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

Memorandum

Date March 17, 2023

From Katie Overbey (Division of Food Ingredients)

Through

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Subject Cell Culture Consultation (CCC) 000001, Cultured *Gallus gallus* cell material

To Administrative File, CCC 000001

Submission Received Date: March 4, 2022

Amendments Received Date: July 8, 2022; August 4, 2022; September 14, 2022; March 2, 2023

Sponsor: GOOD Meat, Inc. (GOOD Meat, the firm)

Summary

- The Food and Drug Administration (FDA, we) evaluated the food that is the subject of CCC 000001 submitted by GOOD Meat.
- For the purposes of this consultation, this food is defined as the cell material at harvest, comprised of cultured *Gallus gallus* cells, with characteristics of fibroblasts, in the form of cell biomass, as produced by the method of manufacture described in CCC 000001.
- The cell line originates from the commercially available chicken cell line, UMNSAH/DF1 (deposited in the American Type Culture Collection as ATCC CRL12203), a chicken fibroblast line initially isolated from mid-stage fertilized chicken eggs. The isolated cells

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are phenotypically characterized using molecular methods to identify fibroblast markers.
- The cell lines are established by selective culture for growth in suspension and reduced bovine serum concentrations.
- The cells are cultured by increasing total cell numbers in a suspension culture proliferation phase.
- The cells are harvested by centrifugation and washed prior to frozen storage.
- The harvested material, following washing, is described as a cell paste. Microbial and toxic heavy metal specifications are provided. Species identity was verified in the harvested material using a species-specific polymerase chain reaction (PCR) assay.
- We evaluated information about the cell lines, the production process (including cell bank establishment), substances used in the production process, and properties of the harvested cell material, including information available in both the disclosable safety assessment as well as supporting, corroborative information in a confidential supplementary appendix.
- Based on the data and information presented in CCC 000001, we have no questions at this time about GOOD Meat's conclusion that foods comprised of, or containing, cultured chicken cell material resulting from the production process defined in CCC 000001 are as safe as comparable foods produced by other methods.

Production Method

GOOD Meat describes an overall production process involving the establishment of a cell bank that provides a standardized source of cells for food production, and a cell culture food production process including proliferation or multiplication of the cells and harvest or collection of the cell material for subsequent conventional food processing.

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

- A current good manufacturing practice (cGMP) program that includes all the items enumerated in 21 CFR 117 subpart B;
- Development of a hazard analysis and risk-based preventative controls (HARPC) food safety plan, including preventive measures and corrective actions for prevention and mitigation of biological, chemical, and physical hazards;
- In-process checks and controls of key process parameters;
- Documentation of the implementation and/or validation of process controls, sanitation and environmental controls, and supply chain controls;
- A product release system involving quality assurance review for incoming raw materials, intermediate products, and finished products;
- Batch record review; and
- Traceability of raw materials and finished products.

GOOD Meat also states that its cell culture production process follows internal standard operating procedures (SOPs) and is performed by authorized and trained personnel. JOINN Biologics US Inc. (JOINN Biologics), the facility performing the cell culture process, lists SOPs

that include: crisis management, document management, employee training, general cleaning procedures, and recall procedures. The firm states that cell culture occurs in a controlled environment that utilizes high efficiency particulate air (HEPA) filters and differential pressure to maintain air quality.

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

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

Process Step	**Potential Issue**	**Management Strategy**
Cell Procurement	Cell identity; contamination from source, reagents, or environment	Aseptic procedures, documentation, genetic testing, supplier management, testing program, filtration of media, controlled temperature conditions
Establishment of Cell Lines	Cell identity; contaminants from materials or environment; appropriate adaptation to culture	Aseptic procedures, documentation, genetic sequencing, process and environmental monitoring, supplier management, testing program
Establishment of Master Cell Banks (MCB) and Master Working Cell Banks (MWCB)	Cell identity; contaminants from materials or environment; media components; appropriate adaptation to culture	Aseptic procedures, documentation, genetic sequencing, controlled temperature conditions, process and environmental monitoring, testing program
Cell Proliferation using Suspension Culture	Cell identity; contaminants from materials, equipment, or environment; contamination during transportation, media components	Aseptic procedures, controlled temperature conditions, documentation, genetic testing, process and environmental monitoring, supplier control, testing program
Harvest of Cell Material	Contaminants from materials or environment; media components	Aseptic procedures, compositional analysis, controlled temperature conditions, documentation, testing program, washing

Cell Banking

GOOD Meat provides information about the establishment of cell banks used in the subsequent cell culture food production process. A cell bank is defined in the firm's manufacturing process as a collection of processed and cryogenically stored cells distributed in a single operation. The firm uses a common system in which there is both a primary cell bank (the master cell bank; MCB) and secondary cell banks (master working cell banks; MWCB) each derived from a subset of cells stored in the MCB. The steps involved include:

- Cell procurement
- Establishment of cell lines
- Establishment of MCBs and MWCBs

Cell Procurement

The cells used to establish the cell banks originate from the commercially available chicken fibroblast cell line UMNSAH/DF1[1,2]. The generation of the parental UMNSAH/DF1 cell lines, including isolation of chicken embryonic primary cells obtained by removing the embryonic torso of 10-day old embryos, characterization, and generation of immortalized cell lines, is described in US Patent 5,672,485 (Attachment A1 of the firm's July 8, 2022 amendment). This cell line exhibits enhanced proliferative capacity due to spontaneous immortalization through selection in culture.

