Premarket Notice for Integral Tissue Cultured

Poultry Meat

SUBMITTED ON BEHALF OF UPSIDE FOODS BY:
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List of Abbreviations

ARTs	assisted reproductive technologies
BLV	bovine leukemia virus
CAPA	Corrective & Preventive Actions
CFU	colony forming units
cGMP	current Good Manufacturing Practice
CIP	cleaning in place
CJD	Creutzfeldt-Jakob Disease
CODEX	Codex Alimentarius
CPM	cultured poultry meat
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
ERP	Enterprise Resource Planning
FCC	*Food Chemicals Codex*
FDA	Food and Drug Administration
FSIS	Food Safety Inspection Service
FSMA	*Food Safety Modernization Act*
FSPCA	Food Safety Preventive Controls Alliance
GFSI	Global Food Safety Initiative
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
HARPC	Hazard analysis and risk-based preventive controls
HEPA	high-efficiency particulate air
LOD	limit of detection
MCB	master cell bank
MoE	margin of exposure
MCB	master cell bank
MWCB	Manufacturer's working cell bank
NCBI	National Center for Biotechnology Information
NOAEL	no-observed-adverse-effect level
PCQI	Preventive Controls Qualified Individual
PCR	polymerase chain reaction
PPE	personal protective equipment
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
QA	quality assurance
RNA	ribonucleic acid
SCAR	Supplier Corrective Action Report
SIP	sanitizing in place
SOP	standard operating procedure
TERT	telomerase reverse transcriptase
Tm	tropomyosintM
U.S.	United States

USDA	United States Department of Agriculture
USP	United States Pharmacopeia
WHO	World Health Organization
WIP	work-in-progress

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1.0 Administrative Information

UPSIDE Foods (formerly known as Memphis Meats) hereby provides the following premarket submission to the United States (U.S.) Food and Drug Administration (FDA) for the company's cultured poultry meat. As discussed further herein, UPSIDE Foods has concluded that cultured poultry meat is as safe as conventional poultry meat from a chicken carcass.

Information presented in this submission constitutes a complete, representative, and balanced submission, and considered all unfavorable, as well as favorable, information known to UPSIDE Foods and pertinent to the evaluation of the safety of cultured meat as described herein.

Signed,

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1.1 Name and Address of Company Submitting Information

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1.2 Freedom of Information Act

This document does not contain trade secrets and confidential commercial information subject to disclosure under the *Freedom of Information Act* [5 U.S.C. § 552(b)(4)], *Federal Trade Secrets Act* (18 U.S.C. § 1905), *Federal Food, Drug, and Cosmetic Act* [21 U.S.C. § 331(j)], and FDA's implementing regulations (codified at 21 CFR part 20).

2.0 INTRODUCTION

UPSIDE Foods (formerly known as Memphis Meats) has developed proprietary food production technologies for the growth of meat tissue using cells isolated from food animals (*e.g*., terrestrial animals such as cows, pigs, and chickens, and aquatic species such as finfish or shellfish). Meat, poultry, and seafood products manufactured by UPSIDE Foods are produced using cells isolated from viable animal tissues and grown under controlled conditions in bioreactors; these products are herein referred to generically as "cultured meat."

In March 2019, Food Safety Inspection Service (FSIS) and FDA entered into a Formal Agreement "*with respect to the oversight of human food produced using animal cell culture technology, derived from cell lines of USDA-amenable species* […]" (U.S. FDA-USDA FSIS, 2019). As explained in the Formal Agreement, FDA will, among other things, "*conduct premarket consultation processes to evaluate production materials/processes and manufacturing controls, to include oversight of tissue collection, cell lines and banks, and all components and inputs*" (U.S. FDA, 2019). FDA also will consult with FSIS and "*share results of premarket consultation processes*" with FSIS, as authorized by law.

This submission describes the identity, production, and safety of a cultured poultry product produced from edible tissues derived from two chicken cell lines. Although cultured meat production represents a new technology for food production, UPSIDE Foods believes that many of the fundamental concepts forming the basis of this technology are rooted in practices that have a long history of safe use in food production. Historical experiences with food fermentation technology, including foods and food ingredients produced from genetically amended microorganisms and plants, are notable examples that have a long history of safe use in the food supply. Scientific approaches to identify and characterize the hazards of a new technology require consideration of potential hazards associated with the new production process. Most importantly, these approaches should focus on objective characterization of the end product; where applicable, reference to conventional comparator products with a history of safe consumption should be leveraged to support safety.

> "*The regulatory status of a food, irrespective of the method by which it is developed, is dependent upon objective characteristics of the food and the intended use of the food (or its components). The method by which food is produced or developed may in some cases help to understand the safety or nutritional characteristics of the finished food. However, the key factors in reviewing safety concerns should be the characteristics of the food product, rather than the fact that the new methods are used*" (57 FR 22984 – U.S. FDA, 1992).

The risk assessment principles applied within this premarket submission draw from FDA's experiences with the safety evaluation of genetically amended plants discussed within the Agency's 1992 policy on Foods Derived from New Plant Varieties (U.S. FDA, 1992), as well as the 2009 Codex Alimentarius (CODEX) guidelines for Foods Derived from Modern Biotechnology (U.S. FDA, 2001; Codex Alimentarius, 2009). Accordingly, the food safety evaluation was conducted using scientific procedures and an emphasis has been placed on obtaining an objective and thorough characterization of the cultured meat identity and composition through comparison to the identity and compositional characteristics of the conventional counterpart, chicken meat from an animal carcass, which has an established history of safety consumption.

UPSIDE Foods' Food Safety and Quality Systems are based on 21 CFR part 117 and the Global Food Safety Initiative (GFSI) standard. The Food Safety Plan addresses potential hazards that may be introduced into food during the production process and the preventive controls that are employed to mitigate such hazards (*e.g*., adventitious agents, microbial and environmental contaminants, culture media components, food contact articles)

Data and information presented herein demonstrates that cultured poultry meat (CPM) products manufactured by UPSIDE Foods are as safe as conventional meat obtained from a chicken carcass.

3.0 Identity of the Food

Cultured chicken (*Gallus gallus*) products are animal cells that are propagated in highly controlled cell culture systems for the explicit purpose of food production. Unlike conventional meat products that are harvested from an animal carcass, cultured poultry products do not depend on completing the entirety of an animal's development cycle to produce an equal or greater mass of food product. UPSIDE Foods has developed a highly controlled aseptic cell production process that recapitulates the naturally occurring cell proliferation and tissue developmental processes that occur within living animals. This controlled system and environment are largely similar to industrial food culture fermentation systems where cell cultures proliferate and mature largely under their own genetic instructions. The process is monitored to support cell growth *via* a control system that supplies media that (1) delivers nutrients, (2) shuttles away cell waste, and (3) facilitates gas exchange, all while essential heat is supplied to keep the cultures viable and to optimize growth rate and productivity.

Meat (*e.g*., muscle) is composed predominantly of cells of mesenchymal lineage, which are differentiated into myogenic, fibrogenic, and adipogenic lineages during the developmental and growth stages of the animal. Similarly, a typical cultured meat production process uses one or multiple cell types found in muscle tissue and often employs multiple approaches to produce cellular phenotypes that are conducive to long-term culture (*i.e.*, delayed senescence/immortalization). Preference is given to cells that have an innate capacity for immortalization, although in certain cell types, UPSIDE Foods uses well-established, safe methods to engineer the cells to intentionally delay senescence through the constitutive expression of endogenous genes within the chicken genome that induce a desired phenotype for extended culture.

The process described in this submission currently uses two cell lines isolated from chicken tissues that are routinely consumed as food, including a myoblast cell line derived from muscle tissue, and a fibroblast-like cell line derived from skin tissue of two mid-stage fertilized eggs. Cell lineages are phenotypically characterized by standard validated methods such as microscopy and immunostaining procedures for verification of the cell type. In addition, chain of custody is verified beginning with cell isolation from the donor animal. These cell lines consist of populations of cells that have been cultivated in a manner that produces a phenotype of delayed senescence, also referred to as immortalization, enabling their extended growth in culture for sufficient durations of time to produce commercial-scale quantities of meat. This phenotype has been achieved through natural processes in the myoblast cell line through continual subcultivation until populations displaying linear growth "break through" the senescent phenotype of the parental population. CPM harvested from the bioreactors are verified for species identity using a cooked meat enzyme-linked immunosorbent assay (ELISA), and the product typically comprises between 70 to 80% moisture, 10 to 20% protein, 1 to 5% fat, and 1 to 5% ash. The CPM will be compliant with specifications outlined in Section 4.4.5.5. In addition to confirmation of species identity, verification of CPM as meat was

conducted in two key ways: (1) its muscle-derived amino acid composition was demonstrated to be similar to meat and (2) the CPM product was demonstrated to contain tropomyosin 1[1], a major protein marker of skeletal muscle.

The tissue harvested from UPSIDE Foods' meat bioreactors is then further processed using permitted food grade ingredients and processing aids to produce various end products (*e.g.*, breaded chicken) that are highly similar to chicken products produced using chicken meat from an animal (Figure 3-1 shows the visual comparison; see Section 5.0 for compositional comparison).

Figure 3-1 Pictorial Example of CPM and Comparison to Conventional Chicken Breast

All CPM images by UPSIDE Foods. Chicken breast image. https://www.amazon.com/Certified-Organic-Chicken-Boneless-skinless/dp/B00Q9A01BI

CPM = cultured poultry meat.

[1] Chicken breast muscle is over 99% fast white muscle, and *alpha*-fast isoforms of tropomyosin are a major characteristic structural protein of chicken breast meat (Matsuda *et al*., 1983).

4.0 Description of the Production Process

4.1 Food Safety and Quality Systems

UPSIDE Foods' Food Safety and Quality Systems are based on 21 CFR §117 and the GFSI standard. These systems include prerequisite programs to mitigate hazards that could affect the safety of UPSIDE Foods' products, including, but not limited to the following:

- Good Manufacturing Practices (GMPs)
    - A GMP Policy that covers employees and visitors to the production facility is in place. The policy is based on 21 CFR §117.10 and outlines the requirements for disease control, personal hygiene, personal protective equipment (PPE) and donning requirements, and foreign material control (U.S. FDA, 2020a).
    - To elaborate on UPSIDE Foods' GMP programs, UPSIDE Foods has assessed the need for different hygiene levels in the company's facility that include varying levels of donning and doffing. To eliminate the transmission of outside biological or chemical agents for cross-contamination, personnel entering UPSIDE Foods' production area will be required to remove all jewelry and watches before changing into captive boots, clean lab coats, hair nets, and beard nets (if needed). Personnel are also required to clean and sanitize hands before entering the production area. If moving from the United States (U.S.) Food and Drug Administration (FDA) production area to the United States Department of Agriculture (USDA) production area, personnel must change out of PPE before leaving and don a new set of captive boots, coats, *etc*. for the USDA uncooked area, as well as a separate set for the ready-to-eat USDA area. Zoning includes the garb and captive boots that are needed in each area. Any visitors allowed into UPSIDE Foods' production areas will be required to follow all of the company's GMPs and must acknowledge them prior to entering the area. In addition to donning and doffing, GMP programs include all of the items as called out in 21 CFR §117 subpart B.
- Validated sanitation processes and environmental monitoring
    - Sanitation standard operating procedures (SOPs) are in place for production areas and production equipment, including validated procedures for cleaning in place (CIP) and sanitizing in place (SIP).
    - Environmental monitoring (EM) at UPSIDE Foods' facility is a control for transmission of biological and chemical hazards that may originate outside of UPSIDE Foods' facility or in non-production areas of the facility. The EM program encompasses Zone 1 indicator organism monitoring and Zone 2 through Zone 4 indicator and pathogen organism monitoring. EM swabs are scheduled based on the hygiene zone map to ensure that all rooms and zones are included in the process on a rotational basis. For any test results above UPSIDE Foods' limits, a process is started to swab adjacent areas in a star pattern to

determine the root cause of the growth. Along with this investigation, additional sanitation will be performed in order to eradicate the growth.

- Supplier approval program
    - UPSIDE Foods' Supplier Management program focuses on managing the risks of incoming raw materials, ingredients, packaging, manufacturing consumables, food contact tools, and services. UPSIDE Foods' program includes requirements for selecting, evaluating, approving, and monitoring UPSIDE Foods' suppliers and the items being purchased. The process includes:
        - Establishing and implementing product specifications, including any physical, chemical, or biological parameters that must be reported on a Certificate of Analysis accompanying each product shipment.
        - Review of Food Safety programs in place by the supplier, which may be in the form of a third-party (such as GFSI) audit report review, review of supplier policies and programs relevant to food safety, or an onsite inspection performed by UPSIDE Foods staff or an agent of UPSIDE Foods. The review includes, but is not limited to:
            - Supplier GMPs and facility/equipment sanitation and maintenance programs.
            - Process hazard analysis and mitigation strategies to control hazards in finished products.
            - Facility or food defense programs, including consideration for food/ingredient fraud and economically motivated adulteration.
            - Supply chain management program.
            - Allergen risk assessment and management program.
            - Separate review and assessment of each supplier location/facility.
    - Supplier evaluation and approval will be completed prior to purchasing. As part of approving the material and the supplier, special handling or analysis may be required at receiving to further mitigate identified risks. Incoming materials will be on quality assurance (QA) hold until all required receiving inspection and analyses are complete and verified to meet specification. The testing frequencies and items for analysis will be conducted according to the specification. After baseline data and results trending for the supplier has been established, the frequency and items for analysis may be modified to align with the identified risk.

- Ongoing supplier management will be performed and feedback will be provided to the supplier in the form of a Supplier Corrective Action Report (SCAR) for any issues pertaining to their materials or services.

- Document and records control, including material and product specifications
    - A document control policy is in place to ensure that all company policies, SOPs, records, and material specifications are reviewed a minimum of annually. Document review and approval records and version control are maintained using a quality management system.

- Measures for prevention of biological, chemical (including allergens), and physical (extraneous material) hazards
    - Each process step is reviewed to determine which potential hazards could be introduced into the product. These hazards are based on product or ingredient history, material origin, production processes, human intervention, and regulatory guidance. A risk assessment is performed to determine the probability and severity of occurrence, and preventive control measures are put in place where risk mitigation is needed. These measures are fully documented in a Food Safety Plan under the guidance of a Preventive Controls Qualified Individual (PCQI).

- Product release system with non-conforming product control
    - Incoming raw materials, intermediate work-in-progress (WIP) products, and finished products are all designated on QA hold until they have been approved for release based on review of production records, testing results, or certificates of analysis/certificates of quality as applicable. These materials may also be placed on QA hold at any time during production in response to a non-conformance. The QA hold status is managed using an Enterprise Resource Planning (ERP) system, which ensures that materials on hold are not able to be added to production batches or open orders. The product release system is managed by the Food Safety team and PCQIs.

- Batch record review by a PCQI
    - Batch records are used throughout the production process to document material usage, production steps, and in-process checks, including food safety critical control point monitoring. Batch records are reviewed by a production supervisor and a final review is conducted by a PCQI to ensure that all product parameters and food safety requirements are met before release of products from QA hold.

- Traceability
    - Complete traceability of all raw materials and finished products is maintained throughout the process using an ERP system. Lot number usage of raw materials, manufacturing consumables, and packaging materials is documented to ensure traceability forwards and backwards, including traceability to one step before and one step after the manufacturing

facility. This traceability is routinely tested in mock recall and traceability exercises performed and documented by a PCQI.

UPSIDE Foods also has supporting programs including, but not limited to:

- Corrective & Preventive Actions (CAPA) Program
    - Non-conformances are documented and managed in a quality management system to ensure continuous improvement of processes and products. Where product safety may have been affected by a non-conformance, the product is placed on hold for disposition assessment by the Food Safety team. The CAPA report consists of a corrective action to fix the issue immediately, a root cause investigation to determine the source of the issue, and implementation of a preventive action to ensure that the issue does not recur.
- Internal GMP & Systems Auditing
    - Periodic inspections of production areas, facility grounds, employee practices, and documentation are performed to ensure compliance with all company policies and Food Safety Plan requirements. These inspections are documented and any identified non-conformances will be investigated through the CAPA program. Inspections are performed by employees outside of the area being audited to ensure impartiality. Inspection performance is trended and periodically reviewed with plant management to ensure continuous improvement of processes and products.
- Raw Materials and Product Sampling, Inspection, and Analysis
    - Incoming raw materials are risk-assessed to determine whether additional analytical testing is needed to ensure the safety of the material for use in food production. The risk assessment is based on historical data of hazards associated with the material, the origin of the material, and regulatory guidance. These materials are kept on QA hold until acceptable results are obtained. The testing frequencies and items for analysis will be conducted according to the specification. After baseline data and results trending for the production facility has been established, the frequency and items for analysis may be modified to align with the identified risk.
    - In-process sampling and analysis of the product is performed to ensure that quality parameters are met and the product has not become contaminated during production.
    - Intermediate product and finished product testing is performed to ensure the safety of the product. The analyses chosen for these products are based on a risk assessment that considers historical data of hazards associated with the material, hazards identified in the production areas and processing steps, and regulatory guidance. Raw material and WIP will be given usage determinants for “best used by” and expiration dates where needed for food safety and quality attributes.

- Specifications are created for incoming raw materials,harvested cultured meat and finished USDA products. Specifications include physical, chemical, and biological characteristics critical in determining the quality and safety of each product.

- Sanitary Design of Equipment and Tools

    - All equipment and food-contact tools and manufacturing consumables are reviewed to ensure that they are made from food-safe materials. Equipment design is performed with sanitation in mind to ensure that all installed equipment is accessible for cleaning and harborage areas are avoided or minimized. Sanitation validation is performed as applicable as part of the qualification process for new equipment for production.

    - Food contact surfaces shall be corrosion-resistant when in contact with food. They shall be made of nontoxic materials and designed to withstand the environment of their intended use and the action of food, and, if applicable, cleaning compounds and sanitizing agents (21 CFR part 1117 Subpart B - Equipment Sec. 117.40) (U.S. FDA, 2020a).

- Crisis Management with Product Recall, Food Defense & Food Fraud programs

    - A crisis management policy is in place to ensure that business continuity can be maintained during adverse events, such as natural disaster, power or network outage, supply disruption, or intentional adulteration. Crisis events and responses are documented and reviewed a minimum of annually with plant management to ensure continuous improvement of the program.

    - A food defense policy is in place to ensure that raw materials and products are protected from intentional adulteration. The policy complies with the *Food Safety Modernization Act* (FSMA) Final Rule for Mitigation Strategies to Protect Food Against Intentional Adulteration and utilizes the checklist in the FDA Food Defense Plan Builder software program. Food defense incidents and responses are documented and reviewed a minimum of annually with plant management to ensure continuous improvement of the program.

    - A food fraud policy is in place to ensure that raw material and finished product provenance is maintained. Raw materials and finished products are risk assessed to determine whether preventive controls are needed to ensure authenticity. This risk assessment is based on historical data of food fraud associated with the product.

- Employee Training Program

    - A comprehensive employee training program is in place to ensure that all employees receive the appropriate initial and ongoing training for their job functions. Training requirements and records are maintained using a training software platform.

UPSIDE Foods' Food Safety Plan meets FDA regulations for GMP and Hazard Analysis and risk-based Preventive Controls for Human Food, codified in 21 CFR part 117, and utilizes the Food Safety Preventive Controls Alliance (FSPCA) Preventive Controls for Human Food Participant Manual v1.2 2016, and the Lead Instructor Guide v1.2 2016 to create a risk-based Food Safety Plan under the guidance of a PCQI. The plan

was created to ensure safe manufacturing, processing, packing, and holding of food. UPSIDE Foods evaluated the processes and ingredients for biological, chemical, and physical agents (including economically motivated hazards) that have the potential to cause illness or injury (21 CFR §117.3). A risk assessment of each potential hazard identified was to establish the probability and severity of the hazard. The risk assessment results were used to determine UPSIDE Foods' monitoring programs and identify those controls elevating to the level of a preventive control (U.S. FDA, 2020a). These preventive controls are intended to minimize or prevent any potential hazards and as necessary will process preventive controls, allergen preventive controls, sanitation preventive controls, supplier preventive controls, or other preventive controls.

Within the biological hazard analysis, UPSIDE Foods evaluated bacteria, viruses, yeasts, and molds. These hazards will be controlled through the enforcement of supplier management for incoming materials, GMPs, and the use of validated and verified sanitation processes to ensure effective cleaning of the facility and equipment.

Chemical hazards identified will be controlled through the enforcement of supplier management for incoming materials and manufacturing consumables, GMPs, chemical approval and usage monitoring, and the use of validated and verified sanitation processes to ensure the complete removal of chemicals used.

Physical hazards will be controlled through the enforcement of supplier management for incoming materials and manufacturing consumables, GMPs, and a glass and brittle plastics program with periodic inspection. The final finished product will be monitored for metallic foreign material with a metal detector.

UPSIDE Foods' Food Safety Plan includes the procedures for monitoring, implementing corrective action (corrections), and verification activities, along with a facility overview, Food Safety team roster, product description (including distribution, intended use, and target consumer information), a verified flow diagram for each process, and a recall plan.

Where material or product testing is identified as a process control in an upstream process that is not duplicated downstream, it is understood that the hazard was not present or not further introduced and the overall risk has been removed, *i.e.*, viral hazards for the original cells that are tested before being banked and no further banking hazard is introduced.

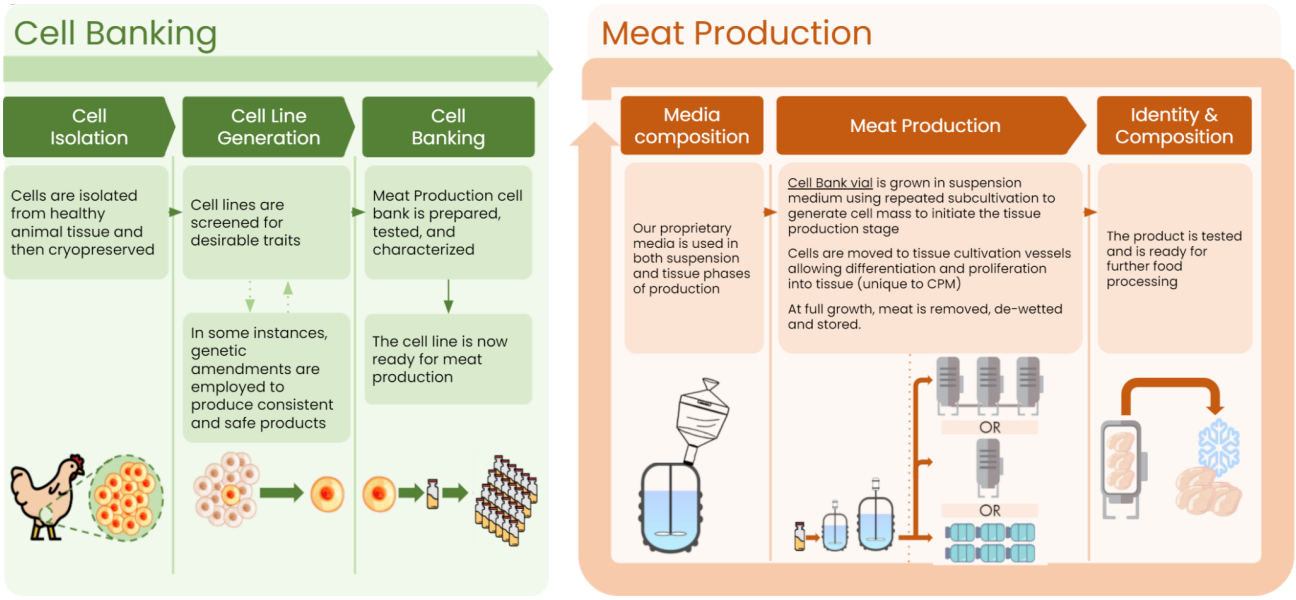
All testing described as periodic in the Food Safety Plan and associated documents will begin with collecting baseline data, evaluating the results, and using a scientific, risk-based approach to determine the frequency needed for ongoing testing. Once the baseline data have been established, frequency of testing and target organisms for testing may be modified to align with the identified risks. UPSIDE Foods' policies and SOPs regarding the Food Safety Plan, Environmental Monitoring Program, Incoming Material Receiving Process, and In-Process Monitoring and Finished Product Monitoring Programs will define the risk assessment and frequency of testing in each program.

The process flow diagrams and hazard analysis as part of UPSIDE Foods' Food Safety Plan is presented on the following pages, biological, chemical, and physical hazards are included in the final Food Safety Plan Hazard Analysis and will be available at the facility.

4.2 Process Overview

The CPM manufacturing process should be considered as two major process stages: (1) **Preparation of Master Cell Banks** and (2) **Cultured Poultry Meat (CPM) Production**. A flow chart overview of the overall processes is provided in Figure 4.2-1.

Figure 4.2-1 Overview of the Cultured Poultry Meat (CPM) Manufacturing Process



4.3 Stage 1 – Preparation of Master Cell Banks

Cell banking[2] assures that a long-term supply of well-characterized cells is stored for use in the production of CPM and that the bank is in sufficient quantity to last over the expected lifetime of the product. Each banked cell line is linked to a certificate of identity and quality characterization of the cell line. This includes confirmation of intended species, phenotypic stability (cell growth and productivity), and absence of detectable adventitious agents (*i.e*., viruses and microbes). All steps in the cell banking process are documented, including identities of all food contact materials used during cell line production. The cell lines that are chosen for CPM production have followed a cell line development process that consist of three stages:

1. Cell line isolation
2. Cell line generation
3. Cell line banking and characterization

Each of these stages is described in more detail in the subsequent sections.

