described by the firm, including UPSIDE's assessment of potential sources of adventitious agents, the significance of the risk posed by each, and the corresponding design of testing strategies for controlling these risks. We also considered UPSIDE's use of cGMPs and supporting programs such as sanitation control, supply management system and environmental monitoring. No information presented by the firm or otherwise available to us indicated that the selected test strategies would be inadequate to control for the presence of adventitious agents or to maintain product quality. We note the self-limiting nature of quality failures related to microbial or viral control in the production process. In summary, we did not identify elements of the process as described that indicate an unaddressed food safety risk resulting from microbial, viral, or other contaminants.

FDA considered the general framework that UPSIDE used for evaluating the safety of each substance for its intended use as well as the complete list of substances provided as supporting, corroborative material in a confidential supplementary appendix, including the identity, intended use, anticipated dietary exposure, and relevant data on safety and existing authorizations. We also considered the information presented by UPSIDE regarding the firm's assessment of the use of recombinant growth factors, including the identity and use of growth factors that are also present in conventional chicken meat, the intended use level, anticipated digestive fate, and corroborative analytical data on residual presence in the cultured cell material. We note that the substances described by UPSIDE have no intended effect in the harvested cell material and if present are expected to be at minimal levels.

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

FDA reviewed the information that was provided on the identity and composition of the harvested cell material, including genetic and cellular identity, batch test data for constituents and contaminants, and specifications. We considered the analytical data provided by UPSIDE on the composition of the harvested cell material from several production runs as one element in characterizing the identity of their product, as evidence of the firm's ability to conform to their stated specifications for food contaminants, and as relevant information in evaluating the relationship between the production process described in CCC 000002 and the properties of the harvested cell material produced through that process. We evaluated the firm's specifications for toxic heavy metals to ensure they were as low as reasonably possible and were consistent with food safety. We also considered information relating to micronutrients that could indicate excessive accumulation of nutrients from the culture medium and potentially represent inappropriate fortification. We note that batch data indicates several nutritional components, including iron, potassium, phosphorus, and cholesterol were elevated either in serum-free or in all cultured cell material compared to comparator data from conventional chicken, but that none of these levels were sufficient to pose a safety concern and did not appear to be the result of intentional manipulation of the medium. The batch data also indicated elevated folate levels relative to conventional ground chicken, because of its use in culture to support primary metabolism of the cells. FDA has previously authorized fortification of several foods with folic acid through 21 CFR 172.345. The level of folate found in UPSIDE's product is much lower than levels of folic acid authorized by this regulation; the bioavailability of folate is significantly lower than that of folic acid, that a variety of other commonly consumed foods contain comparable levels of folate, and that the level is a result of nutritional support for cells in culture experiencing high metabolic demand. In all cases, levels were within the range of those found in commonly consumed foods. We did not identify the establishment of exact equivalence of all nutrients and components relative to a particular conventional comparator as a necessary component of UPSIDE's safety conclusion, nor did we interpret the analytical data provided by the firm as definitive nutritional information regarding either harvested cell material produced through the process defined in CCC 000002 or food products that contain this material.

Conclusions

Based on our evaluation of the data and information that UPSIDE provides in CCC 000002, as well as other information available to FDA, we did not identify a basis for concluding that the production process as described would be expected to result in food that bears or contain any substance or microorganism that would adulterate the food. We have no questions at this time about UPSIDE's conclusion that foods comprised of, or containing, cultured chicken cell material resulting from the production process defined in CCC 000002 are as safe as comparable foods produced by other methods.