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

- Introduction of adventitious agents present in the parental cell line; and
- Phenotypic properties of the parental cell line.

Documentation for the cell line includes supplier certification that the line is negative for Avian Influenza (Type A), Avian Reovirus, Avian Adenoviruses (Groups I-III), Avian Encephalomyelitis Virus, Fowl Pox, Newcastle Disease Virus, Paramyxovirus (type 2), *Mycoplasma* spp.*, Salmonella* serovars, and other infectious agents known to infect poultry stock.

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[1] GOOD Meat states that the cell line is deposited in the cell line collection of the ATCC (Manassas, VA) with the deposit designation ATCC CRL12203. http://www.atcc.org/Products/All/CRL-12203.aspx (last accessed: May 2022).

[2] GOOD Meat notes that the product sheet for cell line UMNSAH/DF1 contains the disclaimer that "this product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use. Any proposed commercial use is prohibited without a license from ATCC." GOOD Meat states the ATCC disclaimer is not relevant in this context due to the safety and quality characterization performed on this cell line as adapted and cultured using the methods described by the firm.

GOOD Meat describes a study[3] characterizing the parental cell line that reports higher expression of genes associated with cell cycle proliferation and progression, downregulation of cell death pathways, and accelerated capacity for molecular transport compared to primary chicken embryo fibroblast cells. [4]

Establishment of Cell Lines

GOOD Meat induces the ability for its adherent cell line to grow in suspension ("suspension adaptation") through selective culture in the presence of bovine serum. The cells are further selected for the ability to grow in lower levels of bovine serum. As previously noted, the cell line described in CCC 000001 displays enhanced proliferative capacity originally acquired in the development of the parental cell line, conferred by spontaneous immortalization in selection culture. Reagents used at this stage include cell culture media and media components (including animal-derived bovine serum) which are used to expand the parental cells and adapt them to suspension culture conditions.

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

- Introduction of adventitious agents from contaminated non-animal sourced reagents or the local environment; and
- Introduction of adventitious agents from animal-derived reagents (bovine serum).

Bovine serum is tested for species-specific viruses, including bluetongue, bovine adenovirus, bovine parvovirus, bovine respiratory syncytial virus, bovine viral diarrhea virus, rabies, reovirus, and cytopathogenic[5] and hemadsorbing[6] agents, as well as tests for general sterility, endotoxin, and *Mycoplasma* spp., and is subject to a filtration and heat inactivation step. Bovine serum is verified to be sourced from bovine spongiform encephalopathy (BSE)-free/risk-negligible herds. GOOD Meat states that cell lines are monitored for cell density and cell viability and documentation of passage number, and population doubling time is used to monitor health of the cell line. Adapted suspension cell lines are documented, expanded, and cryopreserved before creation of MCBs.

GOOD Meat states that internal SOPs are followed, and describes controls in place to prevent

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[3] Kong, B.W.; Lee, J.Y.; Bottje, W.G.; Lassiter, K.; Lee, J. and Foster, D.N. (2011) Genome-wide differential gene expression in immortalized DF-1 chicken embryo fibroblast cell line. BMC Genomics. 12:571. https://doi.org/10.1186/1471-2164-12-571.

[4] GOOD Meat also notes that the developers of the UMNSAH/DF1 cell line assessed the potential for *in vivo* unregulated proliferation through both indirect and direct measures and found no indication of transformation or tumorigenic capacity. Further, as described in US Patent 5,672,485, the cell line has undergone greater than 100 population doublings and greater than 160 passages. No chromosomal aberrations were found after cytogenetic examination of 100 cells.

[5] Infectious Bovine Rhinotracheitis

[6] Parainfluenza Type 3

environmental contamination during suspension adaptation, including the use of authorized and trained personnel, aseptic procedures, and the use of biosafety cabinets during cell handling, cell passaging, and change of culture media. Additionally, throughout the culture process, the firm implements supply chain preventive controls, filtration of growth media, and cold storage to manage potential contamination risk from reagents used during cell culture, as well as sanitation controls (including water quality monitoring, equipment cleaning, and facility sanitation).

Establishment of MCB and MWCB

GOOD Meat states that individual cell lines are prepared for storage in an MCB after adaptation to suspension and low serum culture media. An MCB is defined by the firm as a collection of cryogenically stored cells distributed in a single operation. GOOD Meat states that each MWCB is established directly from aliquots of the MCB which are expanded by serial subculture, and that MWCB are used in the production of cells for the manufacturing of commercial products following cGMP-compliant procedures.