4.3.1 Cell Line Isolation

This section describes the procedure for procuring cells used in CPM and the process for ensuring their safety and quality for use in human food consumption. To mitigate safety risks, UPSIDE Foods documents all steps from animal sourcing to cell isolation. The culture history of each cell line includes animal source documentation, methods used for originating tissue isolation, subculturing history, and materials used, including cell culture media or culture substrate Isolated cell subpopulation lineages are confirmed through morphological, functional, and cell marker expression-based assessments.

UPSIDE Foods obtains cells from animals destined for human consumption. For CPM, UPSIDE Foods uses two sources of animals for the cell isolation samples. The digested tissue is then filtered and washed multiple times with a wash media solution until the tissue has dissociated into a single cell suspension (Motohashi *et al*., 2014; Liu *et al*., 2015; Kisiel and Klar, 2019). Once a single cell suspension is achieved, cells are cryopreserved or cultured immediately for characterization. The distinct morphology of differentiated myofibers, which is well-documented in the literature, further confirms their identity as cells that consist of tissues routinely consumed as meat.

Animal source documentation includes records identifying time and place obtained, as well as any relevant inspection of the animal ante- or post-mortem if the animal was intended for the human food supply. Materials and reagents used in the isolation, development, and bank of cells are recorded, including media components, exposure to cell culture media proteins, antibiotics/antimycotics, culture duration, and

[2] A cell bank is stored at a safe physical location and has a set of quality documentation for every cell line that has met UPSIDE Foods' rigorous screening standards. Only cells that pass UPSIDE Foods' hazard characterization and quality evaluation procedures may be stored in meat production banks used for commercial production. A cell bank typically consists of a collection of cells from a given cell line to produce a master cell bank culture. A collection of working cell banks is typically produced from the master cell bank, and these working cell banks are then used to support the meat manufacturing production runs. As each cell bank is "certified" to be safe and suitable for food use, cell banks are critically important to ensuring that cultured meat products produced by UPSIDE Foods are high quality products that are safe for consumption.

information on tissue extraction location. Experimental and cell line banking registry data are collected using an electronic lab notebook system with date stamps and version control.

4.3.2 Cell Line Generation

UPSIDE Foods' cell line generation process selects cells for population expansion and qualification into a master cell bank (MCB). This selection process includes a number of steps:

1. Cells must be selected for, or induced, to exhibit a stable phenotype with a known replicative lifespan and linear growth to enable continuous propagation of the cells in culture for sufficient durations to produce commercial quantities of high quality meat tissue. This can be achieved using primary, stem, or intentionally immortalized cells.

2. Where necessary, cells are verified for phenotypic expression of molecular markers that define their tissue origin. For example, cells of muscle origin are verified to display active production of myosin heavy chain.

3. Cells must be induced to grow in suspension within the media without the need for surface adherence to maintain cell viability. This step is referred to as "suspension adaptation"[3] and is necessary to produce large volumes of cells.

4. Following suspension adaptation, verification that the cells can transition to growth on solid substrates and produce an intact/integral tissue of high quality meat represents a final requirement for UPSIDE Foods' cell lines used in integral meat production.

Each of the aforementioned steps is described below in more detail.

4.3.2.1 Immortalization

Predictable cellular proliferative capacity is a cornerstone feature of industrial cell culture technologies. Primary cell cultures (*i.e.*, minimally manipulated cells isolated and grown directly from animal tissues) are difficult to culture at large scale, often require specialized media for growth, often enter senescence prematurely, and critically, are not conducive to large-scale culture, as primary cells have limited proliferative capacity that varies greatly by tissue type. This replicative limit is referred to as the Hayflick limit, a concept proposed by Leonard Hayflick in 1965 (Hayflick, 1965), which states that a normal cell can only replicate and divide a fixed number of times (*e.g.*, less than 40 to 60 population doublings) before it cannot divide anymore and enters a state of permanent senescence and eventually dies *via* programmed cell death/apoptosis. To overcome limitations in proliferative capacity of primary cells, a state of delayed senescence or functional immortalization must be induced in the cell line.

[3] Like most cells in an animal's body, cells typically grow attached to each other to form what UPSIDE Foods calls a tissue (*e.g.*, muscle). Cells grow in this manner to enable cell-to-cell communication and coordinated functional activities as a tissue (*e.g.*, muscle contraction) . Cells can be induced to run latent programming that allows them to grow as single cells or as small groups, called aggregates. This adaptation to "suspension culture" and "individual growth" is reversible and allows UPSIDE Foods to grow many cells in a smaller volume much faster in order to produce tissue (*i.e.*, meat).

This delayed senescence is a process found in normally functioning animal and plant cells. Certain cells possess a natural self-renewal ability while in the body or while in culture. This “functional immortality” in response to cellular senescence can be found in certain plant and animal cells (such as stem cells, germ cells, or transiently amplifying populations). Escape from this replicative limit is routinely observed to occur in many normal and cell types are consumed routinely as a result. Induced immortalization is a safe and reliable process used to delay cellular senescence of cells in culture, and UPSIDE Foods intends to delay senescence in the cells using gene amendments derived from the chicken genome. UPSIDE Foods notes that cells that possess functional immortality or delayed senescence can and still have finite viability in culture or in the body. Immortality here is completely dependent upon the cells being highly supported and monitored by staff at all times as the cells cannot function outside of the body without the aid of UPSIDE Foods’ culture systems.

In contrast to immortalization, transformation is a rare, aberrant cellular state whereby cells often possess an unrestricted growth ability and no longer are able to function within their cellular niche (Chow, 2010). Unlike immortalization, transformation is a distinct cellular state defined by the loss of cellular regulation, specifically in relation to growth and differentiation. While not known to be inherently hazardous in the context of meat consumption, the ability to produce phenotypically stable cells with predictable growth rates and cellular composition are paramount to the safety evaluation processes. Transformed cells are phenotypically unstable and therefore highly undesirable for use in food production. UPSIDE Foods has implemented preventive controls to ensure against this outcome. UPSIDE Foods has identified two central ways UPSIDE Foods can monitor cell populations for (1) a lack of an ability to respond to inductive cues and (2) growth rates outside of specification. Cells within normal specifications will use nutrients predictably in culture, proliferate at a predictable rate, and be responsive to inductive cues by staff.

UPSIDE Foods uses established culture techniques to induce a stable/predictable phenotype of delayed senescence or “functional immortalization” in the cell lines, a process that is achieved through two primary methods: spontaneous immortalization or bioengineering. Cell lines that achieve delayed senescence or functional immortality are documented as new entities, cryopreserved, and progress to downstream applications such as suspension adaptation. It should be noted that cells used in culture are not propagated beyond an established number of cell doublings to ensure that cells used for meat production are used within a “lifespan” range that is known to be phenotypically stable (*i.e*., UPSIDE Foods restricts their working lifespan to a fixed proliferative capacity based on observed performance).

4.3.2.1.1 Immortalization by Spontaneous Method

Certain cell types are amenable to intentionally reactivating endogenous senescence resistance mechanisms, and UPSIDE Foods has developed procedures for selection of these cell types within a population of primary cells.

4.3.2.1.2 Intrinsically Immortalized Cells

UPSIDE Foods may use intrinsically immortal cells (*e.g*., stem cells), as these cell populations typically possess a naturally occurring functional immortality due to an actively maintained chromosomal telomere network. These cells possess an inherent capability to proliferate indefinitely in culture under the correct conditions and only require standard culture adaptations described elsewhere in this submission.

4.3.2.1.3 Immortalization by Bioengineering

Although preference is given to innately immortal cell populations developed through spontaneous or natural mechanisms, some cell types are resistant to spontaneous immortalization techniques (*e.g*., chicken fibroblasts). In these cases, UPSIDE Foods uses well-established, safe methods to bioengineer the cells to intentionally delay senescence through genetic amendments that result in the constitutive expression of the chicken telomerase reverse transcriptase (*TERT*) gene and the subsequent maintenance of the cellular telomeres. To verify successful genetic integration and stability of the introduced genes, several genetic characterization events are conducted on the cell line (see Table 4.3.2.1.3-1). These characterization steps ensure that the intended genes are introduced into the cell at a defined location in the genome and ensure the absence of extraneous DNA (*e.g*., antibiotic resistance genes and other vector backbone components) from the working plasmids.

4.3.2.2 Suspension Adaptation

Primary cell lines and their immortalized progeny are anchorage-dependent, meaning they rely on cell-to-cell and cell-to-substrate interactions for growth. In general, anchorage-dependent growth is a function of available surface area within the culture vessel, while anchorage-independent growth is approximated as a function of volume of the culture vessel. This anchorage-dependence creates surface area limitations that are not conducive to large scale cell culture compared to growth that can be achieved when cells are grown in suspension. While the cells are adapting to suspension cell culture, viable cell density and cell viability (% viability) are monitored until the cells start to expand. Once this occurs, these adapted cell lines are documented, expanded and cryopreserved, and progress to downstream applications.

4.3.2.3 Tissue Formation Screening of Candidate Cell Lines

Manufacturing candidate cell lines are screened for desired stage of growth. Cells are prepared for storage in a cell bank at this point.

Further, cell banks are characterized for adventitious agents, cell species verification, and phenotypic and passage stability. Biomass quantification and growth rate monitoring methods are utilized to measure phenotypic stability.

4.3.3 Cell Line Banking

Once a cell line has passed all of UPSIDE Foods' cell line generation selection criteria, a portion of it is cryopreserved in UPSIDE Foods' cell bank. The MCB is defined as a collection of cryopreserved cells derived from a single tissue source from a single animal. Cell lines in the MCB are determined to be of uniform composition. UPSIDE Foods' MCB system consists of two tiers to allow for the most efficient long-term deployment:

1. The MCB, which is the primary bank
2. A secondary bank termed the Manufacturer's Working Cell Bank (MWCB)

Storage, maintenance, characterization, and culture of cells or cell cryobanks are conducted in-line with the general principles of 21 CFR §610.18(a), specifically, "*in a secure and orderly manner, at a temperature and by a method that will retain the initial characteristics of the organisms and insure freedom from contamination deterioration*" (U.S. FDA, 2020b). These freezers are monitored for any temperature changes to control appropriate storage temperatures for preservation. Cell banks are stored both in a secured location at UPSIDE Foods' production facility and at an off-site location to avoid cell bank losses from local natural disasters or equipment malfunction. The locations, identities, and inventory of banked cell lines are documented and recorded.

The qualification of cell banks is performed either on cryopreserved cells from vials or cell cultures derived from an aliquot of the banked cells, as appropriate. Testing to qualify the primary MCB includes the following:

1. Demonstrating freedom from adventitious agents such as microorganisms/bacteria and zoonotic viruses (Section 4.3.3.1).

2. Verifying species identity of MCB *via* cytochrome oxidase I gene sequencing (DNA barcoding method) (Section 4.3.3.2).

3. Characterizing banked cells for stable DTs and protein yields that are acceptable for meat production (Section 4.3.3.3).

4.3.3.1 Adventitious Agent Testing

The necessary use of animals as a primary source of "raw materials" for the production of CPM requires consideration of the potential transmission and propagation of viruses, microbial pathogens, and prions from the source animal to the CPM. For example, infectious diseases, such as bovine leukemia virus (BLV), are prevalent in cattle and may be transmitted to humans through consumption of infected meat (Ong *et al*., 2021). Prions are infectious neuropathogenic agents responsible for Creutzfeldt-Jakob Disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in cows. Prions have been detected in tissues isolated from the brain, spinal cord, lymphoid tissues, tonsil, appendix, enteric nervous system, and the blood of afflicted animals. These tissue sources are not used for the production of CPM suggesting that the risk of capturing and propagating prions is low; however, as the propagation mechanisms and infectivity thresholds for prions are poorly understood, risk mitigation procedures should be implemented for raw material derived from bovine sources (Ong *et al*., 2021). Animal-derived materials include the biopsy samples used for isolation of primary cells, bovine serum, and animal sources of trypsin used during cell culturing.

Safety considerations for microbial and viral "adventitious agents" as human health hazards originate from the use of mammalian and avian cells in biological drug development. The World Health Organization (WHO) defines adventitious agents within the context of biological products as:

> "*Contaminating microorganisms of the cell culture or source materials including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, Rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents, and viruses that have been unintentionally introduced into the manufacturing process of a biological product. The source of these contaminants may be the legacy of the cell line, the raw materials used in the culture medium to propagate the cells (in banking, in production, or in their legacy), the environment, personnel, equipment or elsewhere*" (WHO, 2013).

UPSIDE Foods has implemented an adventitious agent testing plan into the company's food safety risk framework. In the absence of formal guidance on adventitious agent testing of food, UPSIDE Foods considers the general principles outlined in the WHO and FDA Guidance on testing of adventitious agents (U.S. FDA, 2010; WHO, 2013)[4]. Further discussion of the specific testing rationale for adventitious agent screening is described below as it applies to the safety of cultured meat.

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. Cell banks must be free from microbes, especially food-borne pathogens and zoonotic viruses known to be threats to human health.

[4] Guidance for Industry- Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications (fda.gov)
https://cdn.who.int/media/docs/default-source/biologicals/documents/trs_978_annex_3.pdf?sfvrsn=fe61af77_3&download=true

The MWCB is established directly from the thoroughly characterized MCB. MWCB testing includes sterility and mycoplasma testing but not the full adventitious agent panel and is analyzed each time a new MWCB is created. Cell banks that test positive for any of the listed adventitious agents are immediately destroyed and discarded. These testing results will be used, among other characteristics, to create the Certificates of Analysis for the MCB and will be a preventive control at receiving for the company's plant Human Food Preventive Control Food Safety Plan.

4.3.3.1.1 Microbial Testing Rationale

The testing rationales are detailed in UPSIDE Foods' FDA Preventive Controls (see Appendix A for Process Flow and Hazard Analysis). Sources of risks and rationales noted for each potential microbial concern were chosen from traditional food manufacturing organisms until UPSIDE Foods has baseline data indicating what might be expected during a non-traditional manufacturing process without digestive hazards; human pathogens of clinical importance from these sources include *E. coli*, *Salmonella sp.*, *Campylobacter sp*., and *Listeria monocytogenes* (USDA Chicken from Farm to Table). Since the acceptance criteria for these major pathogens are limited to "non-detect," further discriminatory evaluation for pathogen serovars (*e.g*., *E. coli* O157:H7) is not necessary. In addition to human pathogens, the cell banks are screened for APC, *Enterobacteriaceae*, yeasts and molds, and mycoplasma to ensure that the culture banks are absent of microbial contaminants of safety concern, as well as those that would negatively impact the performance of meat production or lead to spoilage.

UPSIDE Foods sent samples to a third-party laboratory for analysis of microbial contamination *via* two methods:

1. Direct inoculation: If contamination was detected via appropriate methods , these were reported by cell counts of bacteria or yeast/mold. Each run included appropriate controls.

2. Polymerase chain reaction (PCR) analysis: Each run contains positive and negative controls alongside internal controls to monitor nucleic acid extraction and PCR efficiency.

   For mycoplasma, samples were sent to a third-party laboratory for testing using highly sensitive real-time PCR assay that can detect as few as 1 to 10 organisms. Every run included positive and negative controls that showed expected positive and negative results.

All results were negative for mycoplasma and microbial testing. Positive controls were positive and negative controls were negative. Overall, UPSIDE Foods detected no microbes or mycoplasma of concern.

4.3.3.1.2 Viral Testing in Pre-Production & Production. Rationale

Viral testing regimens are determined based on (1) the tropism of the virus and (2) the potential for exposures to viral agents. Viruses that are capable of infecting avian cell populations or are capable of crossing the avian-human species barrier are tested in the MCB. In an avian system, human-tropic specific viruses are not tested for, as they are generally incapable of infecting avian cells (*i.e.*, mammalian viruses generally infect other mammals in the same way avian viruses generally infect other avian systems). Since UPSIDE Foods is growing avian cells within an aseptic system, UPSIDE Foods therefore presents the risk as negligible for human-tropic viruses. However, UPSIDE Foods does test for known zoonotic viruses capable of human pathogenesis or latent infection, specifically, avian/human-tropic viruses.

When non-avian animal components are used in the generation of the banked cell lines and/or the production of the tissue itself, UPSIDE Foods tests for appropriate viruses of concern based on the species of origin for the component used, accounting for potential exposure to the component. If only avian cells are used and no other animal-derived component is used during all stages of production, UPSIDE Foods would limit UPSIDE Foods' viral surveillance to avian and avian/human-tropic viral agent surveillance.

4.3.3.2 Species Verification

A small region of the mitochondrial cytochrome c oxidase I (COI) gene is used for species identification in animals (Hebert *et al*., 2003), which the Consortium for the Barcode of Life uses to identify species of origin (Consortium for the Barcode of Life). This species identification method is currently used by FDA to verify different fish species (Single Laboratory Validated Method for DNA Barcoding for the Species Identification of Fish – U.S. FDA, 2011).

Cells are harvested and lysed to extract mitochondrial DNA.

4.3.3.3 Phenotype/Passage Stability Verification

Information demonstrating the phenotypic stability of the cell lines are obtained to ensure that analytical data from finished product testing are reliable and would be representative of long-term commercial production. To determine phenotypic stability of a MCB line over a manufacturing process duration is verified in the following manner. Cells are cultured in three independent cultures to determine if DT and tissue production over time are stable. On a predetermined schedule (*e.g.*, 3 days), cells are counted and expanded into new cultures at a consistent starting cell density. The population DT is calculated at each passage to establish and monitor phenotypic stability. Periodically cells are analyzed for protein yield.

4.3.3.4 Manufacturing Working Cell Bank

The secondary bank or MWCB is a cryopreserved cell bank derived from one or more vials of cells from the MCB, which are expanded by serial subculture. This bank is established as the initial generated manufacturing cell bank used to create UPSIDE Foods' seed train (inoculum). Viruses that could be introduced to the bank from animal exposures have already been conducted on the MCB and no animal

components are used to establish this bank. The banks that meet specifications are stored in liquid nitrogen and can be used, depending on bank size, to manufacture UPSIDE Foods' product.

When new MWCBs are generated, the cell bank generation will follow the general principles outlined in 21 CFR § 211.194 for laboratory records (U.S. FDA, 2020c):

- A description of the thawed MCB
- A description of major processes involved in subculturing and cryobanking of MWCBs.
- A complete record of testing for microbial adventitious agents and species identification.

Until guidance for cultured foods is available, batch information will follow the general principles outlined in 21 CFR §211.188 (U.S. FDA, 2020d):

4.4 Stage 2 – Cultured Poultry Meat Production

The production of cultured meat employs a biologic process to induce integral tissue formation through conversion of media components into edible biomass. This involves successive culturing of small batches of cells, referred to as a seed train, to achieve an adequate number of cells to initiate large scale cell cultures, and then seeding them into a cultivation vessel at a specific density. Tissue is formed and matured by supporting the growth of and protein production by the cells through exposure to, uptake of, and metabolizing of nutrient components in the cell culture media in a process referred to as "bioconversion." After an integral tissue sheet is generated, it is harvested from its culture vessel and prepared for application as the final product. In each step, proprietary cell culture media have been developed by UPSIDE Foods to provide the necessary nutrient raw materials to the cells and optimize expansion of the cells and formation of a high-quality tissue product. The production process can be executed at different scales using different culture vessels for production, based on the need for product quantity.

4.4.1 Culture Media and Food Contact Articles

UPSIDE Foods has developed a proprietary cell-culture medium serving as its base media platform. The cell culture medium consists of common compounds found in animal feed and human food including amino acids, fatty acids, sugars, trace elements, salts, and vitamins. UPSIDE Foods' process requires growing the cells over days, during which time these components are metabolized and used for the fundamental nutritional requirements of the cell for its maintenance, proliferation, or as the fundamental building blocks of the tissues. This is similar to the way animals digest food and distribute the macro- and micronutrients in the bloodstream to cells to both produce and maintain parts of the body, and in this case, muscle tissues. The majority of the ingredients are naturally occurring nutritive substances that are metabolized and biosynthesized naturally by poultry. Additionally, this majority of culture media agents are similar to those used in traditional food fermentation technologies for the production of food microorganisms, algae products, and ingredients produced by fermentation, which have a long history of safe use in food production. Through calculation, UPSIDE Foods demonstrates that media components used in the pre-banking stage dilute out to below the threshold of toxicological concern (if such a limit exists) in the subsequent scaled culture and that the level of any pre-bank media components remaining in the finished product to be well-below the limit of detection (LOD).

Culture media aids will be food grade quality and, where applicable, meet a relevant quality specification standard [*e.g*., *Food Chemicals Codex* (FCC), United States Pharmacopeia (USP)]. Culture media aids that are not yet available commercially as food grade will be subject to UPSIDE Foods' internal quality assurance and food safety systems, including hazard analysis for biological, chemical, and physical hazards, to establish appropriate food grade quality specifications and controls for the ingredient. UPSIDE Foods current Good Manufacturing Practice (cGMP) programs include a robust raw material food safety review for biological, physical, or chemical agents. If the available information characterizing the hazard of a substance results in uncertainty of food-safe consumption, a more thorough review would be conducted, documented, and determination of safety would be required prior to use in the food system. No extracellular matrices/scaffolding materials are added nor required for growth of the meat.

As previously discussed, the majority of media components are highly similar to fermentation nutrients with a long history of safe use in food manufacturing (*e.g*., glucose, minerals, trace elements, vitamins); these culture media, when used for their intended purpose, are generally considered safe for use in food fermentation. In addition to these common macro- and micronutrients used to support cell growth, several media protein components (*e.g*., bovine serum albumin, growth factors) are required for sustaining cell viability and growth during the culture process. These protein components serve the same function as their natural counterparts (*e.g*., growth factors) that sustain growth of muscle tissues in the animal and therefore are expected to be present within the cultured meat tissue at concentrations that are similar to levels of the protein that can be measured within tissues obtained from conventional meat from an animal carcass (*i.e.*, chicken breast). A more comprehensive discussion of the safety of media protein components is presented in Section 6.2.

Media agents have the potential to alter the composition of the finished food to be more similar to conventional meat counterparts or to produce a product that has new attributes that fall outside the normal range of the conventional counterpart. Recognizing the potential impact of the media culture technology on food composition, UPSIDE Foods has developed a food safety framework for evaluating new media components. The primary intent of this framework is to identify changes that may impact the regulatory status and safety of the finished food. Further discussion of this framework is presented in Section 6.0.

4.4.1.1 Mitigating Risks Associated with Cell Culture Media

4.4.1.1.1 Incoming Raw Material Receiving Testing and Handling

All incoming raw materials for use in cell culture media undergo several levels of testing and verification depending on the specific media component, as discussed in Section 4.1.

Following QA acceptance, incoming raw materials are stored in the appropriate location in the component warehouse according to the standard operating procedure. All raw materials are stored under the proper conditions to preserve identity, strength, purity, quality, *etc*. Additionally, expiration dates are tracked using the supplier expiration date, assigned expiration date, or re-test date in the system as an added level of precaution to ensure expired materials are not used in production. A variance program will be in place if materials can be tested and a risk assessment performed to extend raw material usage dates.

4.4.1.1.2 Cultured Media from Third-Party Vendor

UPSIDE Foods will source material for culturing from third-party vendors at certain times to meet production capacity needs. These suppliers of cell culture media and supplements have expertise in cell culture formulation requirements, a history for using quality raw materials, and processes to control and manage those raw materials. UPSIDE Foods qualifies its suppliers by assuring that they meet specific risk-based criteria, as described in UPSIDE Foods' supplier management program (see Section 4.1). UPSIDE Foods' qualification ensures that third-party lots will use components that meet similar specifications to UPSIDE Foods' own raw components. This also ensures consistency in safety and performance between outsourced lots and those made in-house.

4.4.2 Cell Seed Train

For production of the seed train, one or more vials of cryopreserved cells from a qualified cell bank are thawed and placed into sterile culture medium in an appropriately sized vessel for the initial culture step or passage. During this and all future seed train passages, the cells are allowed to divide through cellular mitosis, consuming nutrients from the media to increase cellular biomass through proliferation. Vessels for seed train cultivation can be either pre-sterilized disposable food grade plastic or appropriate food grade reusable vessels that are cleaned and sterilized between uses. The suspension seed train cultures are agitated so that the cells are suspended in a homogeneous mixture within the liquid culture medium inside the vessel. When adherent seed train cultures are used for small batch production, cells are seeded at a desired cell density and kept with liquid culture media inside the vessel. Culture vessels for the initial passage may be maintained in an incubator with defined environmental controls such as temperature and external gas concentrations such as CO_2. Culture vessels may also be associated with a controlled bioreactor capable of maintaining suitable ranges for temperature as well as pH and dissolved oxygen concentration.

4.4.3 Integral Meat Tissue Maturation Stage

Up to this point in UPSIDE Foods' meat production process, biomass increases have been a function of cellular proliferation. During the maturation phase, cells also begin to differentiate and add biomass through both proliferation and hypertrophy. Cells obtained from the seed train are used to initiate the tissue maturation stage where they will continue to proliferate as well as differentiate or mature into the final CPM product. During that time, the culture medium is supplemented or exchanged to provide additional nutrients or key factors that aid in the cultured meat formation. During tissue formation, there may be continued cell division, increased individual cell mass, or differentiation in a manner consistent with tissue formation in an animal.

Depending on the desired scale of production, vessels for tissue formation can be either disposable food grade plastic or stainless steel vessels that can be cleaned and sterilized between uses. Smaller pilot-scale culture vessels may be maintained in an incubator with defined environmental controls such as temperature and external gas concentrations.

For the largest scale production, UPSIDE Foods has developed a proprietary meat cultivation vessel to allow for efficient scaling of tissue formation.