Jeremiah M. Fasano -S

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Jeremiah Fasano

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

Process Step	Potential Issue	Management Strategy
Cell Isolation	Cell source (animal health, species-specific considerations)	Documentation
	Cells from different line or species inadvertently used	Documentation, visual observation of morphology
	Carryover of adventitious agents such as bacteria, fungi, viruses, parasites, and prions during isolation	Animal health documentation, antibiotics application, testing program
	Introduction of contaminants in laboratory reagents	Supplier management program, sterilization
	Introduction of contaminants from animal-derived reagents (e.g., bovine serum, trypsin)	Sterilization, BSE-free certification, testing program
	Facility environment contamination	Aseptic procedures, antibiotics, testing program
Establishment of Cell Lines	Cells from different line or species inadvertently used	Genetic testing
	Mycoplasma spp. and other adventitious agent contamination	Testing program
	Introduction of contaminants in laboratory reagents	Supplier management program, sterilization
	Cells do not display expected growth profile	Measure and discard
	Unintended effects of immortalization	Monitor growth and viability, hazard analysis, food safety assessment
	Contamination with adventitious agents such as bacteria, fungi, viruses, parasites, and prions from original source	Hazard analysis, testing program
	Animal-derived reagent (e.g., bovine serum, trypsin) contamination	Sterilization, BSE-free certification, testing program
	Facility environment contamination	Aseptic procedures, antibiotics, testing program
	Introduction of TERT cisgene	Food safety assessment

Process Step	Potential Issue	Management Strategy
Establishment of Cell Lines	Presence of residual unintended material from genetic engineering	Sequencing to confirm removal
Establishment of MCB	Contamination during transfer	Aseptic procedures, sterilization, environmental monitoring
	Cells from different line or species inadvertently used	Genetic testing
	Cells do not display expected growth profile	Measure and discard
	Contamination with adventitious agents such as bacteria, fungi, viruses, parasites, and prions from original source	Testing program
	Animal-derived reagents (e.g., bovine serum, trypsin) contamination	Sterilization, BSE-free certification, testing program
	Contamination with adventitious agents from culture media components	Sterilization, batch records
	Facility environment contamination	Environmental monitoring
Proliferation Phase	Contamination during transfer	Aseptic procedures, sterilization, environmental monitoring, culture monitoring
	Contamination with adventitious agents from culture media components	Supplier program, sterilization, batch records, culture monitoring
	Animal-derived reagent (e.g., bovine serum, trypsin) contamination	Sterilization, BSE-free certification, testing program, culture monitoring
	Contamination with adventitious agents through inadequate sterilization of bioreactors	Validated sanitation processes and environmental monitoring, equipment design, culture monitoring
	Facility environment contamination during transfer of cells between bioreactors	Validated sanitation processes and environmental monitoring, culture monitoring

Process Step	Potential Issue	Management Strategy
Proliferation Phase	Introduction of media components that could persist as residues in harvested cells	Food safety assessment
	Introduction of media components that could accumulate in the cells before harvest	Food safety assessment, compositional analysis at harvest
Differentiation Phase	Contamination during transfer	Aseptic procedures, sterilization, environmental monitoring, culture monitoring
	Contamination with adventitious agents from culture media components	Supplier program, sterilization, batch records, culture monitoring
	Contamination with adventitious agents through inadequate sterilization of bioreactors	Validated sanitation processes and environmental monitoring, equipment design, culture monitoring
	Facility environment contamination during transfer of cells between bioreactors	Validated sanitation processes and environmental monitoring, culture monitoring
	Introduction of media components that could persist as residues in harvested cells	Food safety assessment, testing
	Introduction of media components that could accumulate in the cells before harvest	Food safety assessment, compositional analysis at harvest
Harvest of Cell Material	Presence of bacterial or viral contaminants from culture process	Culture monitoring, testing, specifications
	Migration of contaminants from food contact materials	Use of authorized food contact materials, supplier program
	Presence of residual media components after harvest	Wash steps, food safety assessment, testing program
	Presence of elemental contaminants (toxic heavy metals) after harvest	Testing program, specifications

Process Step	Potential Issue	Management Strategy
Harvest of Cell Material	Facility environment contamination	Equipment cleaning procedures, environmental monitoring, controlled temperature conditions