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

- Use of an unintended cell line due to documentation or handling errors;
- Introduction of adventitious agents from the animal-derived bovine serum;
- Adventitious agent contamination of the cell line used in establishing the cell bank;
- Biological, chemical, and physical contamination from growth media; and
- Use of cells that do not exhibit desired growth characteristics.

GOOD Meat states that each MCB and MWCB is assigned its own batch record document and number, and that cryopreservation and thawing of cells are performed according to SOPs and good manufacturing practice chain of custody documentation (i.e., vial identity confirmation) during retrieval to ensure that the appropriate vial(s) are retrieved from the MWCB for cell bank release testing and cell culture production.

GOOD Meat describes quality and safety testing for each MCB and MWCB. These include tests and minimum specifications for cell viability and proliferation rate, for species and cell lineage identity, and for sterility and absence of adventitious agent contamination, which the firm states are validated for their intended purposes. The firm confirms the lineage of the cells in the MCB by gene expression analysis of RNA for fibroblast-specific protein 1 and the species identity by PCR analysis and sequencing of mitochondrial DNA. GOOD Meat also measures parameters related to cell proliferation and viability to confirm stability of the cells used in the cell banks.

GOOD Meat states that the firm's adventitious agent testing is intended to address common public health hazards that have the potential to propagate in cell culture and cultured animal cells. Testing will include using a direct aliquot of the primary cell bank to screen for the presence of aerobic, microaerophilic, and anaerobic bacteria, yeasts, and molds via liquid culture. Testing of MCBs is also stated to include tests for *Mycoplasma* spp. and other

adventitious agents, including human and avian viruses.[7] GOOD Meat states that human bacterial pathogens of clinical importance associated with conventional chicken include *Campylobacter* spp., *Salmonella* serovars, and *Escherichia coli*. The firm explains that MCBs and MWCBs are screened for these adventitious agents. GOOD Meat discusses details of adventitious agent testing, which is conducted using validated methods by a third-party laboratory using either observation of potential microbial growth under permissive conditions (sterility testing) or real-time PCR analysis (*Mycoplasma* spp. and select human and avian bacteria and viruses).

GOOD Meat describes controls to prevent environmental contamination during suspension adaptation, including the use of trained personnel, aseptic procedures, and the use of biosafety cabinets. Additionally, as noted above, GOOD Meat implements supply chain controls, filtration of growth media, and cold storage to manage potential contamination risk from reagents used during cell culture, as well as sanitation controls. GOOD Meat ensures that each MCB and MWCB meets standards for cell viability, cell proliferation, identity, purity, safety, and stability before being released for use in cell culture food production.

Cell Culture Food Production Process

GOOD Meat provides information about its cell culture food production process including:

- Cell proliferation using suspension culture
- Harvest of cell material

GOOD Meat states that the cell culture food production process is performed by a third party, JOINN Biologics. GOOD Meat states that the firm's safety systems are based on the requirements of 21 CFR parts 117 and 121 and has confirmed that JOINN Biologics will appropriately mitigate food safety risks and will follow the food safety plan as outlined by the firm.

Batch records will be maintained to provide traceability of all raw materials used, operations, and testing during the production process. GOOD Meat also states that all incoming dry powdered culture media as well as other raw materials used for culture media undergo testing and verification appropriate to the specific media component and are stored under appropriate conditions. Liquid media is filter sterilized through 0.2-micron filters and stored at 2-8°C. GOOD Meat states that the firm uses appropriate and authorized food contact materials throughout the production process.[8] The firm further states that single use disposable sterile components are used for the seed train expansion and cell growth processes. The process also

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[7] These are listed in Table A6 of the firm's July 8, 2022 amendment.

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

uses stainless steel bioreactors that are cleaned-in-place (CIP) and sanitized-in-place (SIP).

GOOD Meat states that the production process is a highly controlled aseptic process. The firm explains that the cell culture environment was designed for the biopharmaceutical industry and that sterile procedures are used at all stages of cell culture. Cell handling, cell passaging, and change of culture media are described as being performed in biosafety cabinets. GOOD Meat states that single use disposable sterile components or CIP/SIP stainless steel are used for all bioreactor surfaces that come in direct contact with cultured chicken cells. Cultures are described as being sampled under sterile conditions at both pre- and post-harvest and tested for adventitious agent contamination. The firm also states that an environmental monitoring program is in place to assess the effectiveness of overall hygienic practices in the manufacturing facility.

Cell Proliferation Using Suspension Culture

Cells from a qualified cell bank are thawed and placed in sterile culture medium under sterile conditions. The culture is transferred to subsequently larger vessels to accumulate the desired quantity of cells. GOOD Meat states that vessels used for cell expansion and growth are single-use, disposable sterile systems and that bioreactor components which come into direct contact with cultured cells are either single-use, disposable, and sterile or CIP/SIP stainless steel bioreactors.