4.4.4 Integral Meat Tissue Harvest

At the conclusion of the tissue formation batch stage, the integral tissue is ready for harvest. The process of "harvest" occurs when the tissue/cells "*are removed from a sealed growth environment and prepared for traditional food processing*," *FSIS, Food Made with Cultured Animal Cells*[5]. The process occurs when the closed vessel is opened for drying, at which point the tissue/cells are removed from a closed environment and prepared for processing. This step is the transition from FDA manufacturing to USDA oversight of the processing integral poultry. The meat is removed from the bioreactor system using washes and mechanical processes and collected in a wash basin. These meat tissues are then washed to remove remaining culture media. Following the wash, the moisture level of the meat tissues is reduced to render them most suitable for consumer product formulation. All operations will be performed in clean equipment under temperature-controlled conditions.

4.5 Culture Process Controls

4.5.1 Contamination Controls

During all stages of cell cultivation for seed train and the tissue formation stage, UPSIDE Foods ensures that the cultures remain free of contaminating bacteria, yeast, molds, or fungi. Cultures grown in small-sized vessels may be taken into an appropriate, high-efficiency particulate air (HEPA)-filtered, biological safety cabinet for any open operations such as sampling or subcultivation procedures. Operators performing these operations will have been trained on the appropriate aseptic practices. Cultures maintained in suspension bioreactors or the meat cultivation vessels use aseptic procedures that effectively minimize the risk of introducing contaminants. Samples will be taken from the cultures periodically and tested using a qualified assay to inspect for contamination. 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.

4.5.2 Growth Environment Controls

The environmental conditions of the cultures are controlled to ensure efficient growth of the seed train cells and production of the final meat tissue.

4.5.3 Harvested Product Controls

Following removal from the meat cultivation vessels, the CPM product will be maintained in clean equipment under cold processing conditions for the moisture adjustment and storage. Samples of the meat product will be taken periodically and tested using a qualified assay to inspect for contamination.

4.5.4 Batch History Controls

Appropriate records will be completed to provide traceability of all raw materials used, operations executed, and samples tested throughout the manufacturing process.

[5] https://www.fda.gov/food/food-ingredients-packaging/food-made-cultured-animal-cells

All UPSIDE Foods' Food Safety and Quality Systems comply with 21 CFR part 117 and the GFSI standard. These systems include a Food Safety Plan that has a foundation in UPSIDE Foods' prerequisite programs to mitigate hazards that could affect the safety of UPSIDE Foods' food.

4.5.5 Specifications

Batch release specifications have been developed for UPSIDE Foods' CPM product to ensure that a safe and suitable product is released for further food processing in a USDA regulated facility. Qualitative and quantitative attributes define the ingredient identity and composition are consistent from lot to lot.

5.0 Identity Verification, Composition, and Impurities

A key objective of this submission is to provide information characterizing the compositional identity of CPM relative to appropriate comparator products. Chicken meat is consumed in part for its organoleptic pleasure, and more broadly for its nutritional value as a source of high-quality protein. The compositional analyses therefore focused on measuring the complete nutritional profile of major nutrients (protein and amino acids, fats, carbohydrates, minerals, and vitamins) and also considered analysis for potential environmental contaminants (*e.g*., heavy metals, microbial contamination), and where relevant, residues of media components in the finished product. When selecting an appropriate comparator, it was recognized that chicken meat is a naturally derived whole food and therefore will display some degree of variability in composition that is influenced by animal breed, age, the environmental conditions of their rearing, and diets of the birds. Comparator data should also consider the reliability of the analytical methods used to generate compositional data and such information should be obtained from validated methods.

For quantitative comparisons to conventional chicken, UPSIDE Foods has constructed a comparator database using data from the USDA Agricultural Research Service (ARS) Food Data Central (FoodData Central, https://fdc.nal.usda.gov/; accessed Q1 2019). This database was used to examine the composition of the company's product against relevant conventional poultry standards. Comparator data were included for 1white and red muscle products, including those with and without skin. Data from offal and bones were not included in UPSIDE Foods' comparator dataset. Where necessary, supplementary data on poultry meat composition was obtained from literature sources. UPSIDE Foods also conducted ELISA-based assays for meat species identification in accordance with USDA FSIS Microbiology Laboratory Guidebook, Title: Identification of Animal Species in Meat and Poultry Products (see Section 5.1) (USDA, 2005).

UPSIDE Foods' cell-culture process can be carried out using serum-free or serum-containing media. UPSIDE Foods has presented compositional data for CPM produced in serum-containing and serum-free media. As the safety evaluation is based to a large degree on compositional comparisons of CPM relative to conventional comparator products, analytical data on CPM grown with and without serum are presented to evaluate the impact of growth media on cultured meat composition. Additional data is presented using a larger set of samples taken from historical production runs throughout UPSIDE Foods' product development experience produced across multiple cell lines and media composition to further provide an understanding of the inherent variation associated with the technology.

Overall, findings from UPSIDE Foods' compositional testing have demonstrated a high degree of similarity across all components between UPSIDE Foods' product and USDA nutritional measures obtained for conventionally produced skinless white meat chicken. The results of compositional analyses for protein and

amino acids demonstrated the most significant degree of consistency between cultured meat products and USDA data on comparator products, a finding that is expected and is attributed to the fact that the muscle composition (*e.g*., myosin and tropomyosin) is genetically defined. In contrast, greater variability in lipid composition was observed with changes to the media composition. As chicken does not provide a meaningful source of fat in the diet, slight variations in lipid composition are not expected to be of nutritional significance. Slight differences in various vitamins and minerals were noted between the cultured meat products and the USDA database samples; however, the levels fell within the normal ranges for conventional chicken meat or within levels that have been reported in other commonly consumed foods. No nutritional deficiencies or levels of nutrients in excess were observed.

5.1 Meat Species Verification

To confirm the animal species identity of the finished product. The goal was to demonstrate, using the FSIS gold standard method as outlined in Identification of Animal Species in Meat and Poultry Products (USDA FSIS Microbiology Laboratory Guidebook), and to verify the species specificity of CPM by analyzing the tissue against ELISA assays for other food species (USDA, 2005).

Samples included the three non-consecutive lots of CPM that were produced using serum (serum-containing CPM) and the three non-consecutive lots of CPM that are serum-free (serum-free CPM). A ground raw chicken sample was assayed alongside the CPM samples. A saline control was also prepared under the same conditions as the meat samples.

Like conventional chicken, the CPM samples all yielded a positive result for poultry and a negative result for others. All assays were valid per USDA criteria as indicated by the positive and negative controls. Overall, the CPM yields the appropriate species response using the gold standard FSIS test for animal species determination of cooked meat samples. Thus, the CPM is poultry.

5.2 Meat Protein Identity (Myogenicity)

The identity of chicken meat is determined using meat biomarkers. Chicken breast muscle is over 99% fast white muscle, and *alpha*-fast isoforms of tropomyosin are a major characteristic structural protein of chicken breast meat (Matsuda *et al*., 1983). Tropomyosin chain is a protein that in chickens is encoded by the TPM1 gene. This gene is a member of the tropomyosin (Tm) family of highly conserved, widely distributed actin-binding proteins involved in the contractile system of striated and smooth muscles and the cytoskeleton of non-muscle cells. UPSIDE Foods therefore uses the expression of fast-tropomyosin in CPM as a signifier that CPM has protein expression that is enriched in meat.

5.3 Proximate Composition

The moisture, total protein, crude fat, ash, carbohydrate content, and total calories of the CPM were evaluated. The pH of the product was also measured (see Table 5.3-1). The macronutrients of CPM are compared to published data on conventional chicken meat cuts found in the USDA ARS FoodData Central database. In this section (see Figure 5.3-1), CPM data are shown across two major categories, which include (1) data collected from lots of CPM generated using “serum-containing CPM,” and (2) data collected from lots of “serum-free CPM.”

Data observed in lots of CPM produced using serum and the lots of serum-free CPM products and comparing CPM results to the USDA ARS FoodData Central database, UPSIDE Foods chose two categories from conventional chicken: (1) All USDA chicken (no organs), which includes data from 27 different published samples ranging across 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; and (2) USDA chicken white meat (no skin), which includes data from four published samples specified as light meat, meat only.

Table 5.3-1 Proximates, Calories, and pH Results for Triplicate Lots of Serum-containing CPM and Serum-free CPM

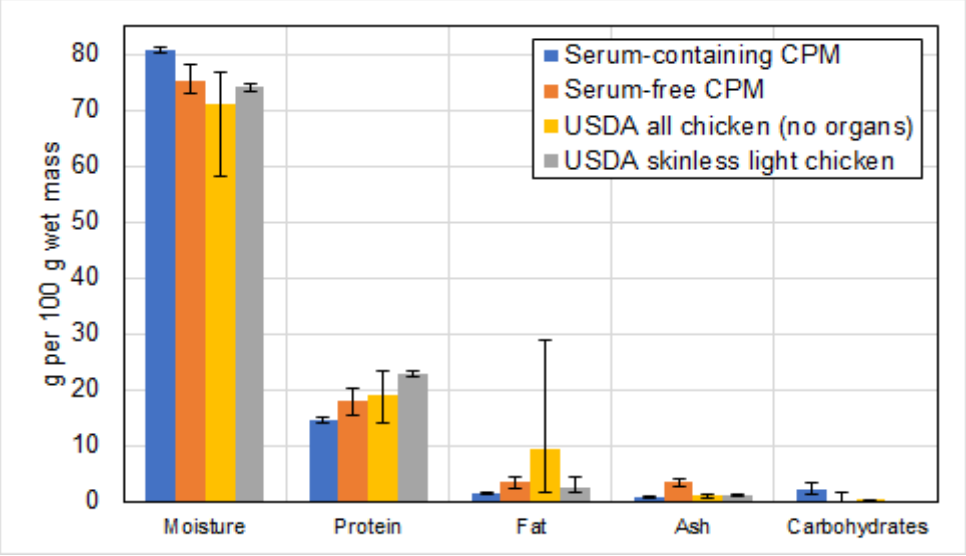
Parameter	Units	Serum-containing CPM						Serum-free CPM					
		Lot 1	Lot 2	Lot 3	Avg.	Min.	Max.	Lot 1	Lot 2	Lot 3	Avg.	Min.	Max.
Moisture	g per 100 g	80.36	80.99	81.31	80.89	80.36	81.31	78.13	73.07	74.11	75.10	73.07	78.13
Protein	g per 100 g	13.94	15.10	14.55	14.53	13.94	15.10	15.28	20.14	18.65	18.02	15.28	20.14
Fat	g per 100 g	1.59	1.64	1.30	1.51	1.30	1.64	2.21	4.37	3.96	3.51	2.21	4.37
Ash	g per 100 g	0.78	0.98	0.92	0.89	0.78	0.98	2.79	4.09	3.24	3.37	2.79	4.09
Carbohydrates	g per 100 g	3.33	1.29	1.92	2.18	1.29	3.33	1.59	0.00	0.04	0.00	0.00	1.59
Calories	kCal per 100 g	83.39	80.32	77.58	80.43	77.58	83.39	87.37	119.89	110.40	105.89	87.37	119.89
pH	-	6.40	6.09	6.39	6.29	6.09	6.40	5.76	6.02	5.95	5.91	5.76	6.02

CPM = cultured poultry meat.

Comparing CPM to Conventional Chicken

Analytical results for serum-containing CPM production lots, serum-free CPM production lots, as well as chicken data from the USDA FoodData Central database [both “all USDA chicken (no organs)” and “USDA skinless light meat”], are observed within the range of “all USDA chicken (no organs)” while the lean nature of CPM indicates a fat content closer to skinless white meat chicken. The pH of the CPM samples observed indicated a chicken-like pH, where the pH of fresh chicken meat post-slaughter is 5.3 to 6.5 (Hertanto *et al*., 2018).

Figure 5.3-1 **Proximate Results Shown as the Average for the Triplicate Serum-containing Production Runs, Triplicate Serum-Free Production Runs, USDA All Chicken, and USDA Light Chicken**



CPM = cultured poultry meat; USDA = United States Department of Agriculture.
Error bars represent minimum/maximum values.

Overall, there is low lot-to-lot variability for triplicate non-consecutive production runs (both serum-containing and serum-free). CPM exhibits macronutrients that are within the range of conventional chicken, specifically closer to a lean cut. The CPM is also within the pH range of conventional chicken.

5.4 Nutrient Composition

Nutrient composition of UPSIDE Foods' cultured chicken has been analyzed and is within expected and is observed within safe ranges.

5.5 Microbial Contaminants and Environmental Impurity Testing

Analyses for microbial and heavy metal contaminants were conducted for 3 lots of CPM produced in the presence or absence of serum.

Table 5.5-1 summarizes the results for microbial and heavy metals testing for CPM samples (triplicate serum-containing and triplicate serum-free lots); data are also shown for a ground chicken sample that was sent for analysis alongside the CPM samples. Overall, the CPM is free from microbes detectable through APC, *E. coli*, coliforms, *Enterobacteriaceae*, yeast, mold, and *Salmonella*, whereas conventional raw chicken yields higher aerobic plate counts, detectable coliforms and *Enterobacteriaceae*, and a confirmed positive result for Salmonella. Regarding the heavy metals lead, arsenic, cadmium, and mercury, levels in the CPM product are below any concentrations that would lead to safety concerns.

Table 5.5-1 Results of All Microbial and Heavy Metals Testing for CPM (Triplicate Production Runs for Each Serum-containing and Serum-free) and a Conventional Chicken Sample

Parameter	Unit	Serum-containing CPM			Serum-free CPM			Ground Chicken
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	
Lead (Pb)	µg per 100 g (20% solids)	1.32	1.49	2.27	4.69	5.03	4.56	0.25
Arsenic (As)	µg per 100 g (20% solids)	1.14	1.23	1.00	<0.50	<0.50	<0.50	<0.50
Cadmium (Cd)	µg per 100 g (20% solids)	0.70	1.24	0.49	1.32	1.65	1.46	<0.13
Mercury (Hg)	µg per 100 g (20% solids)	<1.30	<1.30	<1.30	<1.30	<1.30	<1.30	<1.30
Aerobic plate counts	cfu per g	<100	<100	<100	<100	<100	<100	8600
Coliforms	cfu per g	<10	<10	<10	<10	<10	<10	10
E. coli	cfu per g	<10	<10	<10	<10	<10	<10	<10
Enterobacteriaceae	cfu per g	<10	<10	<10	<10	<10	<10	50
Mold	cfu per g	<10	<10	<10	<10	<10	<10	<10
Yeast	cfu per g	<10	<10	<10	<10	<10	<10	<10
Salmonella	Positive/negative per 25 g	Negative	Not measured	Not measured	Negative	Not measured	Not measured	Confirmed positive
Salmonella	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured
Enterobacter cloacae complex	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured
Influenza Type A	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured
Influenza Type B	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured

cfu = colony forming units; CPM = cultured poultry meat; ICP-MS = inductively coupled plasma mass spectrometry; rt-PCR = reverse transcription polymerase chain reaction.

5.6 Shelf Life

Shelf life of a product as defined by Refrigerated Foods Association (RFA) Standardized Protocol for Determining the Shelf Life of Refrigerated Ready-To-Eat (RTE) Foods, 2009, as "*The period of time at the end of which the quality of a given food product is perceived as significantly, unacceptably different from the expected 'fresh' quality*". When determining shelf life, both microbial and sensory qualities of the product will be taken into consideration. Because this is a product with no established shelf life, UPSIDE Foods is designing the study to look at both quality attributes and microbial characteristics for food safety. This study will take place once the final formulation is completed and set for production.

Sample Preparation: CPM from three different production batches will be prepared for shelf life evaluation. In order to determine shelf life of UPSIDE Foods' CPM, CPM from each production batch will be evaluated separately following the protocols below. Overall shelf life will be determined based on the evaluation from the three different production batches.

To simulate storage conditions, CPM will be individually vacuum packed (25 g/unit and 40 g/unit) for shelf life study.

Determination of Product Microbial Shelf Life: At each sampling will be removed from the storage. The microbial quality of the sample will be measured.

Determination of product sensory quality shelf life: At each sample point, microbial quality of the stored samples will be determined first and, if the product is considered acceptable for food safety (based or regulatory requirements) after microbial analysis, then the control samples will be analyzed for sensory acceptability. To prepare the samples for sensory evaluation, samples will be thermally processed and three trained panelists will analyze the aged product for sensory acceptability. Panelists go through a comprehensive training program established by UPSIDE Foods' Senior Sensory Scientist and are approved to be on the panel, prior to tasting for this study. To determine the acceptability of the samples, the panel will be instructed to rate each sample.

Determination of Product Overall shelf life:

- Overall shelf life of UPSIDE Foods CPM is determined using microbial and sensory quality testing the cultured raw products.
- Initial cut-off point for shelf life is determined by microbial shelf life data collection and review. If the sample passes microbial acceptance for food safety but does not pass sensory acceptance criteria, the cut-off point for shelf life will be determined by sensory quality.
- Considering the variability of CPM and potential temperature fluctuation during storage (during normal allowable processing and storage variability), the shelf life will be set at two-thirds the cut-off point for microbial loads, or limit for sensory quality. For example, if the accepted shelf life is 6 weeks, then the approved shelf life would be 4 weeks. This allows for storage temperature variation, product variation and variation on consumer handling.

6.0 Additional Safety Information

6.1 Risk Assessment Framework for Gene Amendments

DNA is the foundational instructions for all life and is therefore encountered in every foodstuff derived from living organisms unless it is intentionally removed or degraded. Together with its molecular sibling, RNA, DNA itself is a non-trivial fraction of a cell's mass, and digested nucleic acids from food sources are a significant source of important key micronutrients such as phosphorus and nitrogen for humans. Due to its ubiquity and foundational necessity for life to exist, the nucleic acid family is an essential fraction of food and under historical consumption standards is safe and suitable for human consumption. FDA has recognized DNA and other nucleic acids as generally recognized as safe for this reason.

> "*Nucleic acids are present in the cells of every living organism, including every plant and animal used for food by humans or animals, and do not raise a safety concern as a component of food. In regulatory terms, such material is presumed to be GRAS* [Generally Recognized as Safe]" (U.S. FDA 1992).

Gene amendment and expression systems represent a common and powerful method to redirect cellular resources, often for the purpose of seeking a specific cellular phenotype. This desired phenotype may or may not be within the cell's functional repertoire. Broadly, two categories of genes can be thought to drive whether the phenotype will be within the cell's normal functions or if it will be a result of a function new to the cell. For the former, such approaches typically garner enhancement or suppression of phenotypes that are already native to the organism, while the latter

approaches inherently yield outcomes typically outside the natural variation or genetic capabilities of the organism (*e.g*., Yeast expressing leghemoglobin from soybeans or food plants that express herbicide resistance genes from *Agrobacterium* sp.). Desired phenotypes that are within the cell's normal capacity are often derived through the use of genetic elements native to the species, while DNA from other sources can potentially result in new or novel traits added to the cell. Regardless of which method is used, the goal is to produce functions of viable cells and tissues for use as food of high quality and in large quantities.

UPSIDE Foods has categorized the potential hazards associated with genetic insertions into cultured meat production systems. The potential hazards form three distinct categories to define the associated risk of use, termed "A," "B," or "C", defined below. For UPSIDE Foods' purposes, the use of C-category genes is avoided, thus limiting the usage to both A- or B-category genes. Risk of harm is then identified in relation to the ability to (1) be able to predict the direct and indirect effects of the expression product's use, (2) the potential novelty of the expression product within the cell or tissue, and (3) the intentional introduction of proteins that are capable of inducing recombination events. While not necessarily inherently harmful, C-category genes result in the highest potential risk of harm based upon this assessment while both B and A categories represent low risk events.

Categorical Hazards Genetic Changes:

- *A-Category Hazard:* Intentional gene amendment(s), which lead to enriching or depleting expression Zof a cis-protein[6].
    - *Hypothetical Example: Introduction of a gene editing cassetie to delete the wild-type, native EGF locus.*
- *B-Category Hazard:* Intentional gene amendment(s), which lead to protein expression, resulting in an intentionally altered function from its wild-type state (can be either from the same organism or different biological sources).
    - *Hypothetical Example: Introduction of codon-optimized bovine FGF2 to bovine cell population for expression.*
- *C-Category Hazard:* Intentional gene amendment(s), which lead to a virus or other protein expression systems without a known cellular homologue or function capable of inducing recombination events.
    - *Hypothetical Example: Use of viral genes to introduce a foreign gene or intentional insertion of viral genes to add novel function to cells.*

In addition to genes introduced into cells, UPSIDE Foods' risk evaluation requires review of other elements introduced or altered in the cell's genome during the genetic amendment process (*e.g*., regulatory and selectable markers). Most of these elements originating from the insertion vector fall

[6] Cis-proteins, or "endogenous proteins," are proteins that are found naturally in a cell.

into the C category of hazards; these will be removed prior to manufacturing the cell product during the pre-production phase. Evidence of non-target gene removal is performed.

UPSIDE Foods' argument for the risk mitigation to produce meat rests on a few key premises: First, food derived from gene amendments as a tool is federally recognized as not inherently harmful (51 FR 23302). Second, UPSIDE Foods mitigates potential hazards by using genetic elements that are commonly consumed. Third, UPSIDE Foods uses genetic regulatory elements and gene coding sequences that are native to the cell and therefore encode for proteins that are endogenous to the animal. Accordingly, these proteins are identical to their native counterparts in food routinely consumed by humans. And finally, the protein will be denatured and functionally inactivated during the cooking process and further hydrolyzed to its constituent amino acids in the gut during digestion.

UPSIDE Foods notes that current policy is to evaluate foods produced using intentional genetic amendments using a case-by-case process to identify the hazards present and implement controls that mitigate risk derived from those expression events in the finished products.

6.1.1 Genetic Amendment Methods

Amendment and expression practices from within the species of interest (chicken) fall on the same risk spectrum as assisted reproductive technologies (ARTs); that is to say the risk is acceptably low and well-understood due to the manner in which genes move through a population of cells. As is the case for artificial insemination, selective breeding practices, and other human-directed gene flow, risk is controlled by the movement of allele frequency, not necessarily allele identity. Hazards relevant to these practices relate to the tools or the methods, but generally not the genes themselves.

UPSIDE Foods uses approaches that are defined as those alterations that increase gene flow within a single species or population rather than across species boundaries (Schouten *et al*., 2006). Such approaches are used safely and effectively in numerous formats and are most commonly encountered in regulatory aspects such as ARTs. However, in the same way the ARTs methods themselves have the potential to introduce hazards to the finished product, amendment methods too must be subjected to hazard identification.

It is UPSIDE Foods' position that the intentional genomic amendment of poultry cells through introduced genes results in a safe and suitable alternative to conventional poultry meat from a chicken carcass. Intentional amendment of the cells results in the emergence of a single trait of delayed senescence or "functional immortality" as a result of the use of a single gene. UPSIDE Foods' approach reactivates an endogenous, naturally occurring cellular pathway found in normal tissues to maintain tissue homeostasis.

Within typical selective animal breeding, intentional amendment to the genome through breeding results in significant and indeterminate gene flow of both desired and undesired traits in the progeny. If the trait sought in an animal is caused by a single genetic locus, such as that resulting in the

polled cattle phenotype, the goal is selective breeding within an increasingly stable gene pool to ensure a stable target phenotype while minimizing undesirable alleles from flowing in. By omitting breeding and instead using well-established genetic amendment and expression methods, desired phenotypes can be achieved without gene flow. Here, UPSIDE Foods uses this concept to produce functionally immortal cell populations.

Functional immortality (delayed cellular senescence) can be achieved in a number of ways. One way is through adaptive or introduced genotypic changes that result in the constitutive expression of *TERT* (Zhao *et al*., 2009). TERT is a component of the telomerase enzymatic complex, which is responsible for the maintenance and lengthening of telomeres in normal tissue homeostasis (Zhao *et al*., 2009). As such, UPSIDE Foods has chosen to use this native telomere maintenance mechanism to extend cell replicative potential through stable expression of *TERT*. TERT has not been known to induce uncontrolled proliferation as the gene is subject to normal endogenous gene networks to delay, but not eliminate, normal cellular senescence (Holt *et al*., 1996). As discussed previously, this ability does not prevent cell death. The introduced gene is functionally limited to the constraints of the normal cellular control mechanisms of the cell, which have not been altered. The growth requirements, cell-cycle checkpoints and karyotypic stability in telomerase-expressing cells are very similar to comparator cells in their native state. UPSIDE Foods further continues to monitor the cells' phenotype over time, to ensure the cells retain a stable phenotype. The potential pleiotropic effects of the inserted locus are limited to the senescent mechanism inside the cells.

6.1.2 Consideration of Pleiotropy and Off-Targeting

The possibility that introduced genetic events may lead to unintended effects on the phenotypic properties/composition of the organism has been a long-standing concern in food safety risk assessment of gene engineering technologies (Ladics *et al*., 2015; Fernandez and Paoleti, 2018). Unintended changes can, in theory, materialize as a consequence of gene insertion, from random mutations occurring during the tissue culture process or from pleiotropic effects of the introduced protein. Findings over the last 20 years of research, however, have demonstrated that unintended compositional effects that could be caused by genetic amendments have not materialized (Herman and Price, 2013). This conclusion is consistent with observations by UPSIDE Foods that naturally occurring transgenic events routinely occur in poultry without evidence of significant deleterious effects to the animal or its lineage of descendants. For example, in nature, the integration of DNA from retroviruses (known as endogenous retroviral fragments) into the chicken's genome in a manner that is passed vertically from one generation to the next is endemic within poultry consumed as food without known pleiotropic or other deleterious effects; many of these integrations are even translated into protein (Bolisetty *et al*., 2012).

To monitor performance over time and minimize against the chances of a potential off-target event, UPSIDE Foods has determined the copy number and removed vector backbone components, that is, all components remaining in the cells are native to chicken.