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

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

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

Harvest of Cell Material

GOOD Meat states that at the end of cell growth, the chicken cells are pelleted using centrifugation and washed to remove media components. GOOD Meat states that sodium chloride solution is used to wash cells.

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

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

GOOD Meat manages risks associated with the introduction of adventitious agents from personnel or the environment through aseptic procedures and monitoring programs discussed at the beginning of the section "Cell Culture Food Production Process," and through the tests

and specifications discussed in the section "Characterization of Harvested Cell Material." Additionally, the firm states that washing is performed in sterile, single-use centrifuges. Safety considerations associated with the use of media components that could be present as residues after washing are discussed in the subsequent section, "Substances Used in Cell Culture Food Production Process."

The firm states that harvested cell biomass is tested for bovine serum albumin and Pluronic F-68[9] after washing to monitor cell wash efficacy. After washing, the harvested cultured cells are stored in food grade packaging and stored at -20°C (or temperatures below) and are then transported to separate food manufacturing facilities for further processing into finished food products.

Substances Used in the Cell Culture Food Production Process

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

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

For each substance, information about the identity and the basis for its safety conclusion and, in certain cases, information about estimated consumer exposure was provided.[10]

The firm's cell culture medium is described as consisting of a basal media, which includes amino acids, vitamins, inorganic salts, antioxidants, and other components, and states that these substances are metabolized and used for the fundamental nutritional requirements of the cells. Additional substances used by the firm during the production phase of cell culture include anti-clumping agents, fetal bovine serum (FBS), and a sodium chloride wash solution. GOOD Meat explains that most of these substances are already widely consumed in the US food supply and many are present in conventional chicken meat. The firm states that the substances listed above are largely removed from the cell material by washing prior to conventional food processing techniques, that residual levels in the product do not present concerns given available toxicological information and existing use in the food supply, and that the substances have no technical or functional effect in the finished food. No antibiotic agents were identified by the firm as being used during the cell culture process.

GOOD Meat describes its general framework for evaluating substances intended for use as components of the culture medium, including consideration of existing authorizations, prior

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[9] As described in the following section and in Appendix B, bovine serum (of which bovine serum albumin is a major component) and Pluronic F-68 are among the substances used in the cell culture production process, and are used as indicator substances to assess wash efficacy.

[10] A complete list of substances was provided by the firm to FDA as supporting, corroborative information in a confidential, supplementary appendix.

use in or presence in food, and anticipated dietary exposure. The firm states that many of the substance uses, in the complete list provided by the firm in a confidential supplementary appendix, are authorized by existing regulations. Substance uses that are not addressed by an existing, authorizing regulation or other authorization are discussed in the disclosable safety assessment. GOOD Meat discusses information such as identity, existing patterns of exposure, toxicological studies, and estimates of consumer exposure informed by batch analysis of its harvested cell material. In addition to its discussion of relevant safety-related information on these components of the culture medium, the firm also considered the estimated intake level derived from their analytical data for each component with reference to levels present in one or more currently consumed comparator foods. This information provided by GOOD Meat is described in more detail in Appendix 2 of this document.

Characterization of Harvested Cell Material

Identity

GOOD Meat describes measures used to assess the identity and phenotypic stability of the cell material at harvest, including a species-specific PCR assay as well as cytogenic analysis and transcriptomic signature analysis. Multiple production runs were verified as containing chicken (*Gallus gallus*) cells through the PCR assay. GOOD Meat states that cytogenic comparison of the parental and harvested cells indicated only minor changes in modal chromosome number (76 to 72). The firm also conducted a comparative analysis of the transcriptomic signature of the parental and harvested cells to characterize physiological or phenotypic changes that may have occurred during culture. The firm reports that observed changes in gene expression appear to relate primarily to cell adaptation to suspension culture and low-serum conditions (e.g., reduced expression of some genes for extracellular proteins such as integrin that are involved in substrate attachment). The conclusion reached by the firm is that the collected data confirms the species identity and stability of its cell line throughout culture adaptation and manufacturing of its product, and that there was no indication of significant changes in gene expression between parental and cultured cells that would raise questions about safety of cultured chicken cells.

Adventitious Agents and Contaminants

GOOD Meat discusses adventitious agent testing for its cultured cell product at harvest, including specified bacteria and fungi as well as virus testing.[11] The firm noted that a European Food Safety Authority (EFSA) hazard analysis for typical avian contaminants identified *Campylobacter* spp. and *Salmonella* serovars as the most significant biological hazards for conventional poultry, along with *E. coli* bearing certain antibiotic resistance genes.

GOOD Meat provided specifications intended for use in routine microbiological testing of each production batch and negative results from six independent batches. Microbial specifications

[11] GOOD Meat also discusses the possibility of hormones being produced or present in cultured chicken cells and notes that it did not detect any hormones in its testing.

include:

- Aerobic plate count (< 1,000 colony-forming units (CFU)/g)
- Yeast (< 100 CFU/g)
- Mold (< 100 CFU/g)
- Coliforms (< 24 most probable number (MPN)/g)
- *E. coli* (< 3 MPN/g)
- *Enterococcus* spp. (< 10 CFU/g)
- *Salmonella* serovars (negative in 25 g)

Microbial testing was performed using methods validated for their intended purposes, demonstrating conformance with the stated specifications. Six production batches were also analyzed using two standardized PCR screening panels (the same used during the establishment of MCB and MWCB stage) for specified human and avian viruses[12] and bacteria, returning negative results for all batches analyzed.

GOOD Meat concluded that *Campylobacter* spp. were not a meaningful food safety risk in the harvested cell material due to the firm's sourcing and production process. To corroborate its assertion, the firm provided negative results from four production batch analyses using a validated method to detect *Campylobacter* spp.

GOOD Meat also provides specifications for toxic heavy metals that are commonly considered in conventional food manufacturing and could potentially be present as contaminants in the harvested cell material, including:

- Arsenic (< 0.10 ppm)
- Lead (< 0.20 ppm)
- Mercury (< 0.05 ppm)
- Cadmium (< 0.02 ppm)
- Chromium (< 0.20 ppm)

Using analytical methods validated for their intended purposes, the firm analyzed six independent production batches for these toxic heavy metals, demonstrating conformance with

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[12] GOOD Meat states that EFSA's hazard analysis identified avian influenza virus, avian leukosis virus (ALV), hepatitis virus, Newcastle disease virus, and fowl adenovirus (FAV) as viruses most commonly found in poultry. Based on this scientific opinion from EFSA, GOOD Meat selected the comprehensive Human and Avian adventitious agent panel at Charles River Laboratories encompassing the majority of these viruses. Although the avian microorganisms included in the panel are not transmissible to humans (avian reticuloendotheliosis virus, avian encephalomyelitis virus, avian leukosis virus A, avian leukosis virus B, avian leukosis virus J, fowl adenovirus 1, fowl adenovirus 3, chicken anemia virus, avian reovirus) with the exception of avian *Salmonella enterica* subsp. *enterica* serovar Pullorum, GOOD Meat states that this testing was included to demonstrate good cell culture practices throughout research and development, as well as during the production process. Further, as described in the "Cell Banking" section, documentation for the cell line includes supplier certification that the line is negative for avian influenza (type A), avian reovirus, avian adenoviruses (groups I-III), avian encephalomyelitis virus, fowl pox, Newcastle disease virus, paramyxovirus (type 2), *Mycoplasma* spp., *Salmonella* serovars, and other infectious agents known to infect poultry stock.

the stated specifications.

Composition

GOOD Meat conducted a compositional analysis of six independent production batches of harvested cell material, including proximates, amino acids, and fats. The firm provided specifications for moisture, protein, fat, ash, and carbohydrate analyses. The firm also provided some comparative data from conventional poultry products including ground chicken and chicken breast. The harvested cell material was washed with a sodium chloride solution, resulting in moisture and sodium content that were somewhat higher in the firm's independent production batches relative to the conventional chicken breast reference data. Dry weight-normalized data on protein percentage was similar between the harvested cell material and conventional chicken breast, and total fat content was modestly higher in the harvested cell material. The relative proportions of saturated, monounsaturated, and polyunsaturated fat were broadly similar, with a modest increase in the proportion of monounsaturated fat in the harvested cell material relative to reference data. Cholesterol content was comparable in analytical batch data of the harvested cell material and the reference data.

FDA's Evaluation

FDA evaluated the information provided by GOOD Meat with respect to the established cell lines, cell banks, substances used in the production process, and properties of the harvested cell material that collectively are the subject of CCC 000001. The primary focus of FDA's evaluation is the information on which the firm relies to conclude that the harvested cell material is safe for use as food and does not contain substances or microorganisms that would adulterate the food.

GOOD Meat provides information on the establishment of the cell lines used to produce the food that is the subject of CCC 000001. FDA considered the information on the source and lineages of the cell lines and the culture adaptation process. We also considered the information provided by GOOD Meat with respect to the observed behavior of the cell lines in culture; as well as other information available to us with respect to the genetic capacity of animal cells to produce toxins or other potentially harmful substances, and the viability of cells following harvest.

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

Finally, though not discussed by the firm, we note that ectopic expression of egg-specific protein allergens is a theoretical possibility given that each cell contained the complete chicken

genome including genetic code for the relevant egg proteins. However, there was neither evidence of cell differentiation patterns consistent with such expression nor a basis for anticipating it based on the methods used to develop the cell line.