Finally, the possibility that an amendment event may induce expression of a latent toxic substance is limited to microorganisms and plants because of their unique evolutionary history as compared to animals. Plants and microorganisms evolved a diverse strategy of toxin production to defend against predators. Animals by and large did not evolve toxin production as a defense mechanism, settling instead primarily on motility, appendage

tools, evasive or camouflage behaviors, fighting/flight behaviors, and in small fraction, specialized toxin organs such as venom sacs in snakes. In short, animals, and by extension, animal cells, traditionally consumed as food do not typically harbor nor produce toxins. Overall, UPSIDE Foods considers potential harmful effects of off-targeting and potential pleiotropy to be minimal to non-existent in animal cells.

6.2 Risk Assessment Framework for Cultured Media

UPSIDE Foods has developed a proprietary cell-culture medium that contains nutritional ingredients consisting of common compounds found in animal feeds and human food including amino acids, fatty acids, sugars, nucleotides, trace elements and vitamins. Cell culture media proteins also are used at various stages of the cell banking and meat production process. No antibiotics or antifungal agents are used during the meat cell production stage. During the meat production phase, the cells are cultivated for days, during which time the media components are metabolized and used for the fundamental nutritional requirements of the cell for its maintenance, proliferation, or as the fundamental building blocks of the tissues. The metabolism of culture nutrients to sustain growth is under control of the cell in a similar manner to the way animals digest and distribute the micronutrients in the bloodstream to cells to both produce and maintain parts of the body, and in this case, muscle tissues. The majority of the ingredients are naturally occurring nutritive substances that are metabolized and synthesized by poultry. In addition, the vast majority of culture media agents are very similar to those used in traditional food fermentation technologies for the production of food microorganisms, algae products, and ingredients produced by fermentation, and have a long history of safe use in food production (Kampen, 2014; Walker, 2014). The presence of many of the culture media components is reflected in the compositional analyses of the finished product, and this provides verification that levels of various nutrients are not present at levels that are outside of the normal range present in conventional meat products and therefore are safe.

UPSIDE Foods has developed a safety assessment framework for evaluating the use of the company's current media recipes in meat production and for future safety evaluation of new/optimized recipe formulations. A safety assessment is conducted on each component of the cell culture media under the procedure outlined below. Most media components are nutrients and have existing regulatory status for various specified food uses; therefore, the decision tree process begins with a consideration of the regulatory status of each compound. Further overview of the categorical framework for ensuring that culture media components used by UPSIDE Foods are safe and suitable for their intended use in meat production are outlined below according to their category designation.

Category 1. Substance is a food ingredient/additive that is otherwise permitted to be used in food by federal regulation without limitation on use or otherwise Generally Recognized as Safe (GRAS). Compounds in this category would include ingredients such as sugars, pH buffers, water soluble vitamins, and common antioxidants such as tocopherols.

Category 2. These media culture components are common dietary nutrients and are anticipated to have GRAS status for food use or be permitted by regulation for addition to food. Such compounds would include most of the inorganic salts and macronutrients that are present within the media.

Where these compounds are permitted for direct addition to food at use levels comparable to anticipated concentrations that might reasonably be expected in the culture meat product, no safety concerns are anticipated. The majority of nutrients present within the CPM product can be readily measured using common, validated methods for analyzing food composition, and batch analyses of multiple lots of the finished product should be obtained to validate the above assumptions. In some instances, consideration of established safe levels (*e.g.*, acceptable daily intake, UL) derived from a relevant authoritative body (*e.g*., U.S. FDA, European Food Safety Authority, Joint FAO/WHO Expert Committee on Food Additives, FSANZ, United States Environmental Protection Agency, National Academy of Medicine NAM) may be leveraged to support safety. If comparisons of anticipated dietary intakes relative to an authoritative reference intake value is used, consideration of intakes from all dietary sources must be considered. In the absence of an authoritative reference intake value, published no-observed-adverse- effect levels (NOAELs) from animal toxicology studies may be used to evaluate safety using standard scientific procedures for food safety evaluation. A margin of exposure (MoE) of 100-fold or greater between the NOAEL and estimated dietary intakes from food exposures is typically considered adequate to support safety. In situations where the MoE is <100-fold, additional hazard characterization of the compound may be necessary where further reduction of the media component is not possible. These situations also would require careful consideration of the regulatory status of the compound on a case-by-case basis (*e.g*., premarket approval as a food additive or GRAS evaluation).

Category 3. Substances not commonly used in food production (*e.g.*, no express federal regulations or no explicit GRAS determination permitting their use in food identified) but with sufficient information to conclude that the compounds are not present in the fished CPM product of otherwise do not present risk under their specific conditions of intended use in CPM production. In other words, substances that can be shown to be safe for human consumption using generally accepted principles of food safety evaluation or risk mitigation practices. These include, for example, situations where manufacturing controls are in place to ensure that the compound is below the LOD in finished product or is reduced to levels that are equivalent to comparator foods, compounds that are thermolabile and will be denatured during cooking, and/or compounds that are expected to be digested to innocuous compounds following ingestion. Examples of compounds meeting the aforementioned conditions would include bioengineered cultured media proteins and antibiotic/antimycotic compounds used during the upstream cell line development stages.

Category 3 compounds used during **cell line banking** (*e.g.*, antimicrobials, antifungals, sheer protectants, commercial culture media, cryopreservation-aids) are not expected to be present in the finished food at detectable levels, and therefore in the absence of meaningful exposures to such substances from their intended conditions of use can be considered safe.

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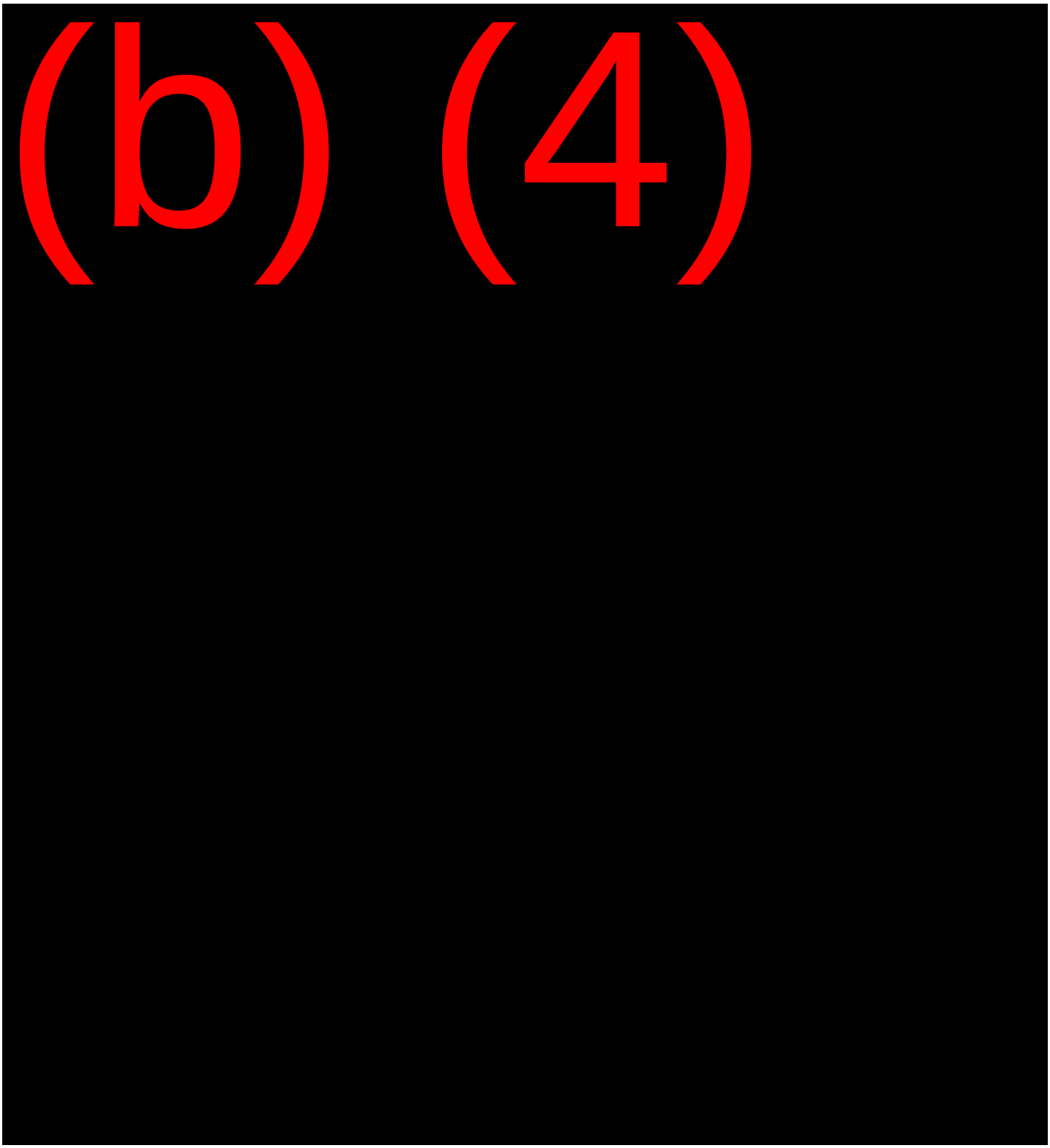
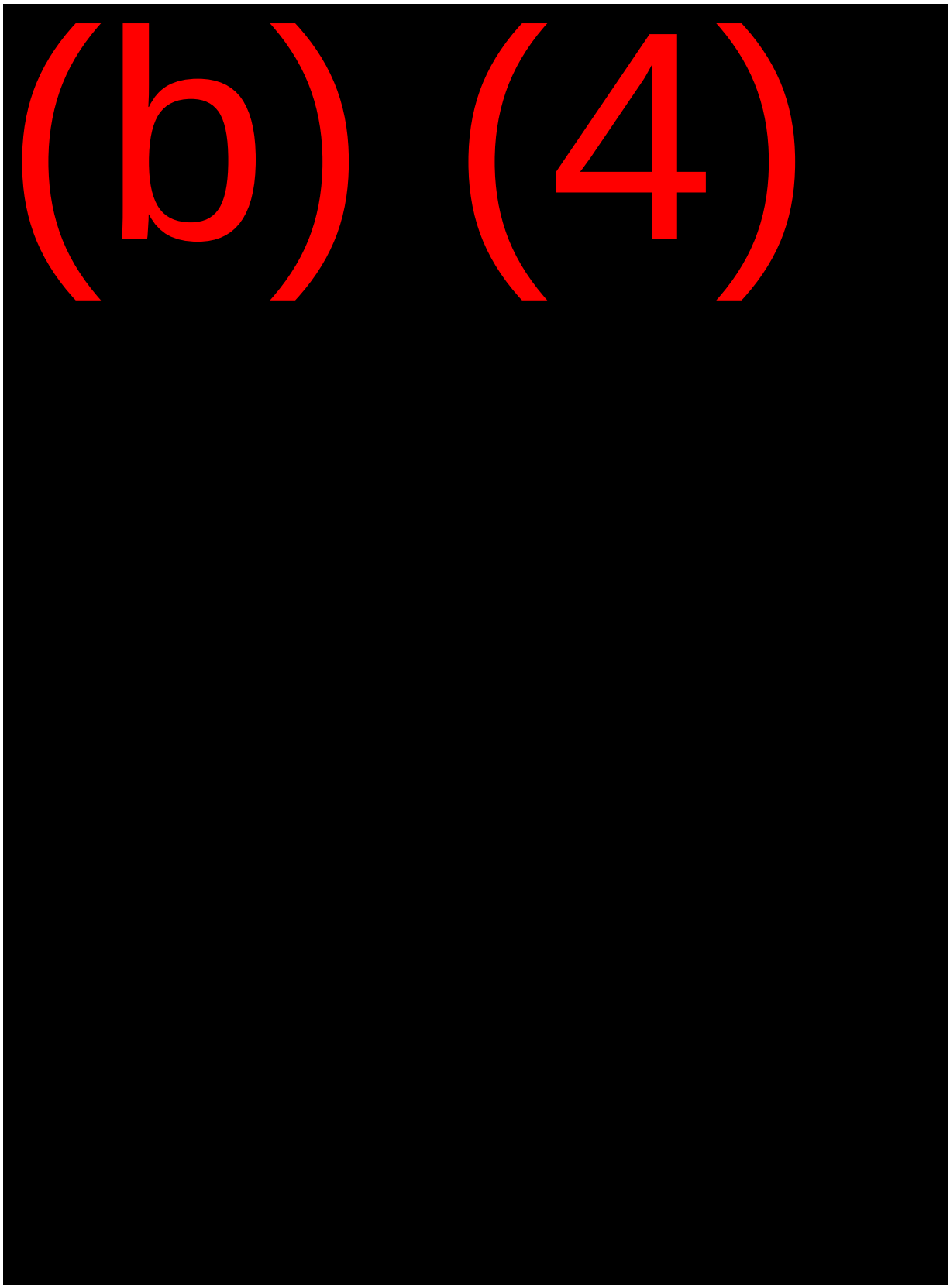
APPENDIX A:
HUMAN FOOD PREVENTIVE CONTROLS FOOD SAFETY PLAN

Appendix A:Human Food Preventive Controls Food Safety Plan

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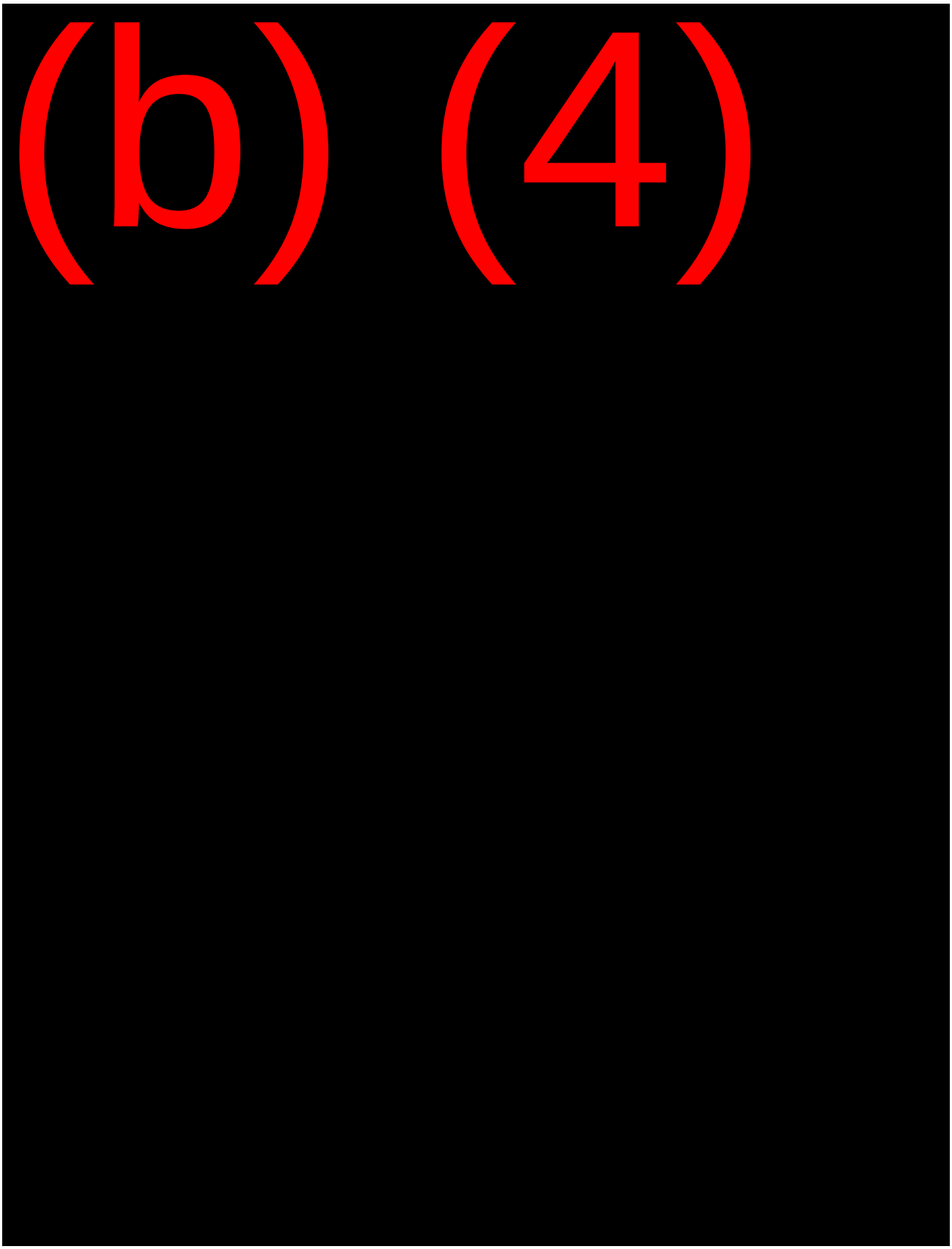
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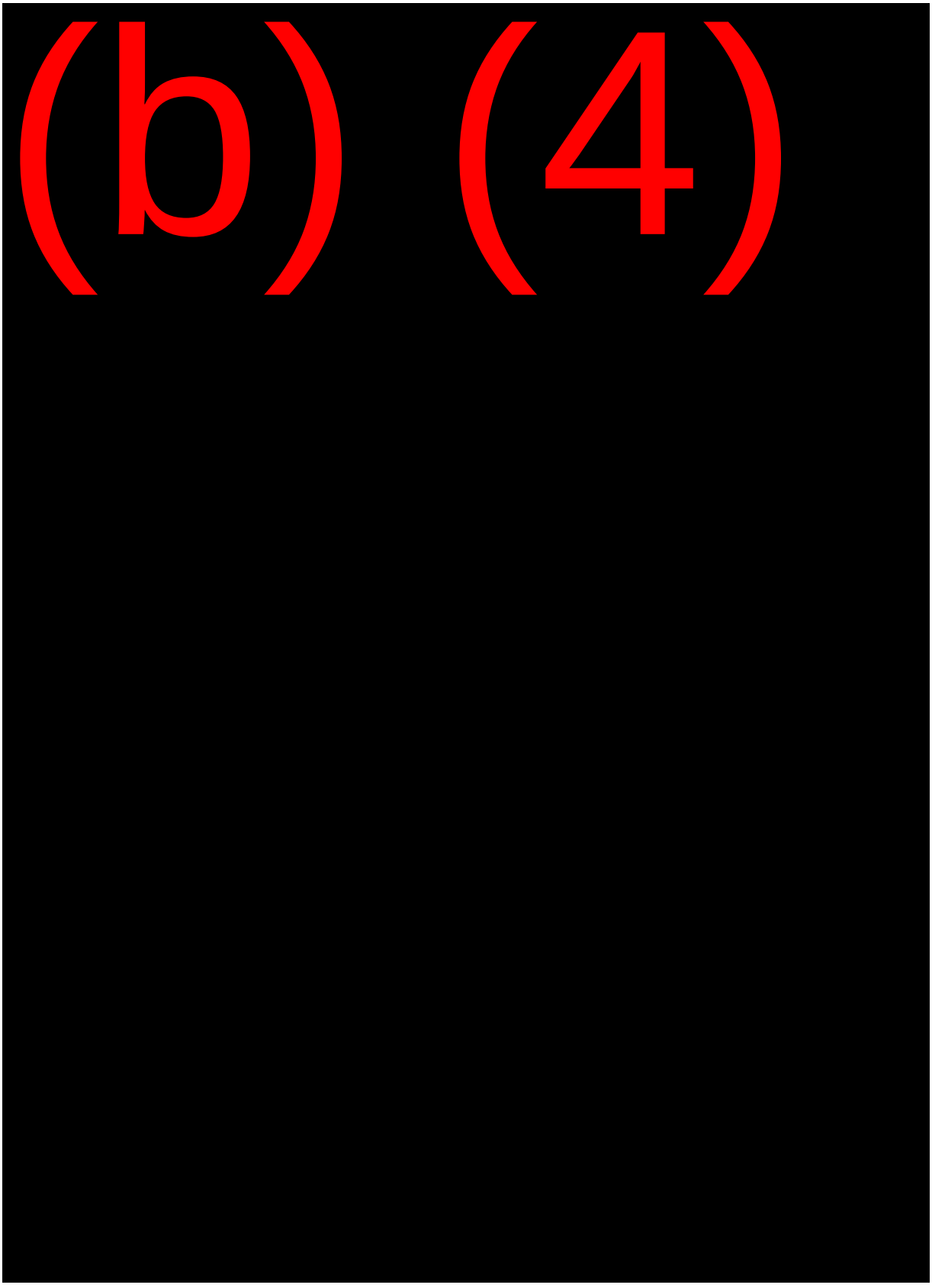
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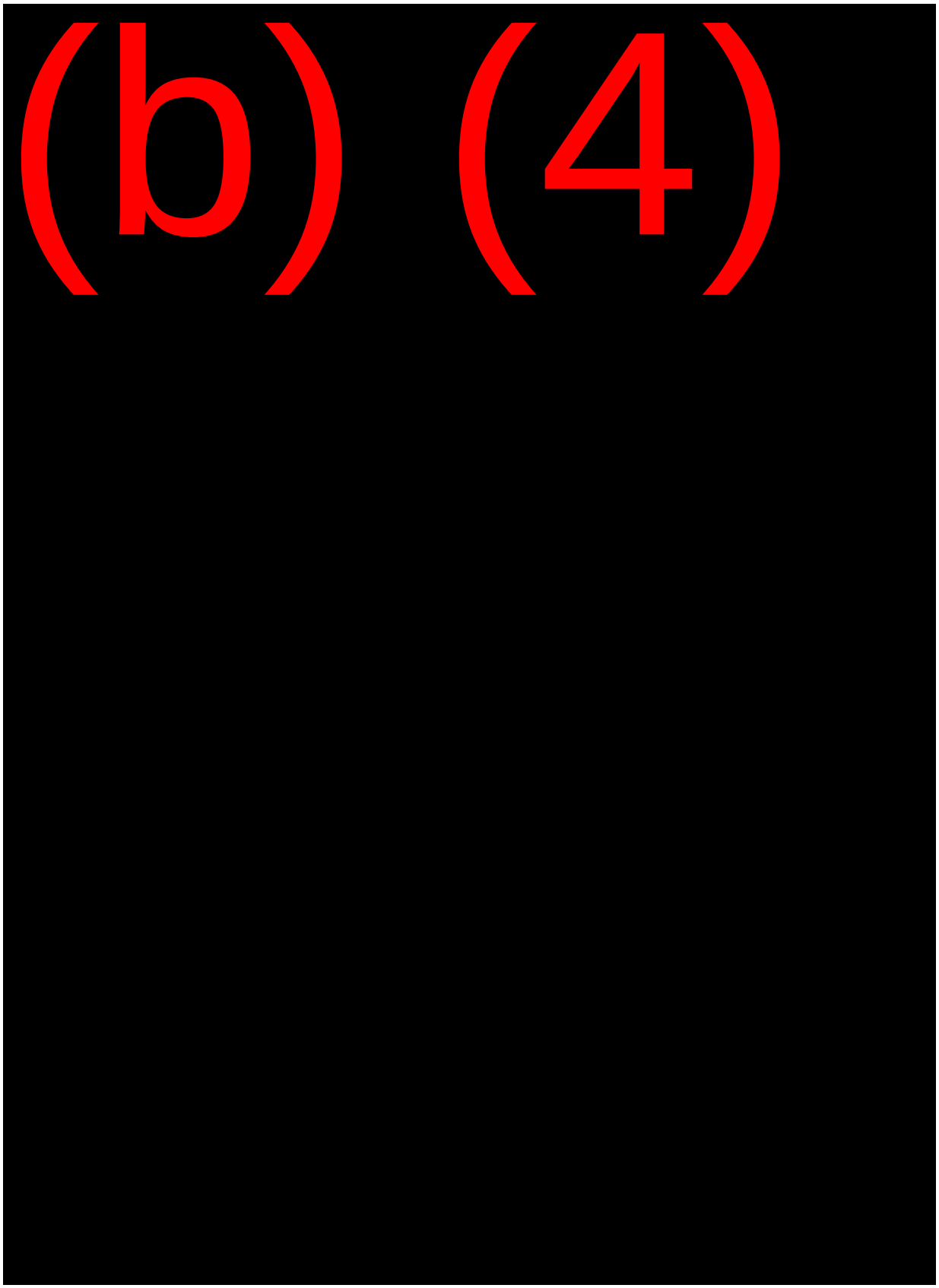
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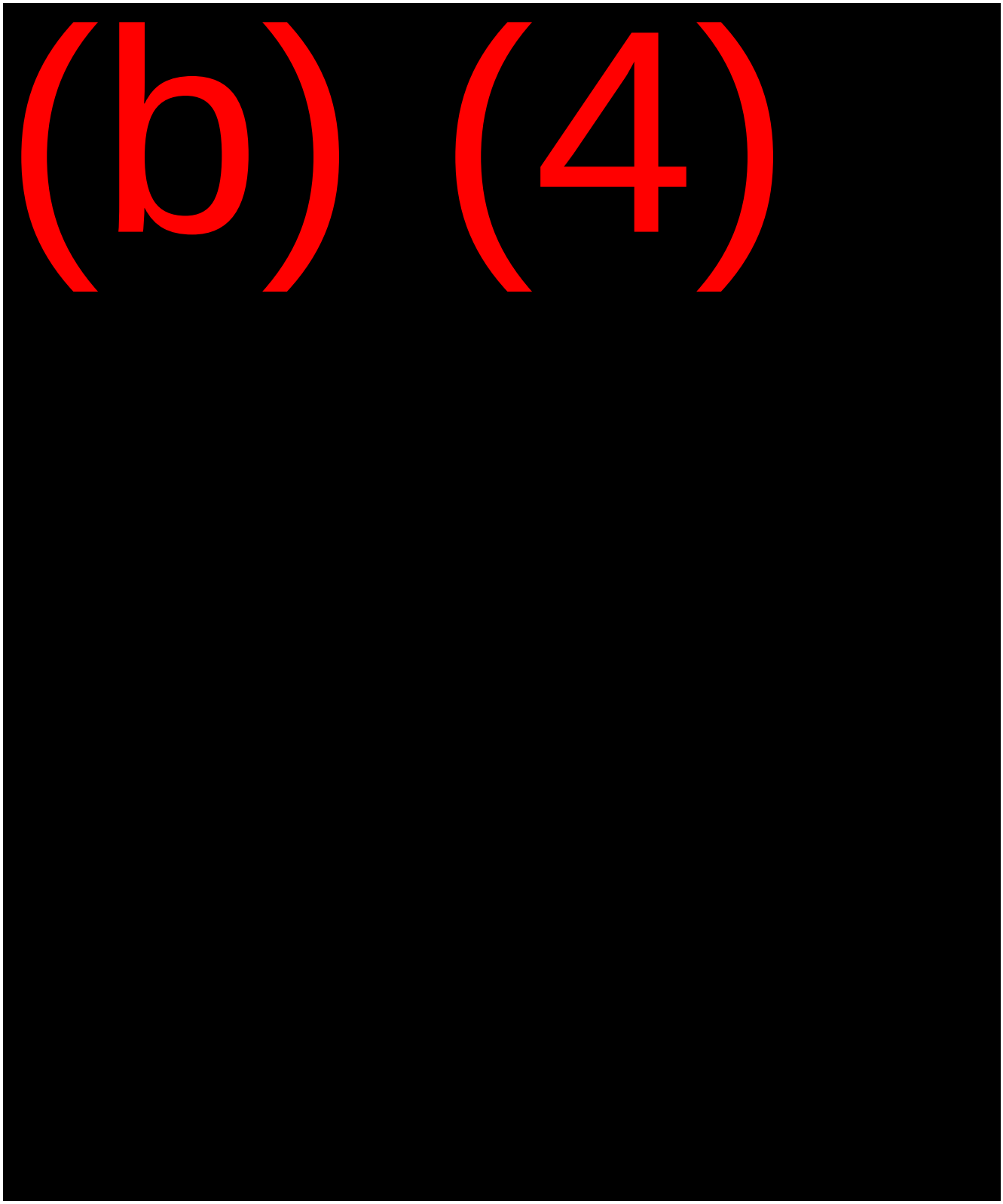
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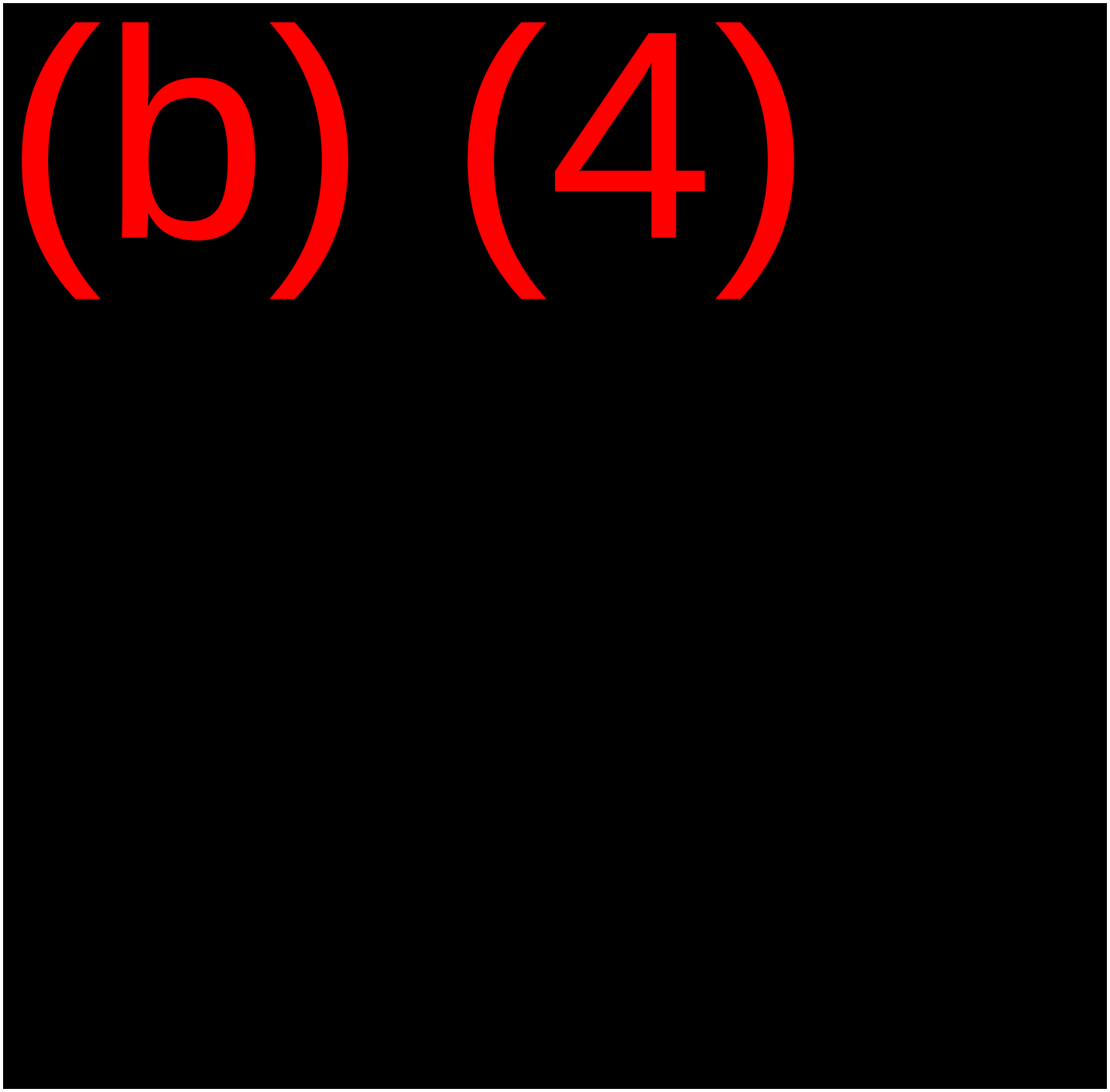
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Jeremiah Fasano, Ph.D.Senior Policy Advisor
Office of Food Additive Safety, CFSAN
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Re: Requests for Additional Information (CCC 000002)