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

We did not identify any substance uses that would lead us to question GOOD Meat's conclusion regarding the safety of its food given available information, existing uses or authorizations in food, and anticipated exposure. We noted moderately elevated levels of several nutritional components relative to conventional chicken meat (discussed below); however, the information available to us from GOOD Meat and from the available scientific literature indicates that these components are being used to support primary metabolism in cell culture rather than for inappropriate or indiscriminate food fortification. Regarding the use of any food contact materials throughout the production process, we note that the production conditions described by the firm during culture for food production and immediately subsequent to harvest are consistent with food type 1 (nonacid, aqueous products; may contain salt or sugar or both [pH above 5.0]) and conditions of use type D (hot filled or pasteurized below 66°C) save for post-harvest storage (conditions F or G for refrigeration or frozen storage, respectively). Thus, any food contact materials authorized for these conditions would be appropriate.[13]

FDA reviewed the information that was provided on the identity and composition of the harvested cell material, including genetic and cellular identity, batch test data for constituents and contaminants, and specifications. We considered the analytical data provided by GOOD Meat on the composition of the harvested cell material from several production runs as one element in characterizing the identity of its product, as evidence of the firm's ability to conform to its stated specifications for food contaminants, and as relevant information in evaluating the relationship between the production process described in CCC 000001 and the properties of the harvested cell material produced through that process. We evaluated the firm's specifications for toxic heavy metals to ensure they were as low as reasonably possible and were consistent with levels that are considered safe in food. We also considered information relating to compositional analysis. In all cases, levels of analytes were within the range of those found in commonly consumed foods. We did not consider the establishment of exact equivalence of all nutrients and components relative to a particular conventional comparator as a necessary component of GOOD Meat's safety conclusion, nor did we interpret the analytical data provided by the firm as definitive nutritional information regarding either harvested cell material produced through the process defined in CCC 000001 or food products that contain this material.

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

Conclusions

Based on our evaluation of the data and information that GOOD Meat provides in CCC 000001, as well as other information available to FDA, we did not identify a basis for concluding that the production process as described would be expected to result in food that bears or contain any substance or microorganism that would adulterate the food. We have no questions at this time about GOOD Meat's conclusion that foods comprised of, or containing, cultured chicken cell material resulting from the production process defined in CCC 000001 are as safe as comparable foods produced by other methods.

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Katie Overbey

Appendix 1: Summary of potential identity, quality, and safety issues

Process Step	Potential Issue	Management Strategy
Cell Procurement	Cell identity	Supplier documentation
	Cells are contaminated with bacteria or adventitious viruses from source animal, reagents, or environment	Aseptic procedures, supplier certification, supply chain control
Establishment of Cell Lines	Cell identity	Genetic testing
	Facility environment contamination	Aseptic procedures, environmental monitoring, validated sanitation procedures
	Introduction of contaminants from animal-derived reagents (e.g., bovine serum)	Aseptic procedures, BSE-free certification, sterile filtration, supply chain control, testing program
	Cells do not display expected growth profile	Monitor growth and viability
	Unintended effects of immortalization	Supplier documentation
Establishment of MCB and MWCB	Cells from different line or species inadvertently used	Documentation, genetic testing
	Cells do not display expected growth profile	Monitor growth and viability, stability testing
	Introduction of contaminants from animal-derived reagents (e.g., bovine serum)	Aseptic procedures, BSE-free certification, sterile filtration, supply chain control, testing program
	Facility environment contamination	Aseptic procedures, release testing, environmental monitoring, validated sanitation procedures
	Contamination with adventitious agents such as bacteria, fungi, viruses, parasites, and prions from original source	Aseptic procedures, release testing
	Cell bank stability	Stability testing
Cell Proliferation using Suspension Culture	Contamination during transfer	Aseptic procedures, validated sanitation procedures
	Contamination with adventitious agents from culture media components	Aseptic procedures, sterile filtration, supply chain control, validated sanitation procedures
	Contamination with adventitious agents through	Sterile equipment, validated sanitation procedures,

Process Step	Potential Issue	Management Strategy
	inadequate sterilization of bioreactors	environmental monitoring
	Introduction of media components that could persist as residues in harvested cells	Final wash step, food safety assessment, residual testing
	Introduction of media components that could accumulate in the cells before harvest	Food safety assessment, compositional analysis at harvest
	Facility environment contamination	Validated sanitation procedures, environmental monitoring
Harvest of Cell Material	Presence of bacterial or viral contaminants from culture process	Culture monitoring, sterility testing, specifications
	Migration of contaminants from food contact materials	Use of authorized food contact materials
	Presence of residual media components after harvest	Final wash step, food safety assessment, residual testing
	Presence of elemental contaminants (metals) after harvest	Testing, specifications
	Facility environment contamination	Validated sanitation procedures, environmental monitoring

Appendix 2: Additional Information Regarding Certain Substances Used in the Cell Culture Food Production Process