Dear Dr. Fasano

This letter responds to the Food and Drug Administration's (FDA) request for additional data and information relating to UPSIDE Foods' disclosable safety narrative submitted to the agency on October 1st, 2021 and filed under Cell Culture Consultation No. CCC 000002. Responses to the FDA's substantive information requests and points of clarification are provided below. Questions from FDA are presented below in blue text and corresponding responses from UPSIDE Foods are shown in black.

FDA's Substantive Information Requests

Cell Line Establishment

#1 Cell Line Generation

Please provide, for addition to the disclosable safety narrative, information about the bioengineering immortalization method as follows: the additional detail about genetic characterization events (referred to on page 20 of the disclosable safety narrative) described in Table 4.3.2.1.5-1 of the confidential supplementary material, your statement categorizing the telomerase reverse transcriptase (TERT)-based strategy on page 90 of the confidential supplementary material within the tiered hazard characterization system described on page 37 of the disclosable safety narrative, the assessment of the potential for pleiotropy and off-target effects on pages 91-93 (Section 6.1.1.2) of the confidential supplementary material, as well as additional discussion of where, when, and at what levels TERT is expressed in animals (including regulation during cell differentiation (*e.g.*, Wang *et al*., NAR 37, 2618) and its consistency with your assessment of the potential for pleiotropy and off-target effects resulting from constitutive expression, as well as your assessment of the relevance of reported changes in TERT expression in the context of human cells found in the literature (e.g., Yuan *et al*., Oncogene 38: 6172) discussed both in the context of food safety generally and with respect to your safety conclusion about your cultured animal cell product.

UPSIDE Foods' Response:
Characterization of Gene Amendments

UPSIDE Foods has broadly applied well-documented methods that result in the constitutive expression of the chicken telomerase gene (TERT), having been inserted in well-characterized genomic regions without rearrangement or fragmentation (Leighton *et al*., 2008). UPSIDE Foods then ensures the removal of vector backbone components and follow-on analysis to both determine the copy number and confirm the successful removal of the vector backbone (see table below).

Characterization of Introduced Genes	
Potential Hazard Introduced by Genetic Engineering Events	**Process Control Preventative Measures**
Location of inserted gene in genome (off-targeting)	PCR-based sequencing to identify insert location.
Copy number of insert	Determination of copy number.

Characterization of Introduced Genes	
Potential Hazard Introduced by Genetic Engineering Events	**Process Control Preventative Measures**
Expression product of inserted gene	Expected phenotype is confirmed through passage assay (monitoring growth and viability of immortalized cell lines).
Vector backbone components	Confirm vector backbone removal using standard PCR analysis.
Function of expressed product characterized	Phenotypic stability determined through growth curve analysis and demonstration that growth had surpassed its primary cell counterparts.
Pleiotropic effects	Inserted gene is self-limiting in function: usage restricts function of gene product to native telomere maintenance.
Transformation potential	Telomere maintenance does not induce transformation when used as intended and monitored. Extended passaging of a single vial thaw in manufacturing is limited to the demonstrated stability duration (*i.e.*, cells are "retired" from culture routinely to always ensure that phenotypically stable cells are used to produce food).

To determine the genomic integration site, genomic DNA from three independent clone cell pellets was extracted using a DNA extraction kit. A primer specific for the inserted exogenous DNA (*i.e.*, plasmid DNA) was used alone or in combination with a degenerate primer to amplify the junction between the exogenous and endogenous DNA. Polymerase chain reaction (PCR) amplification products were confirmed by agarose gel electrophoresis, purified, captured into a plasmid vector, and transformed into *Escherichia coli* cells for amplification and subsequent antibiotic selection. *E. coli* colonies containing junction-PCR amplicons were then Sanger sequenced with a primer specific for the exogenous DNA that read toward the endogenous DNA junction. Sanger sequence reads were compared to a chicken reference genome and exogenous DNA sequence(s) to identify junctions. The location of genomic integration was examined for features such as the closest endogenous gene and whether the inserted gene resides in coding or non-coding regions.

Integrated *TERT* copy numbers were determined for each transfected cell population using PCR for its sensitivity and specificity in detecting targeted sequences (Deprez *et al*., 2016; Lu *et al*., 2020). Genomic DNA was extracted and digested with a suitable restriction enzyme. A DNA probe for *TERT* was used for detection of the amplified gene and Endogenous actin *beta* (*ACTB)* served as a reference gene with two known copies in the chicken genome.

To confirm that vector backbone components were removed after the establishment of stable immortality, primers were designed to amplify a region of the plasmid comprising the plasmid origin and the antibiotic resistance gene. Recombinant cells with successful backbone excision lack the antibiotic resistance gene but retain *TERT*. UPSIDE Foods found that *TERT* was present in all four lines, but the antibiotic resistance gene was only present in the parental line confirming successful excision of vector backbone components in the subsequent clones. Positive PCR controls yielded the expected outcome supporting the validity of the assay.

Cisgenic Engineering Methods

UPSIDE Foods' risk assessment for gene amendments rests on a few key premises: First, food developed using GE tools is both federally recognized as not inherently harmful and at least as safe as food from non-GE sources (51 FR 23302). Second, potential hazards are mitigated through the use of cisgenic approaches that are well-characterized. Third, both gene promoters and the expressed proteins routinely consumed by humans are used. And finally, chicken TERT is a protein that, like almost all proteins consumed, will be denatured and functionally inactivated during the cooking process and further hydrolyzed in the gut during digestion. As noted elsewhere, chicken TERT is not known to be a food allergen.

It is UPSIDE Foods' view that cisgenic GE practices (A-category Hazard) fall on the same risk spectrum as assisted reproductive technologies (ARTs); that is to say, the risk is acceptably low and well-understood due to the manner by which cisgenes move through a population of cells. Similar to the way cloning or modern selective breeding techniques in animals (and thus their cells) moves naturally existing alleles through a population, cisgenic engineering practices (cGEPs) also move naturally occurring alleles throughout a cell population rather than introduce novel genes. As is the case for artificial insemination, selective breeding practices, and cloning, cisgenic engineering risk is controlled primarily by the movement of allele frequency, not allele identity (*i.e.*, the gene identity in cisgenics is native to the organism). Hazards relevant to cisgenic practices relate to the tools or the methods used to introduce them into the cell or genome, but generally the genes or their expression products themselves do not present a significant risk to human health as it relates to consumption[1].

Cisgenic approaches are defined as those alterations that increase gene flow within a single species or population rather than across species boundaries (Schouten *et al*., 2006). Cisgenics are used to elicit specific phenotypes in animals that humans traditionally consume as food and in companion animals. UPSIDE Foods notes that cisgenic practices that comprise ARTs have similar risk profiles for the expression products since they are identical regardless of the method used to produce the cell line. In the same way the ARTs method itself has the potential to introduce hazards to the finished product, cisgenic engineering methods too must subject its methodologies to hazard identification.

UPSIDE Foods has concluded that the intentional genomic alteration of poultry cells through introduced cisgenic events results in a safe and suitable alternative to conventional poultry meat from a chicken carcass. Intentional amendment of the cells results in the emergence of a single trait, delayed senescence, or "functional immortality" as a result of the use of a single endogenous chicken gene, *TERT*. UPSIDE Foods' cisgenic approach reactivates an endogenous, naturally occurring cellular pathway found in normal tissues to maintain tissue homeostasis, the chromosomal telomere maintenance pathway.

Within typical selective animal breeding, intentional cisgenic alteration to the genome through breeding results in gene flow of both desired and potential undesired traits in the progeny. If the trait sought in an animal is caused by a single genetic locus, such as that which causes the polled cattle phenotype, the goal is selective breeding within an increasingly stable gene pool to ensure a stable target phenotype while minimizing deleterious alleles from flowing in. By omitting breeding and instead using well-established cisgene engineering methods, desired phenotypes can be achieved that limit undesired gene flow. Here, this concept is used to produce functionally immortal cell populations.

[1] CVM Cloning Risk Assessment, FDA, CVM (U.S. FDA, 2008) - https://www.fda.gov/media/75280/download

Safety Assessment of *TERT*

UPSIDE Foods has introduced a chicken gene, *TERT*, that produces a chicken protein, telomerase reverse transcriptase. TERT itself is a ubiquitous, highly conserved protein found in plants and animals consumed by humans (Procházková Schrumpfová *et al*., 2019). A brief background overview of the functional role of TERT in animal cells is presented below.

Telomeres are TG-rich nucleoprotein sequences capping/protecting the ends of linear chromosomes from chromosome fusion, exonuclease degradation, and recombination events that could lead to genomic instability; these protective caps are maintained by TERT, an RNA-dependent DNA polymerase that synthesizes telomeric DNA sequences. Normal animal somatic cells display a limited proliferation capacity *in vitro*, with the maximum number being referred to as the Hayflick limit (Cong *et al*., 2002). The progressive shortening of the chromosome acts as a molecular clock that triggers a permanent growth arrest known as replicative senescence or mortality (Cong *et al*., 2002). Telomerase expression is a defining feature of pluripotent stem cells and some somatic cells as telomerase activity is required for the self-renewal capacity of these cells (Wang *et al*., 2009). Telomerase is therefore expressed during embryonic development to sustain cellular proliferation. This effect is transient and is followed by strong transcriptional repression of TERT during differentiation of many somatic cells such that TERT expression is low to non-detectable in most somatic tissues following early embryogenesis. TERT activity, however, continues to be maintained in renewable tissues (*e.g*., epithelial linings of the skin and gastrointestinal tract) and stem cells (in tissues such as bone marrow) (Wang *et al*., 2009).

The process of replicative senescence limits the growth of most somatic cells in culture, and therefore cells used for meat culture are selected from sources that have a natural delayed senescence phenotype, or from tissues where delayed senescence is achieved through spontaneous induction, or *via* the use of gene amendments such as expression/ “reactivation” of endogenous TERT. The “reactivation” of TERT through genetic amendments replicates the effects of natural TERT induction and induces an extended proliferation capacity that is representative of embryonic tissues. Since TERT expression is a phenotype that is native to the cell, the use of genetic amendments to induce TERT expression is not expected to produce unintended pleiotropic effects that would be a safety concern provided the integrated DNA was well characterized and stably integrated; this conclusion is supported by data discussed in this submission demonstrating that the Cultured Poultry Meat (CPM) is qualitatively and quantitatively highly similar to poultry meat from a chicken carcass.

UPSIDE Foods has not identified chicken TERT protein as a food safety hazard. TERT, as an expression product, is denatured in the cooking process, is hydrolyzed further by normal digestive processes, and as a component in food, is ubiquitous in nature in plants and animals that humans consume. TERT is a highly conserved protein in animals and is not known to be a food allergen. Based on its ubiquity in animals that humans routinely consume, chicken TERT can be concluded to be safe for food use. The usage of chicken TERT therefore falls into the A-category as the native function of the gene and expression product is well-known and characterized, the gene is enriching the expression of the native gene and its native function, and its inserted location in the genome is known.

Consideration of Pleiotropy and Off-Targeting

Identifying and assessing potential unintended effects introduced by genetic events on the phenotypic properties/composition of the organism has been long-considered a critical aspect of the food safety risk assessment of GM technologies (Ladics *et al*., 2015; Fernandez and Paoleti, 2018). Unintended changes can, in theory, materialize as a consequence of gene insertion, from random mutations occurring during the tissue culture process, or from pleiotropic effects of the introduced protein. As there is no single direct test for such effects, evaluation for pleiotropy is typically conducted using compositional analyses. Although the potential for unintended effects to arise from the use of genetic engineering techniques has been suggested (Ladics *et al*., 2015), findings over the last 30 years of research in plant biology, and microbial engineering have failed to demonstrate evidence that the use of targeted genetic engineering techniques can produce pleiotropic effects that are of meaningful significance from a nutritional or toxicological perspective (Herman and Price, 2013). This conclusion is consistent with observations by UPSIDE Foods that naturally occurring transgenic events routinely occur in poultry without evidence of significant deleterious effects to the animal or its lineage of descendants. For example, integration of exogenous DNA from retroviruses (known as endogenous

retroviral fragments) into the chicken's genome in a manner that is passed vertically from one generation to the next is endemic within poultry consumed as food without known pleiotropic or other deleterious effects; many of these "trans-integrations'' are even translated at the protein level (Bolisetty *et al*., 2012).

The potential off-target effects of the insertion of TERT are limited to the senescence mechanism itself due to the cisgenic nature of the expression system. Driving expression of the TERT gene is a cisgenic promoter sequence. This sequence is a commonly found housekeeping gene for constitutive expression and is not expressed in cells, rather its sole function is to drive transcription and translation of the TERT locus in the production cell lines. UPSIDE Foods finds no evidence of the promoter being a potential hazard in this context, as it is itself not expressed and cannot move within the genome. The native promoter is already found within chicken cells that humans routinely consume, and thus is unlikely to be a hazard to human health. To monitor performance over time and minimize against the chances of a potential off-target event, UPSIDE Foods has determined the copy number and removed vector backbone components.

Species differences in TERT expression are noted between humans and rodents. In mice most somatic tissues display low-levels of TERT, and consequently mouse cells have longer telomeres and do not undergo telomere- dependent proliferative senescence (Wang *et al.*, 2009). In chicken, TERT activity is similar to that observed in humans where high-level TERT activity occurs during embryogenesis, followed by diminished activity in most somatic tissues shortly after embryogenesis (Swanberg *et al.*, 2010). Differences in TERT activity of somatic cells has been postulated to play roles in age and cancer susceptibility observed between various species and may explain the elevated susceptibility of mouse cells to immortalization (Wang *et al.*, 2009; Yuan *et al.*, 2019). However, the biological outcomes of TERT expression *in vivo* are less relevant to the normal functional state of euploid cells undergoing limited population doublings in cell culture environments. For example, as reported by Simonsen *et al.* (2002), induced expression of telomerase in human mesenchymal stem cells resulted in elongation of telomeres, extended life span, and enhanced differentiation potential, suggesting its role in normal tissue formation and homeostasis in euploid cells under normal cellular conditions such that would be found in both the animal and in culture. The authors also reported that overexpression of TERT in mesenchymal cells did not produce numerical or structural chromosomal abnormalities and maintained a normal diploid male karyotype. Subcutaneous transplantation of TERT immortalized cells in immunodeficient mice for 6 months did not result in tumor formation. These findings are in line with phenotypic effects of TERT overexpression reported in seminal studies by Bodnar *et al.* (1998) demonstrating that induction of TERT endows unlimited replicative potential to primary human cells; however, these cells maintained a normal karyotype, normal cellular morphologies, and showed no malignant properties. Similarly, Morales *et al.* (1999) reported the *in vitro* growth requirements, cell-cycle checkpoints and karyotypic stability of telomerase-expressing cells were similar to those of untransfected controls. Recent studies conducted using chicken mesenchymal cell lines immortalized by overexpression of TERT were not malignantly transformed and maintained the morphologic features and differentiation characteristics of the original cell-lines (Wang *et al.*, 2017). Taken together, these findings demonstrate that overexpression of TERT is unlikely to induce undesirable pleiotropic changes to the cell as the introduced TERT genes are functionally limited to the constraints of the normal cellular control mechanisms of the cell, which have not been altered. The growth requirements, cell-cycle checkpoints and karyotypic stability in telomerase expressing cells are very similar to comparator cells in their native state (Bodnar *et al.*, 1998; Morales *et al.*, 1999). UPSIDE Foods further continues to monitor the cells' phenotype over time, specifically the population doubling limit and maturation ability of the cells into meat to ensure the cells remain under staff control. The potential pleiotropic effects of the inserted TERT locus are limited to the senescent mechanism inside the cells. TERT is known to delay senescence in multiple cell types across the plant and animal kingdom. It is not known to function beyond telomere maintenance under normal cell culture conditions, which are employed here. As a mitigation step, UPSIDE Foods actively monitors the phenotypic stability and growth rate to ensure acceptable specifications in the finished product.

Finally, consideration of the possibility that a genetic engineering event may induce expression of a latent toxic substance is limited to microorganisms and plants because of their unique evolutionary history as compared to animals. Plants and microorganisms are organisms that have evolved to employ a diverse strategy of toxin production to defend against predators. Animals by and large did not evolve toxin production as a defense mechanism, settling instead primarily on motility, appendage tools, evasive or camouflage behaviors, fighting/flight behaviors, and in small fraction, specialized toxin organs such as venom sacs in snakes. In short, animals, and by extension, animal cells, traditionally consumed as food in the western world do not typically harbor nor produce toxins. Overall, UPSIDE Foods

considers potential harmful effects of off-targeting and potential pleiotropy to be minimal to non-existent in animal cells.

#2 Phenotype/Passage Stability

Information Requested

Please provide, for addition to the disclosable safety narrative, the description of the results of the phenotypic stability testing found in the last two paragraphs of page 43, Section 4.3.3.3 of the confidential supplementary material.

UPSIDE Foods' Response:
Phenotype/Passage Stability Verification

Information demonstrating the phenotypic stability of the cell lines is obtained to ensure that analytical data from finished product testing is reliable and representative of long-term commercial production. To determine phenotypic stability of a Master Cell Bank (MCB) line over a manufacturing process, duration is verified in the following manner. Cells are cultured in 3 independent cultures to determine if doubling time (DT) and tissue production over time are stable. On a predetermined schedule, cells are counted and expanded into new cultures at a consistent starting cell density. The population DT is calculated to establish and monitor phenotypic stability at each passage. Periodically, cells are analyzed for protein yield.

Stable doubling time DT is defined as ± 30% change in the average of the first 3 DTs of the study (*e.g.*, Day 3, 6, 9). Stable protein production is defined as ± 30% change from the first time point (*i.e.*, Day 0) of protein production in the study.

Three independent cell line clones were cultured in triplicate for more than 90 days to demonstrate operational excellence and product quality that will allow at least a 90-day manufacturing cycle. After the passage stability time course, cell banks collected every 30 days were thawed and recovered in culture. From these cultures, cells were plated on a small-scale tissue production format (*e.g.*, 12-well tissue culture dish), and protein yield was measured after 4 days in culture. BCA measures protein yield. Both DT and protein yield were consistent over time for Clone 1 and Clone 3, showing less than 30% variability in both assays. Clone 2 had 2 timepoints that had more than 30% faster DT of the initial average DT but otherwise exhibited stable DT and protein yields.

3 Cell Bank Establishment
Cell Bank Characterization
Information Requested

Please provide, for addition to the disclosable safety narrative, a version of the cell bank characterization table (Table 4.3.3.3.1-1, page 45 of the confidential supplementary material.

UPSIDE Foods' Response:

UPSIDE Foods' cell banks are characterized for adventitious agents, cell species verification, and phenotypic and passage stability. Cell lines that pass all 3 criteria are used for master cell banking purposes. The table below provides an example of cell bank characterization which does not have any adventitious agent detected, is identified as chicken, and has stable phenotypes.

Summary of Cell Line Bank Characterization		
Testing Category	**Parameter Tested**	**Compliance Standard**
Adventitious agent testing (microbial)	Aerobic plate count and yeast/mold	Negative
	Mycoplasma	Negative
	Enterobacteriaceae	Negative
	Campylobacter	Negative
	E. coli	Negative
	Salmonella	Negative
	Listeria monocytogenes	Negative
Adventitious agent tested with animal component exposure	Bovine species suitable analysis	Negative
	Porcine species suitable analysis	Negative
	Avian (Chicken) species suitable analysis	Negative
Cell species verification	*COI* PCR and sequencing	Chicken
Phenotypic and passage stability	Doubling time variation	Conforms
	Protein yield variation	Conforms
Proceed with MCB	Yes	

COI = cytochrome c oxidase I; MCB = master cell bank; PCR = polymerase chain reaction.