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

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

GOOD Meat describes its general framework for evaluating substances intended for use as components of the culture medium, including consideration of existing authorizations, prior use in or presence in food, and anticipated dietary exposure. A complete list of substances was provided by the firm in a confidential supplementary appendix, and the firm states that many of the substances are authorized for use in food by existing regulations. Substance uses that are not addressed by an existing, authorizing regulation or other authorization are discussed in the disclosable safety assessment. GOOD Meat discusses information such as identity, existing patterns of exposure, toxicological studies, and estimates of consumer exposure informed by batch analysis of its harvested cell material. In addition to its discussion of relevant safety-related information on these components of the culture medium, the firm also considered the estimated intake level derived from their analytical data for each component with reference to levels present in one or more currently consumed comparator foods.

Nutrients used to support primary cell metabolism

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

Folic acid is used as a nutrient to support primary cell metabolism in culture. Folic acid is an oxidized form of folate, a class of water-soluble B vitamins that play a key role in DNA and RNA synthesis and in DNA methylation. Folate is present in all cells and many foods, including various vegetables and legumes, which provide substantial quantities of folate in the diet. Folic acid is a regulated food additive for use in fortification of specified foods at limited use levels. GOOD Meat notes that folic acid is converted to folate by the cells in culture, reports that neither folic acid nor folate are detectable above the limit of quantitation, and that if it was conservatively assumed that folate was present at the limit of quantitation, its harvested cells would contain levels similar to conventional chicken breast, which is considered a poor source of folate.

Ferric nitrate is used as a nutrient to support primary cell metabolism in culture. While ferric nitrate itself is not the subject of an existing food ingredient authorization, the firm notes that the compound dissociates in aqueous solutions to ferric (iron (III)) and nitrate ions which are components of other substances permitted for use in food and are naturally present in many

foods. Average iron intake from foods and supplements is more than 13 mg/d for all age groups. The firm's analytical data indicates that a typical iron value for 100 grams of its harvested cell material is 80 micrograms and that this value is substantially lower than the iron content of comparable conventional chicken products. The firm also analyzed the nitrate content of its harvested cell material and found the result (2 mg/100 g) comparable to the level reported for fresh poultry meat. The firm notes that nitrates are present in green leafy vegetables at 250 mg/100 g or higher, and that the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) has established an acceptable daily intake (ADI) for nitrates equivalent to over 200 mg/d for an adult.

Hypoxanthine, monosodium salt, a purine derivative, is used as a nutrient to support primary cell metabolism in culture. GOOD Meat states that hypoxanthine is present in a wide variety of foods including chicken, citing a reported aggregate value of 80.6 mg/100 g for poultry products. The firm analyzed the hypoxanthine content of its harvested cell material and reports an average value of 46.87 mg/100 g, consistent with the value cited above as well as other reported literature values for conventional raw chicken.

Lipoic acid is used as a nutrient to support primary cell metabolism. GOOD Meat notes that lipoic acid plays a role in mitochondrial energy metabolism, is synthesized by plants and animals endogenously, and may also be absorbed from dietary sources. The firm discusses several toxicological studies, including a 2-year rat study that reported no adverse effects at the highest administered level of 60 mg/kg body weight (bw)/d.[14] The firm cites a study reporting an endogenous lipoic acid level of approximately 20 mg/kg in chicken thigh meat. The firm's analytical data indicates that the lipoic acid content of its harvested cell material is below the limit of quantitation of the assay employed. The firm further states that based on mean lipoic acid content in chicken broiler thigh meat reported in the literature and assumption of lipoic acid being present at the limit of quantitation of the assay in its harvested cell material, the lipoic acid content would be equivalent in both conventional chicken meat and GOOD Meat's harvested cell material.

Putrescine dihydrochloride, a polyamine, is used as a nutrient to support primary cell metabolism. GOOD Meat notes that putrescine is widely present in foods, including vegetables, meat products, cheese, and seafood. The firm cites a subacute rat study that identified a no observed adverse effect level of 180 mg/kg bw/d[15] for putrescine. An exposure assessment conducted by EFSA reported that the background cumulative 95th percentile one-day intake of putrescine from all food sources, calculated across several European countries, ranged between 35 and 138 mg/d. Raw conventional chicken was reported to contain an average of 0.286

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[14] After application of a 100-fold safety factor, this would be equivalent to an intake of 36 mg/d for an adult.

[15] After application of a 100-fold safety factor, this would be equivalent to an intake of 108 mg/d for an adult. FDA notes that it may be appropriate to use an additional safety factor in some cases where subacute studies are used to assess chronic exposure, as occurs here. However, in this particular case, other considerations include the assumption that all detectable polyamines would be putrescine, as well as existing patterns of exposure from the diet.

mg/100 g putrescine, and 7.14 mg/100 g of total polyamines. The firm analyzed total polyamines in its harvested cell material and reported an average value of 1.54 mg/100 g. The firm notes that the total polyamine content of its harvested cell material is well below that of conventional chicken products, and states that even if all the polyamine content was conservatively assumed to be putrescine, the contribution to total intake would be both small and well below an ADI derived from the cited study.