#4 Species Verification
Information Requested

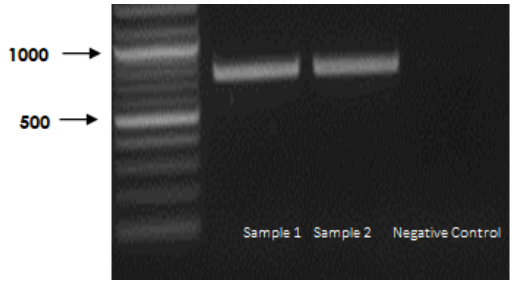
Please provide, for addition to the disclosable safety narrative, the additional information about species verification contained in the 2nd and 3rd paragraph of Section 4.3.3.2 of the confidential supplementary material.

UPSIDE Foods' Response:

Cells are harvested and lysed to extract mitochondrial DNA using an enzymatic DNA extraction kit. Next, the DNA is PCR amplified with a universal primer cocktail for the cytochrome c oxidase I (COI) gene in animals (Hebert et al., 2003), which the Consortium for the Barcode of Life uses to identify species of origin (Consortium for the Barcode of Life). A fraction of the PCR reaction is used to verify the PCR amplicon product by running on an agarose gel. Then the remaining samples are subject to DNA sequencing by Sanger sequencing *via* a third-party testing laboratory. The sequence is compared to a known reference genome from validated sequence databases. This species identification method is currently used by the FDA to verify different fish species (Single Laboratory Validated Method for DNA Barcoding for the Species Identification of Fish – U.S. FDA, 2011).

UPSIDE Foods' production cells are positively identified as chicken. PCR analysis showed the expected size of PCR product size for *COI* PCR analysis. The PCR product was sent for Sanger sequencing, and the sequencing results returned as a 100% match with chicken.

Example of *COI* PCR Assay Results



bp = base pairs; *COI* = cytochrome c oxidase I; PCR = polymerase chain reaction.
Genomic DNA samples were analyzed by PCR using the universal primer cocktail (658 bp). Water was used in place of genomic DNA for the negative control. DNA ladder is a 100 bp ladder.

#5 Adventitious Agent Hazard Assessment
Information Requested

Please provide, for addition to the disclosable safety narrative, additional discussion of your hazard assessment for adventitious agents, including the second full paragraph and the second part of the final paragraph on page 37 of the confidential supplementary material as well as the categorization scheme described on page 39 and the information contained in Table 4.3.3.1-1.

UPSIDE Foods' Response:
Adventitious Agent Testing

UPSIDE Foods recognizes that the human safety risks associated with the presence of adventitious agents in biologic drug products differ from those relevant to food safety. The primary reason for such differences are based upon the fact that many biologics are produced using human cell-lines, most human pathogenic viruses require human hosts for propagation and biologic products are not orally administered and therefore are not exposed to the digestive process. 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. For example, mammalian

and poultry retroviruses are endemic and consumed in food from animals without apparent harm (DiGiacomo and Hopkins, 1997). It should also be recognized that viral propagation of adventitious agents within a meat cultivator unit would typically kill the cell or otherwise siphon-off nutrients for their reproduction as a latent infection. The net result of a lytic or latent viral infection would be a negative impact on the productive capacity of the cell culture system well-prior to any tissue harvest (Barone *et al*., 2020) preventing introduction to the food supply; therefore, the presence of adventitious agents is inherently self-limiting, as the resulting poor growth performance of the cell culture would trigger quality control checks of the bioreactor.

As safety is of paramount importance to UPSIDE Foods, the company has incorporated an adventitious agent testing plan into the company's food safety risk framework. In the absence of formal guidance on adventitious agent testing for foods developed using animal cell culture technology, the general principles outlined in the WHO and FDA's Guidance on testing of adventitious agents were considered: FDA *Guidance for Industry Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications;* WHO *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks* (U.S. FDA, 2010; WHO, 2013). Virus risk mitigation measures used by UPSIDE Foods to prevent adventitious agent contamination of CPM uses 2 complementary approaches: 1) Prevention of virus entry into the meat production system by selecting low-risk raw materials (*e.g.*, healthy animals intended for food use, sterile media, recombinant trypsin) used for the production of cell lines and CPM production process; and 2) testing of in-process materials (*i.e.*, cell banks, bovine serum, finished products) used during manufacturing to verify the absence of relevant adventitious agents. Although not a risk mitigation measure, the requirement for cooking CPM products to safe temperatures (*i.e.*, 165°F) provides a third level of assurance that CPM products used as food will be safe from viral and bacterial contaminants that would be inactivated at these temperatures.

Rationale for Viral Testing During Pre-production & Production

Viral testing regimens are determined based on (1) the tropism of the virus and (2) the potential for exposures to viral agents. Viruses that are capable of infecting avian cell populations or are capable of crossing the avian-human species barrier are tested in pre-bank and are monitored for in production. In an avian culture system, human-tropic specific viruses are not tested for, as they are incapable of infecting avian cells. UPSIDE Foods therefore presents the risk as negligible for human-tropic viruses.

If non-avian animal components are used in the generation of the banked cell lines and/or the production of the tissue itself, UPSIDE Foods tests for appropriate viruses of concern based on the species of origin for the component used, accounting for potential exposure to the component. For example, if trypsin has been sourced from porcine origin and used solely in pre-banking, UPSIDE Foods tests for porcine-human tropic viral agents of concern in the cell banking stage, but not during post-production phases. If only avian cells are used and no other animal-derived component is used during all stages of production, UPSIDE Foods would limit UPSIDE Foods' viral surveillance to avian and avian-human tropic viral agent surveillance.

A brief summary of the virus classification for selection of a particular virus testing panel is presented below and in the following table.

Class A Viruses:

- Latent viruses that become lytic on recrudescence from latency and viruses that can establish non-lytic or low-level lytic infections that impact fermenter performance or cellular differentiation.

Class B Viruses:

- Zoonotic viruses known to be transmissible from animals to humans. Amongst those viruses considered as zoonotic include approaite avian viral analysis

- Animal viruses that replicate in human cells *in vitro*. Some animal viruses replicate in human cells in vitro but evidence of their zoonotic potential is weak or absent. Some avian retroviruses replicate in human cells and one group is probably derived from a mammalian retrovirus. Studies reported to date have failed to demonstrate either human infection with poultry retroviruses or an association between human diseases and these viruses (DiGiacomo and Hopkins, 1997).

- Virus families that have shown a propensity to change host range, such as parvoviruses and coronaviruses and certain single-stranded DNA viruses like anelloviruses, circoviruses, gyroviruses, and cycloviruses.

- Human viruses that replicate in avian cells. Avian cells are used in the production of various vaccines including Vaccinia, Measles, Rubella and Influenza.

Class C Viruses:

- Primary concern for the biosecurity of domestic animal populations. In the past, significant epidemics and pandemics of virus diseases have occurred through recycling of animal meat, *e.g.*, vesicular exanthema of swine and swine fever. These events are increasingly unlikely given modern practice and regulations.

UPSIDE Foods confirms that all cell lines have been tested for, and determined to be free of, the above microbial and viral contaminants using validated assays by qualified third-party experts with experience in testing of cell-lines for adventitious agents.

Potential Hazards Associated with Viral Tropism in Avian Cell Culture		
Viral Tropism	**Tested (yes/no)**	**Rationale**
Avian	No	CPM production systems do not come in contact with live poultry.
Avian-Mammalian	Yes	Avian viruses capable of infecting mammalian cells are a potential human health hazard and are therefore tested by UPSIDE Foods.
Mammalian	No	Mammalian viruses are not a human health hazard in the context of an avian culture system due to the inability of mammalian-tropic viruses to infect avian cell populations.
Mammalian*	Yes	Porcine or bovine viruses capable of infecting mammalian cells are a potential human health hazard and are therefore tested by UPSIDE Foods.

CPM = cultured poultry meat.
* Viral tropism is mammalian with rare case reports of infectivity in avian hosts.

6 Microbial and Viral Testing

Information Requested

Please provide, for addition to the disclosable safety narrative, the information contained in the 2nd full paragraph on page 38 of the confidential supplementary material, including both a positive statement that you are testing for viruses you have identified as the result of your hazard assessment, as well as a statement that all analytical methods are validated for their intended purpose. Please include the identity of the viruses you are testing for, as discussed on page 38 of the confidential supplementary material.

UPSIDE Foods' Response:

Reagents, raw materials, and MCBs used for CPM production are assured to be free of adventitious agents through a series of fit-for-purpose validated tests and documentation. Records are retained for all reagents and biological raw materials used for CPM production. Bovine sera are also verified to be sourced from BSE-free/risk-negligible herds. 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. Cell banks must be free from microbes, especially food-borne pathogens and zoonotic viruses known to be threats to human health. MCB testing includes the following list of microorganisms identified through UPSIDE Foods' hazard assessment:

- For microbes:

    1. Aerobic plate count
    2. Yeast/mold
    3. Mycoplasma
    4. *Enterobacteriaceae*
    5. *E. coli* panel
    6. *Campylobacter* species screen
    7. Salmonella
    8. *Listeria monocytogenes*

- For viruses:

    1. Bovine Animal Component Containing (ACC) viruses
    2. Porcine ACC virus
    3. Chicken ACC viruses

As the working cell banks (*i.e.*, the MWCBs) are produced from the MCB, reevaluation for adventitious agents is not conducted for qualification of the MWCB; however, for quality purposes, the MWCB is tested for sterility and mycoplasma prior to banking, as it is critical that the MWCB is sterile prior to the initiation of a production run. Cell banks positive for any of the listed microbial or viral agents are immediately destroyed and discarded.

Microbial Testing

Sources of risks and rationales are documented for each potential microbial concern that is linked to conventional chicken, and, where applicable, bovine origin when serum is used. Human pathogens of clinical importance from these sources include *E. coli*, Salmonella *sp*., *Campylobacter sp*., and *Listeria* (USDA Chicken from Farm to Table – USDA, 2019). Since the acceptance criteria for these major pathogens are limited to "non-detect," further discriminatory evaluation for pathogen serovars (*e.g.*, *E. coli* O157:H7) is not necessary. In addition to human pathogens, the cell banks are screened for aerobic plate counts (APC), *Enterobacteriaceae*, yeast and mold, and mycoplasma to ensure that the culture banks are absent microbial contaminants of safety concern, as well as those that would negatively impact the performance of meat production or lead to spoilage. All tests for adventitious agent contamination are conducted using validated fit-for-purpose assays.

#7 Material Inputs
Culture Media
Information Requested

Please provide, for the addition to the disclosable safety narrative, additional information on the classes (e.g., surfactants) and characteristics of substances used in the culture medium which are not metabolized and are not used for the fundamental nutritional requirements of the cells.

UPSIDE Foods' Response:

The cell culture process requires the use of processing aid components that are not used for fundamental nutritional requirements for the cells or otherwise are not metabolized. These components fall within two general categories. In the first category are upstream culture media aids (*e.g.*, antibiotics/antimycotics, anti-foaming aids, dissociation reagents, cryoprotectants, sheer protectants, anti-clumping aids) that are used to manage certain physical or chemical properties of the medium, including those used in the generation of the cell lines. These components are determined to be of suitable purity for their intended use and will not be present in the finished food at significant levels due to washing and dilution effects that occur during downstream culture processes, as previously discussed. In the second category are downstream culture media aids that will be in contact with the food product, including emulsifiers/surfactants, antioxidants, and wash buffers. These components are determined to be non-genotoxic substances, of suitable purity for use in food processing, and are largely removed from the CPM product by washing during the harvesting step. Residual concentrations that may be present have been estimated or analyzed empirically and determined to not be present in significant levels using traditional scientific procedures safety evaluation (*i.e.*, exposures to residues are safe relative to the hazard profiles of the substance) and have no technical or functional effect in the final ready-to-consume product.

#8 Cell Culture Proteins

Information Requested

Please provide, for addition to the disclosable safety narrative, UPSIDE's analysis of the factors involved in evaluating the safety of culture media proteins on pages 96-100 (Section 6.2.2 through Subsection 6.2.2.3) of the confidential supplementary material.

UPSIDE Foods' Response:
Culture Media Proteins

The recombinant growth factors are produced by fermentation using safe laboratory strains of bacteria (*e.g.*, *E. coli* K-12) or yeast (*e.g.*, *Saccharomyces cerevisiae*, *Pichia pastoris*) with a history of safe use. In these cases, the safety of the production organism can be evaluated by using general principles established by Pariza and colleagues for the safety evaluation of recombinant enzymes (Pariza and Foster, 1983; Pariza and Johnson, 2001). As the gastrointestinal tract is adapted for exposure to foreign protein and microbial endotoxins are endogenous within the gastrointestinal tract, lower purity preparations of growth factors could be commercialized for cultured meat production provided they were sourced from safe production. The use of human cell culture systems and/or human recombinant growth factors are avoided. With respect to quality standards for recombinant growth factors, a product from a production organism derived from a safe strain lineage or equivalent standard and that is appropriately manufactured would be suitable for food use. Commercial growth factors used for large scale production will be custom manufactured. In-house quality standards should be developed to ensure the quality and safety of the product. Quality standards should be based on food safety paradigms rather than biologic drug paradigms, as safety concerns for parenterally administered drug products (*e.g.*, endotoxin) are not relevant to food substances. To develop a current Good Manufacturing Practice quality standard, an understanding of the general manufacturing process may be necessary to ensure that specifications for residual processing aids (*e.g.*, elution solvents) are included, where relevant.

Growth factors fall into the Category 3 segment of UPSIDE Foods' risk assessment framework (described on pages 40-41 of the public-facing document for CCC 000002) on the basis that they are not currently permitted for food

use by an appropriate federal regulation or previous (Generally Recognized as Safe) GRAS evaluation under 21 CFR 170.30. These substances are present in all animals (and many plants) and are consumed as food. As growth factors are proteins, UPSIDE Foods has drawn upon the principles for protein safety evaluation developed for the safety evaluation of novel proteins used in the context of agricultural biotechnology (Delaney *et al*., 2008). This safety evaluation paradigm consists of a two-tiered weight of evidence strategy that places an emphasis on thorough hazard identification under Tier I and requires evidence demonstrating history of safe food use, findings from bioinformatic analyses, information on the mode of action, in *in vitro* digestibility and stability, and information on the expression level and dietary intake. In situations where information provided by the Tier I assessment is insufficient to assess the safety of introducing the protein to the food supply, additional Tier II hazard characterization studies would be initiated and may include acceptable toxicity studies, and potentially, hypothesis-based evaluations. For application of the two-tiered testing strategy to the safety evaluation of growth factors used in UPSIDE Foods' cultured meat production process, emphasis was placed on weight of evidence establishing the following 3 criteria:

1. History of Safe Use
2. Expression level and dietary intake
3. *In vitro* digestibility and stability

Less emphasis was placed on information provided from bioinformatic comparisons to known toxins and allergens as proteins used in cultured meat production are well-defined with known modes of action and would not be homologous to protein toxins or cross-reactive with major food allergens.

History of Safe Use

The functional use of growth factors in cultured meat production replicates the natural autocrine/paracrine signaling processes that operate within animal tissues and regulate a variety of complex homeostatic processes necessary for maintenance of normal cellular functions. With respect to establishing the safety of recombinant proteins used in cultured meat production, the existence of reliable, documented evidence substantiating the natural presence of the protein at equivalent levels in foods routinely consumed in the human diet, would, by definition, demonstrate prima facie evidence of their safety for food use.

UPSIDE Foods notes that FDA references the concept of history of safe use in food safety evaluation as an extension of the GRAS provision; this concept as it relates to protein safety evaluation is further discussed in the Agency's 1992 Statement of Policy: Foods Derived from New Plant Varieties. In the 1992 policy, the Agency states:

> "*When the substance present in the food is one that is already present at generally comparable or greater levels in currently consumed foods, there is unlikely to be a safety question sufficient to call into question the presumed GRAS status of such naturally occurring substances and thus warrant formal premarket review and approval by FDA*" (57 FR 22990 – U.S. FDA, 1992).

Implicit within the concept of history of safe use are assumptions that the "new proteins" used in food are qualitatively comparable to those with a history of food consumption. The safety assessment of a new protein used in food production should consider homology of the protein to that with a history of safe use and potential impacts of species-specific post-translational modifications arising from the production organism from which the protein was derived. In this regard, UPSIDE Foods only uses growth factors with protein sequences that are 100% homologous to those from agriculturally important animals with a history of safe consumption (e.g., bovine, porcine, chicken).

Expression Levels and Dietary Intake

As discussed, in situations where it can be demonstrated that a growth factor used in cultured meat production can be detected within finished food products at levels that are quantitatively comparable to those in an appropriate comparator food, it is UPSIDE Foods' view that such levels are safe to consume. Since cultured meat products are intended for use as a 1:1 substitution for meat from an animal carcass, dietary intake modeling will typically be unnecessary, and the safety evaluation can rely on comparisons of concentrations within the cultured meat product to those present within tissues of meat products with a history of consumption. Care should be taken to ensure that reference values for background concentrations of a protein are obtained using peer-reviewed studies or validated internal analytical methods. Potential differences in intra-laboratory variability, assay type, and regional differences in concentrations of growth factors within tissues should also be considered during the safety evaluation.

In situations where a growth factor used during cell culture is detectable at levels that are significantly outside the historical variation that can be measured, or have been reported for comparator foods, further hazard characterization using *in vitro* digestibility and stability assays would typically be necessary. *In vitro* digestibility studies also would be appropriate for further characterization of proteins that differ slightly from their natural counterparts (*e.g.*, slight amino acid differences or differences in post-translational modifications). Growth factors used by UPSIDE Foods as culture media aids are used at physiological levels and are homologous to those present in agriculturally relevant species.

***In Vitro* Digestibility and Stability**

Proteins are essential nutrients in the diets of humans, and the mammalian digestive system efficiently hydrolyses dietary proteins to their amino acid components, which are absorbed and metabolized for energy or incorporated into new proteins. Most proteins consumed in the diet are sensitive to digestive processes and, due to the minimal potential for the absorption of intact proteins, proteins consumed in the diet have virtually no potential for systemic toxicity (Delaney *et al*., 2008); this physiological barrier is particularly relevant to growth factors since systemic absorption of such compounds could be anticipated to have undesirable effects. Historical experiences of investigational attempts at oral delivery of somatotropins and insulin as active drug substances have been unanimously unsuccessful, and abandonment of these efforts by the pharmaceutical industry further underscores the high efficiency of the gastrointestinal digestion of dietary proteins (Delaney *et al.*, 2008). In this regard, UPSIDE Foods recognizes that the digestion of dietary protein to amino acids is generally considered to be complete; however, there are examples of proteins that have been reported to resist digestion and may be absorbed at least partially "intact" such as ovalbumin (Delaney *et al*., 2008). Other examples include protein families that have originated through evolutionary pressures to resist oral digestion, such as anti-nutritional proteins from plants (*e.g*., lectins, and protease inhibitors) and microbial enterotoxins. Notwithstanding these exceptions, it should be recognized that the inherent functions of growth factors as regulators of cellular function within tissues suggests that most growth factors are likely to display stability profiles that are aligned with their acute/transient functions in tissues. Growth factors are highly unlikely to retain biological activity outside of their evolutionarily adapted environments and are not expected to be active following even moderate denaturation or partial hydrolysis. Based on UPSIDE Foods' understanding of the biological effects of growth factors in mammalian tissues, safety considerations of consuming growth factors in the diet will be largely limited to the potential for effects on the gastrointestinal tract. Only under circumstances where the concentration of the growth factor within a cultured meat product was demonstrated to be significantly increased relative to the upper range of historical safe levels established from comparator foods would a potential hazard arise. In these instances, further characterization of protein stability is necessary to characterize the potential hazard of the elevated levels in the food. Partial denaturation of proteins from cooking is well-known to increase susceptibility of most proteins to digestion (Hammond and Jez, 2011). As poultry is typically cooked to an internal temperature of 165°F, experimental data demonstrating the poor thermal stability of a growth factor would be considered sufficient to support a conclusion that elevated levels of growth factors in CPM beyond those observed in a conventional food product would not be a safety concern. Additional studies evaluating digestive stability using *in vitro* simulated gastric digestion models would provide additional corroborative evidence for safety.

To date, all growth factors used by UPSIDE Foods in cultured meat production are present in store-bought commercial chicken meat samples. Growth factors used in the production of UPSIDE Foods cultured chicken products and those

detected in commercial samples are thermolabile and cannot be detected using appropriate sensitive assays following sufficient cooking of the chicken to achieve safe internal temperatures for food use. UPSIDE Foods also notes that growth factors also are expected to be susceptible to acid and protease hydrolysis conditions within the gastrointestinal tract, thereby providing a secondary barrier for assurance that uses of growth factors as described in this submission are safe.

#9 TERT Protein

Requested Information

For addition to the disclosable safety narrative, please elaborate on your discussion of the safety of the expressed TERT protein itself as a constituent of the cells, per pages 29-30 and 90-91 (Section 6.1.1) of the confidential supplementary material, including considerations of digestibility, prior exposure and existing expression, functionality, and anticipated expression level for the recombinant protein.

UPSIDE Foods' Response:
See response #1 for this discussion.

(b) (4)

[redacted]

#11 Information Requested

On page 167 of the confidential supplementary material, you mention a "visual inspection of media" step that is employed to identify potential environmental biological hazards introduced during the media preparation step of your processing stream. This language was used to describe other steps in your processing stream, such as step 5, media hydration, (page 172 in the confidential supplementary material of your risk assessment). How could a "visual inspection of media" help to identify potential environmental biological hazards? Please provide, in addition to the disclosable safety narrative, your response in the context of using proper aseptic practices, as discussed in the safety narrative on page 28. Please revisit these steps to ensure that the information is accurately presented.

UPSIDE Foods' Response:
UPSIDE has robust controls in place to prevent contamination from environmental and biological hazards during media preparation. In addition to environmental controls to ensure that processing occurs in a clean, appropriate environment, UPSIDE's manufacturing is generally performed in a closed system, and processing is monitored to confirm appropriate parameters are met. Visual inspection of the liquid media is intended as a 'top-level' control to detect contamination were it to occur during processing and is considered a routine best practice for aseptic practice. When properly designed, milled, and hydrated, cell culture media is translucent since the nutrients therein are soluble. Contaminating biological agents such as bacteria or fungiform films and larger clump-like or filamentous objects that

are visible to the human eye in refracted light. Personnel are trained to identify potential microbial contamination under Standard Operating Procedures (SOPs), the specific criteria: "*Media should be clear, yellowish clear, or reddish clear, with no sign of cloudiness, biofilm, or floating objects.*"

Visual inspection of media by persons trained in recognizing unusual visual appearance, provides an initial indication in the event that environmental contaminants have been introduced into media. If contamination is detected, further analysis may be performed to identify the type of contamination and investigate the source. For example, turbidity indicative of potential bacterial or fungal contamination can be confirmed by microscopy, and confirmed contamination may be further identified to determine the likely sources of contamination.

With respect to good laboratory practice, lab personnel are trained in cell culture aseptic techniques, ensuring no cross-contamination between cell plates, cultivators, or instruments. This includes training on applicable procedures and on-the-job coaching on topics such as techniques to ensure cell cultures are free from cross-contamination, sanitizing and cleaning areas, proper techniques and handling of materials, and environmental controls.

#12 Microbial and Viral Testing

Information Requested

For addition to the disclosable safety narrative, please elaborate on your adventitious agent testing protocols for each adventitious agent identified, including the following: identification of the specific adventitious agents tested for, the analytical methods employed (including complete citations, as appropriate), specification and sample size, and the stage testing is performed in the processing stream. In your discussion, please describe how your processing stream controls for the presence of adventitious agents. Please also include a statement that all analytical methods were validated for their intended purpose. This information should be provided in a tabular format with the aforementioned sections as columns within the table (*i.e.*, adventitious agent, analytical method, sample size, stage in processing stream). As an example, an entry in the table might look like: Salmonella serovars; *Bacteriological Analytical Manual* (BAM) Chapter 5; (a) non-detect, (b) absent in 25 g; (a) establishment of master cell bank, (b) specification for harvested cellular material).

UPSIDE Foods' Response:

Tabulated data detailing UPSIDE Foods' adventitious agent testing procedures are provided below. UPSIDE Foods confirms that all analyses were conducted using validated assays by qualified third-party experts. For additional details on the rationale for adventitious agent testing see response #5.

Adventitious Agent Testing Protocols					
Parameter Tested	**Method**	**Sample Size**	**Specification**	**Stage in Processing Stream**	**Result**
Sterility – Aerobic cell count	Growth in 8 media direct inoculation culture/cell count when positive	1 million cells in 1 mL media	Negative	MWCB	Negative
Sterility – Yeast/mold	Growth in 8 media direct inoculation culture/cell count when positive		Negative	MWCB	Negative
Mycoplasma	Real-time PCR assay on cells with spent media or cell pellets	1 million cells in 1 mL media	Negative	MWCB	Negative
Enterobacteriaceae	Real-time PCR assay on cell pellets	5 – 10 million cells frozen	Negative	MCB	Negative

Adventitious Agent Testing Protocols					
Parameter Tested	**Method**	**Sample Size**	**Specification**	**Stage in Processing Stream**	**Result**
E. coli panel	PCR assay on cell pellets		Negative	MCB	Negative
Campylobacter species screen	Real-time PCR assay on cell pellets		Negative	MCB	Negative
Salmonella	Real-time PCR assay on cell pellets		Negative	MCB	Negative
Listeria monocytogenes	Real-time PCR assay on cell pellets		Negative	MCB	Negative
Bovine animal component containing (ACC)	Real-time PCR assay on cell pellets		Negative	MCB	Negative
Porcine ACC	Real-time PCR assay on cell pellets		Negative	MCB	Negative
Chicken ACC	Real-time PCR assay on cell pellets		Negative	MCB	Negative

#13 Product Characterization
Folic Acid Content
Information Requested

Please provide additional information, for addition to the disclosable safety narrative, preferably incorporating analytical data, regarding the anticipated folic acid content of the cultured cell material at harvest.