Sodium pyruvate is used as a nutrient to support primary cell metabolism. In aqueous solution, sodium pyruvate dissociates to sodium ions and pyruvate ions, the latter a key metabolite in the glycolytic pathway present in all cells. GOOD Meat notes that while pyruvic acid is present in all cells, particularly high concentrations can be found in certain plants such as onion, leek, shallot, and garlic. The firm also cites a study reporting pyruvate concentration in chicken blood of approximately 19.5 µg/g. The firm states that pyruvic acid is approved as a food additive by FDA for use at a flavoring substance, has been consumed in multiple clinical studies at levels of approximately 5 g/d without reported adverse effects, and that an EFSA review considered pyruvic acid intake of up to 3.4 g/d "not of safety concern." The firm analyzed pyruvic acid content of its harvested cell material and reported an average value of 1.22 mg/100 g, noting that typical flavor use levels are similar.

Thymidine is used as a nutrient to support primary cell metabolism. Thymidine occurs naturally in all cells, is used in DNA synthesis, and consists of thymine attached to the sugar deoxyribose. GOOD Meat notes that as a component of DNA, thymidine is consumed in a broad array of foods and its absorption, metabolism, and excretion are well understood. The firm cites data indicating that thymine, the key component of thymidine, is abundant in plant tissues such as broccoli and cauliflower but much lower in animal tissue products. It also notes that an evaluation by the European Chemicals Agency of thymidine included acute, chronic, and developmental toxicity studies in rats, and that these studies reported no adverse effects at the highest level tested in each case (1000 mg/kg bw/d or more). The firm analyzed the thymidine content of its harvested cell material and reports that thymidine content was near or below the limit of quantitation of the assay and comparable to the thymine content reported in conventional animal tissue products including chicken.

Substances to manage properties of the culture media

Pluronic F-68 is used as a surfactant to reduce clumping in cell culture. Also known as Poloxamer 188, this substance is a non-ionic polyoxyethlyene-polyoxypropylene block polymer commonly used in cell culture to (i) control shear forces in suspension cultures; (ii) reduce cell attachment to glass; and (iii) reduce foaming in the culture. The substance is also used in a variety of other applications such as an excipient in drugs. GOOD Meat cites studies on absorption, metabolism, and excretion of Pluronic F-68 in humans and other species following intravenous exposure, as well as acute and chronic oral toxicity studies in rats. No adverse effects were reported at a chronic exposure of 1500 mg/kg bw/d.[16] The firm analyzed its harvested cell material and was unable to detect Pluronic F-68. In the most conservative scenario conducted by the firm where it is assumed that Pluronic F-68 is present at the level of

[16] After application of a 100-fold safety factor, this would be equivalent to 900 mg/d for an adult.

detection of the assay, no more than 10 mg would be present per 100 grams of harvested cell material. GOOD Meat notes that this amount would represent less than 5% of the ADI that could be derived from the studies it cites.

Substances intended to support cell proliferation in culture

FBS is used to support cell proliferation in culture. Serum is the fraction of blood from which cells and clotting factors have been removed. It contains a variety of proteins and other substances that are present in blood, and which can provide support for cell proliferation in culture. GOOD Meat notes that bovine serum albumin is the major component of FBS, representing 50-60% of the total serum proteins, and was thus selected as a proxy analyte for quantification. The firm notes that serum is an inherent part of blood-based food products such as blood sausages, and that serum components including serum albumin are present in a wide variety of animal-derived foods, including chicken meat. The firm also discusses a variety of food uses for plasma (the blood fraction from which cells but not clotting factors have been removed), which is closely related in composition to serum, including use as a binder and thickener in meat products. The harvested cell material was analyzed by the firm for the presence of bovine serum albumin. The firm reports an average level of 1.57 mg/100 g, comparing this to a reported cow milk concentration of approximately 19 mg/100 g. GOOD Meat notes that FBS is also a source of other proteins as well as electrolytes, lipids, carbohydrates, hormones, enzymes, among other constituents present in low concentrations. The firm states that considering the initial low levels of these components in FBS, the low concentration of FBS used in the culture media and similar consumption and washing out ratio observed for BSA during culture, the residual content of these other components is negligible and does not represent a safety concern for human consumption. The firm also states that any growth factors present in FBS are heat labile molecules, that their activity is reduced during cell culture at physiological temperatures, and that frozen storage and subsequent cooking will affect the stability and activity of any residual growth factor that might be present in the firm's food product. In the remote possibility of residual growth factors preserving high activity levels during these multiple temperature shifts, the firm notes the residual growth factors would lose stability during their passage through the digestive system, especially as they go through the low pH observed in the stomach (pH 1-2.5).