UPSIDE Foods' Response:

It should be noted that estimates for quantities of nutrients present within the CPM product reported in Table 1.1-2, of page 133 of the confidential supplementary material were calculated using a conservative assumption that concentrations of the substances in the media will be present in the CPM at roughly equivalent levels. These estimates were conducted for risk assessment purposes to understand the maximum dietary exposure in the unlikely event that consumption and clearance of the substance does not occur such that all of the substance in the media was to be present in the resulting CPM product. In addition, these estimates were used to identify substances that may require further empirical testing in the resulting CPM product. At the time of preparing this table, empirical data on concentrations of each media constituent actually present in the CPM product were not available. UPSIDE Foods has subsequently performed analytical testing of the folic acid levels in the finished CPM product; the analysis shows actual levels of 36 ± 14 µg of folic acid per 100 g serving of cultured meat. This level is well within the tolerable upper limit for folic acid established by the Institute of Medicine as well as the level specified in FDA's food additive regulation for folic acid, codified at 21 C.F.R. 172.345. It is important to note that folic acid is used by the cells as an enzyme cofactor and is a critical requirement to support one-carbon transfer reactions, including nucleotide synthesis, amino acid synthesis, and methylation. Accordingly, it is necessary to supplement cultures with folic acid at the levels indicated to sustain cellular division during the expansion and differentiation stages of the cell culture process, and the substance is not used to fortify the CPM product. Because large quantities of folic acid are consumed by the cells to sustain cell growth and proliferation, the theoretical estimates for folic acid presented in Table 1.1-2 greatly exceed the actual levels of folic acid in the resulting CPM product.

#14 Specifications
Information Requested

For addition to the disclosable safety narrative, please provide specifications for the final product (with units or sample size, as appropriate), including the information contained in Table 4.5.5-1 of the confidential supplementary material as well as for toxic elements other than lead (e.g., arsenic, mercury, cadmium).

UPSIDE Foods' Response:

Batch release specifications have been developed for UPSIDE Foods' CPM product to ensure the safety and suitability of product that is released for further food processing pursuant to USDA oversight, and that the qualitative and quantitative attributes defining the product's identity and composition are consistent from lot to lot. Specifications for CPM are shown in the Table below.

Specification for CPM – Integral Tissue		
Parameter	**Specification**	**Method**
Name	Cultured poultry meat	-
Item Description	Post separation from cultivator	-
pH	>5.3	pH electrode for meat products
Total Protein	>12 %	AOAC 2011.04 g per 100 g (AOAC, 1920-2013)

Specification for CPM – Integral Tissue		
Parameter	**Specification**	**Method**
Moisture	>70 %	AOAC 2008.06 g per 100 g (AOAC, 1920-2013)
Total Plate Count	<10 Colony-forming units (CFU)	APHA CMMEF CHP 8 CPU
Enterobacteriaceae[2]	<10 CFU	AOAC 2003.01 (AOAC, 1920-2013)
Salmonella[2]	Non-detect in 25 g	BAM Chapter 5
Lead	<2 ppm	FDA EAM 4.7
Arsenic	<1 ppm	AOAC 2013.06-2013 (AOAC, 1920-2013)
Cadmium	<2 ppm	AOAC 2013.06-2013 (AOAC, 1920-2013)
Mercury	<2 ppm	AOAC 2013.06-2013 (AOAC, 1920-2013)

CPM = cultured poultry meat; SOP = standard operating procedures; TQ = tissue quality.
[1] This SOP measures the structural integrity of a tissue, from low (TQ 1) to acceptable (TQ 3) and above.
[2] Analyses only conducted on lots of products that exceed the specification limit for aerobic plate count.

#15 Cell Culture Proteins

Information Requested

Please provide, for addition to the disclosable safety narrative, a general description of the analytical testing strategy and results described in Section 5.4.6 of the confidential supplementary material.

UPSIDE Foods' Response:

A framework for analyzing growth factors is presented to account for the quantification of any growth factor residues in the CPM product due to use in the cell culture media. As the cell culture media formulation may include growth factors, UPSIDE Foods has used commercially available test kits for analyses of residual growth factor(s) in the native product and to confirm destruction during cooking. Residues of growth factors are tested in both raw and cooked CPM (serum-containing and serum-free).

Samples were prepared according to literature protocols provided by a validated commercial ELISA testing kit for extracting proteins from tissue samples. Samples from the 3 non-consecutive lots of CPM that were produced using serum (serum-containing CPM) and the 3 non-consecutive lots of CPM that are serum-free (serum-free CPM) were analyzed. Three comparator chicken samples were assayed alongside the CPM samples for comparison: ground chicken meat, whole muscle meat from a chicken leg, and skin from a chicken leg. For the tissue digestion process, a blank tissue extraction buffer was digested alongside all meat samples. In general, the residual concentrations of growth factors in the raw CPM product were significantly elevated relative to concentrations measured in the comparator products; however, once the samples were cooked, the growth factors were effectively denatured and could no longer be detected by the assay.

#16 Cell Differentiation

Information Requested

Please provide, for addition to the disclosable safety narrative, the discussion of the method of analysis of tropomyosin in the 2nd paragraph of Section 5.2 in the confidential supplementary material.

UPSIDE Foods' Response:

For analysis of tropomyosin, protein from CPM samples produced with or without serum were extracted and prepared as described above for analyses of growth factors. Serum-containing and serum-free CPM tissue samples were collected from multiple production lots. A ground raw chicken tissue sample was included as a baseline comparator. Representative myogenic protein candidate was chosen and validated *via* immunoblotting (Jiang *et al.*, 2019). Immunoblotting method is from well-established protocols (Abcam, 2020). Tropomyosin was expressed in all CPM lots tested as detected by immunoblotting, demonstrating a shared protein signature of UPSIDE Foods' CPM with chicken meat.

#17 Nutrient Composition

Information Requested

Please provide, for addition to the disclosable safety narrative, a brief description of the nutrients tested, the testing methods, and the measured values for each nutrient from both the test articles and comparator articles, based on the discussion in Section 5.4 through Subsection 5.4.4 in the confidential supplementary material. This would include the results from the analysis of the nutrient composition of three non-consecutive batches of (1) cell material produced using serum-based media, (2) cell material produced using serum-free media, and (3) ground chicken conventional comparator.

UPSIDE Foods' Response:

Fatty acids were assayed according to a method based on AOAC method 996.06 (1976), "Fat (Total, Saturated, and Unsaturated) in Foods: Hydrolytic Extraction Gas Chromatographic Method," which is applicable to food matrices (AOAC, 1920-2013). This method includes identification of fatty acids based on gas chromatographic retention time and quantification *via* a multi-component standard curve; recovery is evaluated *via* an internal standard. Samples were sent to a third-party analytical testing laboratory for evaluation; their validated method is based on AOAC 996.06, with five key differences:

1. The AOAC method requires enough sample to extract 100–200 mg of total fat, whereas this method has been scaled back to require as low as 50–500 mg of total sample;
2. A screw-top vial is used instead of the traditional Mojonnier flask;
3. A C19 triglyceride internal standard is used instead of C11;
4. In the extraction process, chloroform is used instead of pyrogallic acid; and
5. For the methylation [to convert fatty acids to fatty acid methyl ester (FAME) derivatives for gas chromatography (GC) analysis], methanolic sulfuric acid is used instead of boron trifluoride.

Essentially, there are many modifications that can be achieved for fatty acids analysis *via* FAME derivatization with gas chromatography with flame ionization detection (GC-FID) analysis (Ostermann *et al.*, 2014); [redacted] has validated their specific method to analyze cells, tissue, plasma and media samples.

Cholesterol was assayed according to AOAC method 976.26 (1996), "Cholesterol in Multicomponent Food," which is also applicable to food matrices (AOAC, 1920-2013).

Tabulated information on the nutrients tested, testing methods and measured values for the test articles and comparator articles for USDA All chicken (no organs and USDA skinless light chicken) are presented below. Ground chicken comparator results were sent in parallel with the CPM material for the Mineral Profiles for the lots of serum-containing CPM and serum-free CPM.

Fatty Acid Profiles and Fat Categories for Triplicate Lots of Serum-containing and Serum-free CPM*												
Fatty Acid or Fat Category	**Units**	**Serum-containing CPM**			**Serum-free CPM**			**USDA All Chicken (no organs)**			**USDA Skinless Light Chicken**	
		Lot 1	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**	**Average**	**Standard deviation**	**Population (N)**	**Average**	**Standard deviation**	**Population (N)**
16:0 Palmitic	g per 100 g	0.259	0.254	0.224	0.265	0.260	0.326	1.184	0.790	27	0.307	0.136	4
16:1w7 Palmitoleic	g per 100 g	0.032	0.031	0.032	0.180	0.266	0.365	0.317	0.219	27	0.069	0.041	4
18:0 Stearic	g per 100 g	0.316	0.336	0.315	0.218	0.194	0.220	0.350	0.190	27	0.119	0.052	4
18:1 Oleic**	g per 100 g	0.311	0.288	0.284	0.612	0.683	0.909	1.903	1.333	27	0.449	0.223	4
18:2w6 Linoleic	g per 100 g	0.108	0.114	0.130	0.028	0.029	0.047	0.934	0.596	6	0.243	-	1
20:4 Arachidonic**	g per 100 g	0.216	0.246	0.218	0.011	0.017	0.020	0.077	0.035	27	0.076	0.055	4
Total Saturated	g per 100 g	0.623	0.640	0.600	0.545	0.513	0.614	1.626	1.040	27	0.449	0.193	4
Total Monounsaturated	g per 100 g	0.396	0.364	0.348	0.858	1.019	1.354	2.287	1.608	27	0.537	0.280	4
Total Polyunsaturated	g per 100 g	0.568	0.640	0.584	0.147	0.187	0.233	1.314	0.744	27	0.427	0.234	4
Total Fat	g per 100 g	1.735	1.786	1.656	1.726	1.889	2.404	5.984	3.561	27	1.934	0.876	4
Cholesterol	mg/100 g	185.3	321.5	289.9	360.8	359.6	429.9	54.8	12.8	27	45.4	8.5	4

*Due to varying moisture content across samples, all CPM and conventional chicken data shown were normalized to a 20 w/w% solids content to show equivalency as g per 100 g per sample wet mass. ** analysis noted as undifferentiated

Individual amino acids were assayed according to a custom validated method employed by a third-party laboratory. Freeze-dried samples were provided to the laboratory for analysis. Sample preparation involves hydrolysis of the lyophilized sample. For cysteine (CYS) and methionine (MET), the sample was stored overnight at 2°C in fresh performic acid. For the remaining acid-stable amino acids, a liquid-phase hydrolysis is performed using 6N hydrochloric acid with 1% phenol at 110°C for 24 hours in vacuo. A separate alkaline hydrolysis is performed to resolve tryptophan (TRP), which is destroyed during acid hydrolysis. Acid-stable amino acids are assayed using ion-exchange chromatography to separate amino acids followed by a "post-column" ninhydrin reaction detection system and analysis using high-performance liquid chromatography. Each amino acid is identified by peak retention time and quantified by the peak area. For calibration, an amino acid standard solution is used. Recovery is evaluated *via* an internal standard. This protocol is based on well-established sample preparation and analytical chemistry for amino acids analysis for lyophilized tissues, among other sample types.

Amino Acid Profiles for Triplicate Lots of Serum-containing and Serum-free CPM												
Amino Acid	**Units***	**Serum-containing CPM**			**Serum-free CPM**			**USDA All Chicken (no organs)**			**USDA Skinless Light Chicken**	
		Lot 1	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**	**Average**	**Standard deviation**	**Population (N)**	**Average**	**Standard deviation**	**Population (N)**
Alanine	g per 100 g	0.793	0.774	0.788	0.701	0.565	0.660	0.784	0.174	27	0.974	0.037	4
Arginine	g per 100 g	1.189	1.159	1.187	0.918	0.725	0.855	0.874	0.209	27	1.090	0.060	4
Aspartic Acid, Asparagine	g per 100 g	1.599	1.518	1.568	1.175	0.958	1.144	1.254	0.317	27	1.586	0.055	4
Cysteine	g per 100 g	0.311	0.291	0.292	0.185	0.163	0.181	0.170	0.042	27	0.215	0.024	4
Glutamic Acid, Glutamine	g per 100 g	2.157	2.048	2.015	1.708	1.448	1.621	2.076	0.545	27	2.623	0.095	4
Glycine	g per 100 g	0.946	0.866	0.996	0.836	0.620	0.700	0.725	0.107	27	0.841	0.059	4
Histidine	g per 100 g	0.455	0.441	0.543	0.432	0.384	0.449	0.422	0.119	27	0.572	0.050	4
Isoleucine	g per 100 g	0.781	0.766	0.738	0.555	0.490	0.544	0.689	0.194	27	0.911	0.052	4
Leucine	g per 100 g	1.243	1.236	1.181	0.973	0.851	0.970	1.057	0.285	27	1.351	0.066	4
Lysine	g per 100 g	1.033	1.050	1.024	0.928	0.793	0.940	1.188	0.326	27	1.540	0.092	4
Methionine	g per 100 g	0.370	0.350	0.384	0.302	0.277	0.161	0.374	0.101	27	0.479	0.026	4

Phenylalanine	g per 100 g	0.703	0.693	0.682	0.550	0.523	0.551	0.542	0.140	27	0.700	0.023	4
Proline	g per 100 g	0.899	0.857	0.896	0.727	0.559	0.639	0.581	0.110	27	0.682	0.092	4
Serine	g per 100 g	0.802	0.780	0.759	0.578	0.480	0.565	0.504	0.126	2727	0.620	0.032	4
Threonine	g per 100 g	1.003	0.973	0.959	0.624	0.515	0.599	0.592	0.157	2727	0.753	0.027	4
Tryptophan	g per 100 g	0.279	0.261	0.233	0.165	0.142	0.172	0.156	0.044	2727	0.209	0.008	4
Tyrosine	g per 100 g	0.742	0.743	0.729	0.475	0.438	0.460	0.470	0.129	2727	0.602	0.022	4
Valine	g per 100 g	0.963	0.933	0.901	0.683	0.577	0.681	0.673	0.176	2727	0.880	0.029	4

Individual elements were analyzed by a third-party laboratory. A panel of 31 elements was assayed by inductively coupled plasma mass spectrometry (ICP-MS) using their protocol for a tissue plug, which is a method compatible with UPSIDE Foods' sample types. A Quality Assurance (QA) standard was run with every batch, which employed a sample of known concentration prepared by an external agency under stringent conditions and was used as an independent check of method accuracy. A spike blank was also run, which employed a blank matrix sample to which a known amount of the analyte has been added from a second source, also to evaluate method accuracy. A method blank employed a blank matrix containing all reagents used in the analytical procedure to identify laboratory contamination. The QA standard limit was 75 to 125% recovery; the spiked blank limit was 80 to 120% recovery. The method blank returned less than the limit of detection (LOD) for all analytes. Moisture normalization was applied to samples as indicated below in the data discussion. Elements are reported in units of mg per 100 g of wet sample mass.

Mineral Profiles for Triplicate Lots of Serum-containing CPM and Serum-free CPM								
Parameter	**Unit**	**Serum-containing CPM**			**Serum-free CPM**			**Ground Chicken**
		Lot 1	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**	
Calcium	mg per 100 g	27.40	29.60	70.80	8.45	5.93	6.19	4.92
Copper	mg per 100 g	0.0588	0.0868	0.0406	0.1410	0.1980	0.1560	0.0442
Iron	mg per 100 g	0.515	0.774	0.397	4.400	6.120	5.400	0.552
Potassium	mg per 100 g	60.4	80.6	45.9	966.0	1130.0	1010.0	288.0
Magnesium	mg per 100 g	8.36	12.20	8.77	18.30	18.80	17.10	22.00

Manganese	mg per 100 g	0.0289	0.0534	0.0383	0.0303	0.0556	0.0483	0.0104
Sodium	mg per 100 g	214.0	263.0	196.0	129.0	92.6	95.6	164.0
Phosphorus	mg per 100 g	159	196	141	593	702	616	168
Selenium	mg per 100 g	0.0174	0.0237	0.0106	0.0314	0.0236	0.0249	0.0155
Zinc	mg per 100 g	1.48	2.47	1.46	1.99	1.88	1.84	1.11
Moisture	g per 100 g	80	72	73	71	72	73	72

CPM = cultured poultry meat.
A store-bought ground chicken sample was assayed alongside the sample for comparison and is also listed. Moisture content for each sample is listed.

Vitamins A, D, B3 (niacin), B5 (pantothenic acid), and B6 (pyridoxine) were analyzed according to the following methods by a third-party laboratory. Vitamin A was assayed according to AOAC 2001.13 (2001) (suitable for foods), and vitamin D was assayed according to AOAC 2011.11 (2011) (validated for infant formula and adult/pediatric nutritional formulas) (AOAC, 1920-2013). R-Biopharm VitaFast assays were used for vitamins B3, B5, and B6; all VitaFast methods are validated for meat and meat products (among other sample types). Due to varying moisture content across samples, all CPM and conventional chicken data shown were normalized to a 20 w/w% solids content.

Vitamin Results for Triplicate Lots of Serum-containing CPM and Serum-free CPM*												
Vitamin	**Units**	**Serum-containing CPM**			**Serum-Free CPM**			**USDA All Chicken (no organs)**			**USDA Skinless Light chicken**		
		Lot 1	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**	**Avg.**	**Std. dev.**	**N**	**Avg.**	**Std. dev.**	**N**
Niacin (B3)	mg per 100 g	1.29	2.03	0.44	4.44	5.39	5.25	4.75	1.77	27	7.79	0.51	4
Pantothenic acid (B5)	mg per 100 g	0.55	0.75	1.88	1.62	1.46	1.31	0.67	0.27	27	0.78	0.24	4
Pyridoxine (B6)	mg per 100 g	0.33	0.35	2.15	1.03	1.70	1.76	0.30	0.12	27	0.48	0.10	4
Vitamin A	mcg per 100 g	39	56	22	23	65	65	17	10	27	9	5	4
Vitamin D	mcg per 100 g	0	0	0	0	0	0	0	0	11	0	0	2

CPM = cultured poultry meat.

*Due to varying moisture content across samples, all CPM and conventional chicken values shown in the table and figures were normalized to a 20 w/w% solids content to show equivalency as g per 100 g per sample wet mass.

#18 Contaminant Analysis

Information Requested

Please provide, for addition to the disclosable safety narrative, a revised version of Table 5.5-1 in the safety narrative that includes the complete citations of the analytical methods used, as in Table 5.4.5-1 of the confidential supplementary material.

UPSIDE Foods' Response:

The following indicator organisms were tested: APC (APHA CMMEF, Chapter 8 – Ryser and Schuman, 2015), [suitable for foods, including meat], *E. coli* and coliforms (AOAC Method 998.08) [for poultry, meat, and seafood], *Enterobacteriaceae* (AOAC Method 2003.01) [selected foods] (AOAC, 1920-2013), and yeast and mold (APHA CMMEF, Chapter 21 – Ryu and Wolf-Hall, 2015) [suitable for foods, including meat]. In cases where enough product was available per lot, Salmonella testing was performed *via* the 25 g AOAC Method 2011.03 method (AOAC, 1920-2013) [suitable for a variety of foods, including raw chicken breast and other meats]. Samples were sent to Zoologix for real-time PCR analysis for Salmonella [suitable for various samples, including bacterial culture or environmental swab] and *Enterobacter cloacae* complex [suitable for various samples, including food]. The heavy metals lead, arsenic, cadmium, and mercury were part of the elemental panel described in Mineral Profiles for Triplicate Lots of Serum-Containing CPM and Serum-Free CPM. For heavy metals, data are reported as µg per 100 g for samples that were normalized to 20% solids. UPSIDE Foods provides a table below that includes citations of the analytical methods.

Results of All Microbial and Heavy Metals Testing for CPM and a Conventional Chicken Sample									
Parameter	**Method**	**Unit**	**Serum-containing CPM**			**Serum-free CPM**			**Ground Chicken**
			Lot 1	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**	
Lead (Pb)	ICP-MS	µg per 100 g (20% solids)	1.32	1.49	2.27	4.69	5.03	4.56	0.25
Arsenic (As)	ICP-MS	µg per 100 g (20% solids)	1.14	1.23	1.00	<0.50	<0.50	<0.50	<0.50
Cadmium (Cd)	ICP-MS	µg per 100 g (20% solids)	0.70	1.24	0.49	1.32	1.65	1.46	<0.13
Mercury (Hg)	ICP-MS	µg per 100 g (20% solids)	<1.30	<1.30	<1.30	<1.30	<1.30	<1.30	<1.30
Aerobic plate counts	APHA CMMEF Ch8	CFU per g	<100	<100	<100	<100	<100	<100	8600
Coliforms	AOAC 998.08 (2002) (AOAC, 1920-2013)	CFU per g	<10	<10	<10	<10	<10	<10	10
E. coli	AOAC 998.08	CFU per g	<10	<10	<10	<10	<10	<10	<10
Enterobacteriaceae	AOAC 2003.01 (AOAC, 1920-2013)	CFU per g	<10	<10	<10	<10	<10	<10	50
Mold	APHA CMMEF Ch21	CFU per g	<10	<10	<10	<10	<10	<10	<10
Yeast	APHA CMMEF Ch21	CFU per g	<10	<10	<10	<10	<10	<10	<10
Salmonella	AOAC 2011.03 (AOAC, 1920-2013)	per 25 g	Negative	Not measured	Not measured	Negative	Not measured	Not measured	Positive
Salmonella	rt-PCR	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured
Enterobacter cloacae complex	rt-PCR	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured

Results of All Microbial and Heavy Metals Testing for CPM and a Conventional Chicken Sample									
Parameter	**Method**	**Unit**	**Serum-containing CPM**			**Serum-free CPM**			**Ground Chicken**
			Lot 1	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**	
Influenza Type A	rt-PCR	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured
Influenza Type B	rt-PCR	Presence/absence	Negative	Negative	Negative	Negative	Negative	Negative	Not measured

FDA Points of Clarification

#19 Cell Line Establishment

Information Requested

For addition to the disclosable public narrative, please clarify, per the statement in the penultimate paragraph on page 19 of the safety narrative and our understanding of the data, that UPSIDE does not use intrinsically immortal cells in the production of cell material as defined in CCC 000002.

UPSIDE Foods' Response:

Although UPSIDE Foods did discuss 3 potential methods of immortalization, the cell material used to provide data in CCC 000002 did not employ intrinsically immortalized cells.

#20 Information Requested

For addition to the disclosable safety narrative, please clarify that Table 4.3.2.1.3-1, referred to on page 20 of the safety narrative, is not present and was not intended to be present in the document.

UPSIDE Foods' Response:

UPSIDE Foods inadvertently omitted Table 4.3.2.1.3-1 in the disclosable safety narrative. The correct table is provided in response #1 as well as below.

Characterization of Introduced Genes	
Potential Hazard Introduced by Genetic Engineering Events	**Process Control Preventative Measures**
Location of inserted gene in genome (off-targeting)	PCR-based sequencing to identify insert location.
Copy number of insert	Determination of copy number.
Expression product of inserted gene	Expected phenotype is confirmed through passage assay (monitoring growth and viability of immortalized cell lines).
Vector backbone components	Confirm vector backbone removal using standard PCR analysis.
Function of expressed product characterized	Phenotypic stability determined through growth curve analysis and demonstration that growth had surpassed its primary cell counterparts.
Pleiotropic effects	Inserted gene is self-limiting in function: usage restricts function of gene product to native telomere maintenance.
Transformation potential	Telomere maintenance does not induce transformation when used as intended and monitored. Extended passaging of a single vial thaw in manufacturing is limited to the demonstrated stability duration (*i.e.*, cells are "retired" from culture routinely to always ensure that phenotypically stable cells are used to produce food).

#21 Information Requested

For addition to the disclosable safety narrative, please provide full reference for Chow (2010) found on page 19 of the safety narrative.

UPSIDE Foods' Response:
Chow AY (2010). Cell cycle control by oncogenes and tumor suppressors: driving the transformation of normal cells into cancerous cells. Nat Educ 3(9):7. Available at: https://www.nature.com/scitable/topicpage/cell-cycle-control-by-oncogenes-and-tumor-14191459/.

#22 Information Requested

For addition to the disclosable safety narrative, please clarify if the animal used to source the cell types that are used to establish cell lines that are the subject of CCC 000002 are destined for the human food supply chain.

UPSIDE Foods' Response:
The animals used to source cell types that were used to establish cell lines that are the subject of CCC 000002 were destined for the human food supply.

#23 Information Requested

For addition to the disclosable safety narrative, please clarify that all cells used to establish cell lines that are the subject of CCC 000002 are isolated from a single animal species.

UPSIDE Foods' Response:
All cell lines that are the subject of CCC 000002 are isolated from a single animal species, i.e., chicken.

Cell Bank Characterization

#24 Information Requested

For addition to the disclosable safety narrative, please clarify, per Table 4.3.3.3.1-1 of the confidential supplementary material, that testing of cell banks includes *Campylobacter* spp.

UPSIDE Foods' Response:
UPSIDE Foods hereby confirms that the testing for adventitious agents in cell banks includes testing for *Campylobacter* spp.

Culture Process

25 Information Requested

For addition to the disclosable safety narrative, please clarify that sterilization procedures are in place for large-scale culture vessels and culture transfer lines, and for liquid nutrient media, as described in Section 4.5.1 of the confidential supplementary material.

UPSIDE Foods' Response:
During all stages of cell cultivation for seed train and the tissue formation stage, UPSIDE Foods employs measures to ensure the cultures remain free of contaminating bacteria, yeast, molds, or fungi.

- Cultures in small-sized vessels may be taken into an appropriate, High Efficiency Particulate Air (HEPA)-filtered, biological safety cabinet for open operations.
- Operators are trained on appropriate aseptic practices.
- Cultures maintained in suspension bioreactors, or the meat cultivation vessels, will be conducted using aseptic procedures demonstrated to be effective for minimizing the introduction of contaminants.
- Stainless steel vessels and culture transfer lines will be sterilized using high temperature steam (>121°C).
- Liquid nutrient media will be sterilized using 0.2 µm membrane filters prior to use.
- Samples will be taken from the cultures periodically and tested/ inspected for contamination.
- Environmental monitoring will be in place to assess the effectiveness of the overall hygienic practices.

#26 Information Requested

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

UPSIDE Foods' Response:
UPSIDE Foods will use appropriate and authorized food contact materials throughout the production process for its products. While UPSIDE Foods did not explicitly state this in the previous document, the document does state: "*All equipment and food-contact tools and manufacturing consumables are reviewed to ensure that they are made from food-safe materials*".

Product Characterization

#27 Information Requested

For addition to the disclosable safety narrative, please provide the information in Table 5.3-1 of the supplementary material regarding the methods used for the proximate comparison data.

UPSIDE Foods' Response:

The methods used to quantify proximate components in UPSIDE Foods' analyses are tabled below.

Methods Used for Proximate Analyses		
Parameter	**Method**	**Reference(s)**
Moisture	AOAC 950.46	AOAC (1950) (AOAC, 1920-2013)
Total Protein	AOAC 981.10	AOAC (1983) (AOAC, 1920-2013)
Crude Fat	AOAC 960.39	AOAC (1960) (AOAC, 1920-2013)
Ash	AOAC 920.153	AOAC (1920) (AOAC, 1920-2013)
Carbohydrates	Calculation: Carbs = 100% - (% protein + % fat + % ash + % moisture)	See combined reference
Calories	Calculation: Calories = 9(Fat) + 4(Protein + Carbs)	See combined reference

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Table of AOAC Official Methods		
Official Method 920.153	Ash of meat	First action: 1920
Official Method 950.46	Moisture in meat	First action: 1950
Official Method 960.39	Fat (crude) or ether extract in meat	First action: 1960
Official Method 976.26	Cholesterol in multicomponent foods, gas chromatographic method	First action: 1976
Official Method 981.10	Crude protein in meat, block digestion	First action: 1981, Final 1983
Official Method 996.06	Fat (total, saturated, and unsaturated) in foods: hydrolytic extraction gas chromatographic method	First action: 1996
Official Method 998.08	*E. coli* and coliforms	First action: 2002
Official Method 2001.13	Vitamin A (retinol) in foods, liquid chromatography	First action: 2001
Official Method 2003.01	Enumeration of *Enterobacteriaceae* in selected foods. Petrifilm™ *Enterobacteriaceae* count plate	First action: 2003; Final 2006
Official Method 2008.06	Moisture and fat in meats microwave and nuclear magnetic resonance analysis	First action: 2008
Official Method 2011.03	Salmonella in a variety of food, Vidas® Salmonella (SLM) easy Salmonella	First action: 2011
Official Method 2011.04	Protein in raw and processed meats. automated	First action: 2011
Official Method 2011.11	Vitamin D in infant formula and adult/pediatric nutritional formula, ultra-high-performance liquid chromatography/tandem mass spectrometry	First action: 2011
Official Method 2013.06	Arsenic, cadmium, mercury, and lead in foods	First action: 2013

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September 16, 2022
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Re: Requests for Additional Information (CCC 000002) Received on July 6, 2022

This letter responds to the Food and Drug Administration's (FDA) request for additional data and information relating to UPSIDE Foods' disclosable safety narrative submitted to the agency on October 1, 2021, and filed under Cell Culture Consultation No. CCC 000002. UPSIDE Foods previously provided responses to FDA's substantive information requests and points of clarification on March 25, 2022. The following responses address additional FDA substantive information requests received on July 6, 2022. Questions from FDA are presented below in blue text and corresponding responses from UPSIDE Foods are shown in black.

FDA's Substantive Information Requests and Points of Clarification

Substantive Information Request

Product Characterization

[#1] Information Requested: We note that your reported analytical data for lead content is substantially lower than your planned specification. If possible, please consider providing, for addition to the public narrative, a revised and lower lead specification.

UPSIDE Foods' Response:

UPSIDE Foods agrees with FDA and has reduced the lead specification, reported on page 19 of UPSIDE Foods' response in the amendment dated March 25, 2022, to ≤ 0.1 ppm.

Points of Clarification

Product Characterization

[#2] Information Requested: For addition to the disclosable safety narrative, please provide the method used for folic acid/folate analysis.

UPSIDE Foods' Response:
UPSIDE Foods analyzed folic acid/folate using a validated third-party testing system (i.e., the VitaFast® Folic Acid microtiter plate test). The test is a microbiological method for the quantitative determination of total folic acid in food. It has been validated for use in meat products and has an intra-assay variability below 10% in this food matrix. The test system meets international standards, and has been certified by the AOAC research institute as being fit for purpose (AOAC-RI 100903).[1]

[1] R-Biopharm AG (2021). VitaFast® [Vitamin B9] (Folic Acid), Art. No.: P1001. Darmstadt, Germany: R-Biopharm AG. Available at: VitaFast® Folic Acid (en) - Food & Feed Analysis [2021].

[#3] Information Requested: For addition to the disclosable safety narrative, please clarify your view on the role of comparative analysis with regard to the presence of growth factors in food in your overall safety assessment, and in particular the sensitivity of your assessment to variability in analytical data for your product with respect to any particular conventional comparator.

UPSIDE Foods' Response:

Growth factors used during cell cultivation include recombinant proteins produced in safe production organisms expressing homologous protein sequences of chicken and bovine origin, which exhibit functional similarity due to their evolutionary history. UPSIDE Foods notes that growth factors produced by vertebrate species tend to display a high sequence and functional homology. BLAST comparisons, conducted by UPSIDE Foods personnel, of the amino acid sequences of bovine and chicken growth factors used during cell cultivation support this biological principle; the comparisons demonstrated a 100% amino acid sequence homology between the growth factors analyzed for these species (UniProt, 2021)[2]. In general, growth factors also demonstrate a highly conserved functionality in vertebrates, which is supported by numerous studies that employ alternate growth factor species that result in the same functional outcome. UPSIDE Foods observes similar proliferation rates and phenotypes of cell lines in the presence of homologous growth factors, which supports the scientific evidence that growth factors exhibit highly-conserved structure and function.

UPSIDE Foods' safety assessment of the presence of growth factors in food is based upon a weight of evidence approach that considered: 1) information detailing the measured quantities of growth factors in cultivated poultry meat (CPM) post-harvest relative to measured quantities within an appropriate comparator (i.e., store-bought chicken breast); 2) analytical data characterizing the poor stability of growth factors to cooking temperatures; 3) published information characterizing the overall poor stability of growth factors; 4) published in vivo data in animals and humans demonstrating that growth factors do not display bioactivity when ingested via the oral route; and 5) limiting use to growth factors from agriculturally relevant species that have a history of safe consumption. Each of these points is discussed further in the subsequent paragraphs.

For UPSIDE Foods' comparative analysis, concentrations of each growth factor used during cell cultivation were analyzed post-harvest using a commercially available ELISA kit. Store-bought chicken breast was used as the comparator. UPSIDE Foods verified that the commercial test kits have been validated for the detection of growth factors from each species source tested. In addition to considerations related to the sensitivity and specificity of the ELISA assays to accurately detect growth factors from different species (i.e., chicken vs. bovine), differences in matrices of CPM in comparison to chicken breast samples were addressed by the use of positive control spiking assays to ensure that there were no differences in the extraction efficiencies between the samples that would impact interpretation of the results. UPSIDE Foods reported an extraction efficiency of ca. 100%, demonstrating an absence of meaningful matrix interactions.

From a safety perspective, UPSIDE Foods' analysis revealed no meaningful differences between the levels of growth factors in cooked CPM and cooked conventional chicken. The very low or undetectable levels

[2] The UniProt Consortium, UniProt: the universal protein knowledgebase in 2021, Nucleic Acids Research, Volume 49, Issue D1, 8 January 2021, Pages D480–D489, https://doi.org/10.1093/nar/gkaa1100

in the cooked samples demonstrate that growth factors in CPM are, in effect, functionally destroyed by the usual temperatures achieved during cooking of poultry (165 °F), as discussed further below.

Differences in growth factor presence between uncooked CPM and uncooked conventional chicken are rendered inconsequential by the cooking process[3]; however, for the sake of comprehensiveness, UPSIDE Foods also notes that an important consideration in the analytical comparisons relates to differences in the age and storage of the CPM and store-bought chicken breast samples that were analyzed. For our comparison, the CPM product was a freshly harvested product, which was then compared to store-bought chicken breast samples. The store-bought samples were procured days to weeks after harvest from the live animal and thus, as a result of the high thermolability of growth factors, lower levels of endogenous growth factors were detected in the store-bought samples compared to levels that would have been detected in a recently slaughtered animal. Accordingly, the observed differences in the concentrations measured in the uncooked store-bought chicken breast relative to uncooked CPM are likely due, in part, to differences in the age of the samples.

In addition, it should be noted that chicken is not consumed as a raw food product. CPM, like conventional chicken, will be subjected to further food processing and cooked prior to consumption. Temperatures achieved during cooking (165 °F) typically denature or inactivate growth factors. As a result, due to the poor thermal stability of growth factors, residual quantities of growth factors that may be present in uncooked CPM will be biologically inert after the CPM is cooked and ready for consumption. Using the aforementioned ELISA assays, UPSIDE Foods has demonstrated that the cooked CPM and the cooked chicken breast samples contain either no detectable levels or only de minimis levels of growth factors. These observations also support conclusions that variability in the concentrations of growth factors observed between the uncooked CPM and uncooked chicken breast samples, whether due to age of the samples or differences in the specificities or sensitivities of the assay methodologies, are inconsequential to the safety assessment. In summary, analysis of growth factor presence in cooked samples demonstrates that the growth factors are susceptible to usual temperatures applied during cooking of poultry to safe temperatures (165°F), and no hazard from their residues in food are identified.

In addition to the above analytical/comparative studies, UPSIDE Foods conducted scientific literature searches to obtain publicly available data and information characterizing the stability of each growth factor used in UPSIDE Foods' process for producing CPM. Where available, published studies in animals characterizing the hazard of consuming growth factors in the diet also were obtained. Studies in animals administered the same growth factors used for the production of CPM have demonstrated no evidence of toxicity or any biological effects following oral consumption of the growth factors; this is consistent with studies demonstrating that growth factors are susceptible to gastrointestinal digestion and thus are not orally bioactive[4].

As noted above, comparative evaluations formed part of the weight of evidence approach to safety. UPSIDE Foods also considered the source and sequence of each growth factor used in the company's production process and its history of consumption in the food supply. Notably, UPSIDE Foods uses only growth factors from agriculturally relevant species (e.g., chickens and cows) that have a history of safe

[3] Growth factors have functional roles to modulate localized changes within tissues, a functional property that is critical for tissue homeostasis. Therefore, most growth factors have functional half-lives of minutes to hours (Cell Access Systems, 2020)

[4] As one example, see Playford RJ, Marchbank T, Calnan DP, Calam J, Royston P, Batten JJ, Hansen HF. Epidermal growth factor is digested to smaller, less active forms in acidic gastric juice. Gastroenterology. 1995 Jan;108(1):92-101. doi: 10.1016/0016-5085(95)90012-8. PMID: 7806067.

consumption. Even so, a greater emphasis was placed on results of analytical studies demonstrating that growth factors in CPM are fully denatured and are present at undetectable or otherwise de minimis levels following cooking, findings that were substantiated by data obtained from the literature demonstrating that the growth factors used for CPM production are unstable, and are generally recognized to be inactive when administered to animals *via* the oral route. Overall, in light of the generally recognized poor stability of these proteins, any variability in the sensitivity of the ELISA assays or statistical variability in the range of concentrations of growth factors that may be present in the samples or in comparator foods did not impact conclusions relating to the safety evaluation of growth factors.

Material Inputs

[#4] Information Requested: For addition to the disclosable safety narrative, please provide a statement that the production process described in CCC 000002 does not incorporate medium constituents that have been modified to be more stable and/or more active than those found endogenously in animal tissue, consistent with your statement on page 14 of the March 30, 2022 amendment.

UPSIDE Foods' Response:

The production process described in CCC 000002 does not incorporate or otherwise include any medium constituents that have been modified to be more stable or more active relative to their native counterparts in food.

[#5] Information Requested: For addition to the disclosable safety narrative, please provide additional context to clarify what aspects of identity (e.g., protein identity, functional activity, protein sequence, species origin) are the basis of your statement on page 14 of the March 30, 2022 amendment about the equivalence of growth factors in commercially available conventional chicken and in your production process, and why those aspects are most important in the context of your overall reasoning on the safety of your use of these proteins.

UPSIDE Foods' Response:

As discussed above, as part of its weight of evidence approach, UPSIDE Foods evaluated the intrinsic characteristics of growth factors, including their functional activity, species origin, and other aspects of their identity. Most importantly from a safety perspective, most growth factors exhibit poor functional stability (i.e., short half-lives)[5] and are generally recognized to be susceptible to denaturation to inert proteins during cooking temperatures.

UPSIDE Foods has demonstrated that all growth factors used during its CPM production are denatured to nonfunctional proteins as evidenced by the inability to detect significant levels of any of the growth factors used during production *via* highly sensitive ELISA assays in fully cooked products. As discussed in the response to request #3 above, the ELISA assays, used to analyze growth factor concentrations in CPM

[5] As one example, see Chen G, Gulbranson DR, Yu P, Hou Z, Thomson JA. Thermal stability of fibroblast growth factor protein is a determinant factor in regulating self-renewal, differentiation, and reprogramming in human pluripotent stem cells. Stem Cells. 2012, 30:623-30. This study notes that FGF2 loses its activity within 24 hours at 37 °C.

in comparison to chicken breast samples, are validated for cross-reactivity to chicken and bovine sources. In addition, it is UPSIDE Foods' view that the function of a protein will be more susceptible to thermal inactivation denaturation than its antigenic binding by immunoglobulins used in ELISA assays, which target small epitopes of the proteins; therefore, the absence of detectable growth factors using an ELISA assay provides a high level of confidence that residues of growth factors remaining in CPM are functionally inactive.

As part of the company's weight of evidence approach, UPSIDE Foods further employed a comparative approach to evaluate the safety of CPM, leveraging the history of safe consumption of growth factors that are naturally present within meat and poultry products consumed in the diet. UPSIDE Foods recognizes that this approach in the safety assessment should establish that the growth factors used for cultivated meat production share a similar functional identity to the comparator growth factor from meat products. In this regard, it should be noted that UPSIDE Foods only uses growth factors from agriculturally relevant species (e.g., bovine and chicken), thereby ensuring that the comparative approach to safety remains a valid comparison for use in the safety evaluation. Moreover, UPSIDE Foods uses growth factors with protein sequences that are fully homologous to their native counterparts in the animal and does not use growth factors with modified amino acid sequences or other modifications that enhance functionality or stability.

[#6] Information Requested: For addition to the disclosable safety narrative, please provide a summary of your reasoning regarding the comparative basis for safety of serum used in your production process as discussed on page 96 of the supplementary confidential material, including a statement on the importance (if any) of the life stage of the serum source with respect to your reasoning.

UPSIDE Foods' Response:

Put simply, serum is animal blood that is absent of cells and clotting factors - the acellular liquid fraction derived from whole blood. Potential hazards associated with the use of animal serum, regardless of life stage, include possible contamination with mycoplasma, viruses, endotoxins, veterinary drugs, and prions. To mitigate such hazards, any serum products used by UPSIDE Foods are tested for human zoonotic viruses, bacterial contamination, and mycoplasma, and filter-sterilized prior to use in commercial production. Importantly, bovine serum will be sourced only from countries that are classified as Bovine Spongiform Encephalopathy (BSE) negligible risk by the Animal and Plant Health Inspection Service (APHIS), in accordance with 9 CFR § 92.5. As part of the company's food safety plan, animal-derived raw materials also are tested for adventitious agents.

On the basis that serum is derived from animal blood and used in culture media at physiologically relevant concentrations optimum for cell survival and growth, UPSIDE Foods concludes that any serum constituents in the culture media will be present at levels that are largely representative of extracellular concentrations bathing animal cells in vivo from circulating blood. During animal slaughter, 40 to 60% of the total blood volume is typically removed from the carcass through exsanguination. The remaining blood is believed to be present within visceral tissue, and the residual blood content of lean meat has been reported to be between 2 to 9 mL/kg of muscle (Warriss, 1984). To the extent that serum residues are present in CPM, UPSIDE Foods expects such levels to be no higher than levels that would be ingested from consumption of conventional meat products already in the diet. UPSIDE Foods also notes that the

bioactive protein constituents of serum are expected to be highly thermolabile and will be denatured during cooking temperatures, providing an additional degree of safety for the use of this product in CPM production[6]. Such denatured proteins would be subject to the typical digestive process and converted to peptides and other common nutrients like other dietary proteins.

UPSIDE Foods is currently optimizing its process to phase out the use of whole animal serum, as well as animal serum constituents, during the meat production phase.

[6] PubMed: Chen G, Gulbranson DR, Yu P, Hou Z, Thomson JA. Thermal stability of fibroblast growth factor protein is a determinant factor in regulating self-renewal, differentiation, and reprogramming in human pluripotent stem cells. Stem Cells. 2012 Apr;30(4):623-30

September 30, 2022

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Re: UPSIDE Foods' amendment to submission CCC-000002: Wash Buffer

Dr. Fasano,

UPSIDE Foods submits the following amendment to the premarket notice filed under Cell Culture Consultation No. CCC 000002, to provide additional information regarding the wash buffer referenced in Section 4.4.4, used during the company's post-harvest media removal process.

Relevant Factual Background

Description of Media Removal Process and Components

UPSIDE Foods' media removal process, described herein, uses a phosphate buffered saline (PBS) solution to remove residual culture media components from the extracellular tissue matrix of the harvested cultivated chicken. Sterile PBS solution, rather than sterile water, is used to maintain the necessary osmotic pressure on harvested cultivated chicken cells to ensure they do not lyse during the rinse. These solutions are widely used in the food industry, with each of the individual components considered to be Generally Recognized as Safe (GRAS) by FDA.

Occurring post-harvest in UPSIDE Foods' small-scale production process, the media removal process will be subject to FSIS inspection as set forth in the 2019 *Formal Agreement Between FDA and USDA Regarding Oversight of Human Food Produced Using Animal Cell Technology Derived from Cell Lines of USDA-amenable Species*.

Upon completion of cultivated chicken production, at small-scale, the tissue and media are placed in a food-grade strainer container, where sterile PBS solution (at up to 0.25x concentration - See Table 1 below) is added to ensure sufficient dilution effect.

After the materials are mixed, the container is drained over a sterilized strainer with the tissue fraction remaining in the strainer to be collected. Thereafter, the drained PBS solution is appropriately discarded.

PBS Wash Buffer Composition

The PBS solution is prepared in sterile water, sterile-filtered, and used during the media removal process at no greater than 0.25x concentration as specified in Table 1. 0.25x or lower is used as 0.25x is the greatest concentration observed that sufficiently removes residual media components while maintaining cell membrane integrity. UPSIDE Foods has observed no further benefit from the use of a PBS solution above 0.25x and therefore does not intend to use the solution above 0.25x for post-harvest media removal for cultivated chicken at this time.

Table 1

	0.25x (g/L)	**Final Concentration (millimolarity)**
POTASSIUM CHLORIDE	0.05	6.7 mM
POTASSIUM PHOSPHATE MONOBASIC ANHYDROUS	0.05	3.7 mM
SODIUM PHOSPHATE DIBASIC ANHYDROUS	0.29	20.3 mM
SODIUM CHLORIDE	2.0	342 mM

Removal of PBS Solution from Cultivated Chicken and Lack of Technical Effect in the Finished Product

Virtually all the PBS solution is removed from the cultivated chicken during this process, with none, or only an insignificant level, present in the finished product. To confirm that the PBS solution is removed from the finished product, UPSIDE Foods measured the sodium, potassium, and phosphorus in the cultivated chicken through processing. Total phosphorus is measured as an indicator of individual phosphates. Measurement was by ICP-OES, FDA EAM 4.4 method, across seven production runs. UPSIDE Foods' analysis reveals that the level of sodium in the final cultivated chicken product is significantly lower than in the pre-wash, harvested cultivated chicken material. The levels of potassium and phosphorus are not significantly different, demonstrating that significant levels of constituents of the PBS solution do not adhere to the cultivated chicken after processing.

Safe Use of the PBS Solution

Each component of the PBS solution is considered to be GRAS by FDA:

- Potassium chloride is listed as a direct food substance affirmed as GRAS in 21 CFR § 184.1622 "with no limitation other than current good manufacturing practice."
- Sodium phosphate is listed as a GRAS substance in 21 CFR §§ 182.1778 and 182.8778 "when used in accordance with good manufacturing practice."
- Potassium phosphate (monobasic) was reviewed by the Select Committee on GRAS Substances (SCOGS) in Report No. 32, PB262651 (ID Code 7778-77-0). The report states that "this substance is included on a list of substances presumed to be GRAS by FDA" and was issued a conclusion of "1" by the Select Committee.
- Sodium chloride is listed as an example of a GRAS substance in 21 CFR §182.1(a)

Furthermore, the safe use of a PBS solution comprised of the same or similar components has been evaluated in GRAS Notices that have received No Question Letters from FDA. For example, in GRN 966, which received a No Questions Letter, the notifier discussed the use of a PBS solution comprised of the

same components as a wash buffer.[1] Each of the components is also found in FSIS Directive 7120.1 *Safe and Suitable Ingredients Used in the Production of Meat, Poultry and Egg Products* with various acceptable uses.

Conclusion

In summary, UPSIDE Foods proposes to use an isotonic, aqueous solution of potassium chloride, potassium phosphate monobasic, sodium phosphate dibasic, and sodium chloride to remove residual culture media components from cultivated chicken tissue after harvest, as referenced in Section 4.4.4 of the submission, as part of its small-scale production process. Each of these components is GRAS when used in accordance with good manufacturing processes, and UPSIDE Foods' use of the solution will be conducted in accordance with good manufacturing practices.

[1] *GRAS Notification for Preparations Containing Three to Eight Bacteriophages Specific to Campylobacter jejuni* (GRN 966), available at https://www.fda.gov/media/152868/download [Oct. 6, 2021 - FDA response - no questions].

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RE: UPSIDE Foods' Responses to Requests for Clarification (CCC 000002)

UPSIDE Foods submits this letter in follow-up to a phone conversation with Stephanie Hice, Ph.D., on November 7, 2022 regarding two clerical errors in the March 30, 2022 amendment and September 16, 2022 amendment relating to UPSIDE Foods' disclosable safety narrative submitted to the agency on October 1, 2021, and filed under Cell Culture Consultation No. CCC 000002. As requested during the November 7, 2022 call, this letter provides clarification regarding (1) the specification for total plate count in the harvested cell material, and (2) the comparator chicken samples used in the tests UPSIDE Foods performed to analyze concentrations of growth factor residues in samples of cultivated poultry meat. For the first item, FDA's specific question is presented in blue text and UPSIDE Foods' response is shown in black.

(1) Specification for total plate count.

Table 4.5.5-1 (page 54) of the confidential supplementary material and the table presented on page 19 of the March 30, 2022 amendment list the specification for total plate count as <10 colony forming units (CFU)/g; however, the results of the microbial analyses of the harvested cellular material are reported in Table 5.4.5-1 (page 82) of the confidential supplementary material as <100 CFU/g. For the administrative record, please clarify this discrepancy.

UPSIDE Foods' Response:

The listing of the specification for total plate count as <10 CFU/g was a typographical error. The correct specification is <100 CFU/g. This number reflects the lower limit of detection (<100 CFU/g) of the Aerobic Plate Count Method used (APHA CMMEF Ch8), as shown in Table 5.5-1 of UPSIDE Foods' disclosable safety narrative, as well as in the table presented on page 27 of the March 30, 2022 amendment.

(2) Comparators for growth factor analysis.

Below we provide clarification regarding the comparator chicken samples used in the tests UPSIDE Foods performed to analyze concentrations of growth factor residues in samples of cultivated poultry meat.

In our response to request #15 (on page 19) in the March 30, 2022 amendment, we identified "ground chicken meat, whole muscle meat from a chicken leg, and skin from a chicken leg" as the comparators used in these tests. However, in our response to request #3 (on pages 2-4) in the September 16, 2022 amendment, we incorrectly stated that "store-bought chicken breast" was used as the comparator in

these tests and referred to the comparator samples as “chicken breast samples.” This error was repeated in our response to request #5 (on pages 4-5) in the September 16, 2022 amendment. In both responses in the September 16, 2022 amendment, references to the comparators used in these tests should be to store-bought ground chicken meat, whole muscle meat from a chicken leg, and skin from a chicken leg, as stated in the March 30, 2022 amendment.